



UNIVERSITI PUTRA MALAYSIA

***PRELIMINARY STUDY OF THE ANTIBACTERIAL ACTIVITY OF
FERMENTED PLANT EXTRACTS AGAINST ESCHERICHIA COLI AND
BACILLUS SUBTILIS***

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CERTIFICATION

This project report entitled 'Preliminary study of antibacterial activity of fermented plant extracts against *Escherichia coli* and *Bacillus subtilis*' was prepared by Nur Fitrah bt Mat Zainal and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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TABLE OF CONTENTS

| | |
|---|------|
| CERTIFICATION | i |
| ACKNOWLEDGEMENT | ii |
| TABLE OF CONTENTS | iii |
| LIST OF TABLES | v |
| LIST OF FIGURES | vi |
| LIST OF ABBREVIATIONS | vii |
| ABSTRACT | viii |
| ABSTRAK | ix |
| CHAPTER 1 | |
| INTRODUCTION | 1 |
| 1.2 Problem statement | 2 |
| 1.3 Objectives of the research | 2 |
| CHAPTER 2 | |
| LITERATURE REVIEW | 3 |
| 2.1 <i>Escherichia coli</i> | 3 |
| 2.1.1 History of <i>Escherichia coli</i> | 3 |
| 2.1.2 Morphology of <i>Escherichia coli</i> | 4 |
| 2.1.2 Biology of <i>Escherichia coli</i> | 4 |
| 2.1.3 Ecology of <i>Escherichia coli</i> | 5 |
| 2.2 <i>Bacillus subtilis</i> | 7 |
| 2.2.1 History of <i>Bacillus subtilis</i> | 7 |
| 2.2.2 Morphology of <i>Bacillus subtilis</i> | 8 |
| 2.2.2 Biology of <i>Bacillus subtilis</i> | 8 |
| 2.2.3 Ecology of <i>Bacillus subtilis</i> | 9 |
| 2.3 Sources of fermented plant extracts | 11 |
| 2.3.1 Source of fermented plant extracts from <i>Citrullus lanatus</i> | 12 |
| 2.3.2 Source of fermented plant extracts from <i>Murraya koenigii</i> | 13 |
| 2.3.3 Source of bioenzyme from of <i>Etlingera elatior</i> | 15 |
| 2.3.4 Source of bioenzyme from <i>Cymbopogon citrates</i> | 16 |
| CHAPTER 3 | |
| MATERIAL AND METHODS | 18 |
| 3.1 Preparation of fermented plant extracts | 18 |
| 3.2 Concentration of the fermented plant extracts by using freeze dry technique | 18 |
| 3.3 Preparation of the bacterial isolates | 19 |
| 3.4 Disc diffusion method | 19 |
| 3.5 Measuring the zone of inhibition | 20 |
| CHAPTER 4 | |
| RESULTS | 21 |
| 4.1 Control Measures | 21 |
| 4.3 Antibacterial activity of fermented plant extracts against <i>Bacillus subtilis</i> | 26 |
| CHAPTER 5 | |

| | |
|------------|----|
| DISCUSSION | 32 |
| CHAPTER 6 | |
| CONCLUSION | 36 |
| REFERENCES | 37 |
| APPENDICES | 46 |



LIST OF TABLES

| Table | Title | Page |
|-----------|--|------|
| Table 1: | Source: Microbiology, An Introduction, 11th edition (authors: Tortora, Funke, and Case) | 3 |
| Table 2: | Source : Microbiology, An Introduction, 11th edition (authors: Tortora, Funke, and Case) | 7 |
| Table 3 : | Mean zone of inhibition for the control plate | 21 |
| Table 4: | Effect of fermentation plant extracts against <i>E. coli</i> | 22 |
| Table 5: | Effectiveness of difference sources of fermented plant extracts in 2 mg/ml compared to 10 mg/ml concentration for <i>E. coli</i> | 23 |
| Table 6: | Dose response of fermented plant extracts on <i>E. coli</i> | 24 |
| Table 7: | Effect of fermentation plant extract against <i>B. subtilis</i> | 27 |
| Table 8: | Effectiveness of difference sources of fermented plant extracts in 2 mg/ml compared to 10 mg/ml concentration for <i>B. subtilis</i> | 28 |
| Table 9: | Dose response of fermented plant extracts on <i>B. subtilis</i> | 29 |

LIST OF FIGURES

| Figure | Title | Page |
|-----------|---|------|
| Figure 1: | Zone of inhibition for <i>E. coli</i> . A) FPECL in 2 mg/ml, B) FPECL in 10 mg/ml, C) FPECC in 2 mg/ml, D) FPECC in 10 mg/ml. | 25 |
| Figure 2: | Zone of inhibition for <i>E. coli</i> . A) FPEMK in 2 mg/ml, B) FPEMK in 10 mg/ml, C) FPEEE in 2 mg/ml, D) FPEEE in 10mg/ml. | 26 |
| Figure 3: | Zone of inhibition for <i>B. subtilis</i> A) FPECL in 2 mg/ml, B) FPECL in 10 mg/ml, C) FPECC in 2 mg/ml, D) FPECC in 10 mg/ml. | 30 |
| Figure 4: | Zone of inhibition for <i>B. subtilis</i> A) FPEMK in 2 mg/ml, B) FPEMK in 10 mg/ml, C) FPEEE in 2 mg/ml, D) FPEEE in 10 mg/ml. | 31 |

LIST OF ABBREVIATIONS

| | |
|--------|--|
| FPE | Fermented plant extract |
| FPEMK | Fermented plant extract <i>Murraya koenigii</i> |
| FPEEEE | Fermented plant extract <i>Etilingera elatior</i> |
| FPECC | Fermented plant extract <i>Cymbopogon citrates</i> |
| FPECL | Fermented plant extract <i>Citrullus lanatus</i> |

ABSTRACT

Fermented plant extracts from 4 sources of plants *Murraya koenigii*, *Etlingera elatior*, *Cymbopogon citrates* and *Citrullus lanatus* were examined for their antibacterial activity against *Escherichia coli* and *Bacillus subtilis* by using disc diffusion technique. Fermented plant extracts from organic waste is a product produced from fermentation of organic solid waste and it can be used as surfactants, liquid fertilizer, antimicrobial agents, treatment of domestic wastewater, municipal and industrial sludge treatment. Among all of the fermented plant extracts tested, all show positive response to antibacterial activity by showing zone of inhibition after incubation period towards those bacterial strains except for *E. elatior* that did not show any activity towards *B. subtilis*. The results shown suggested a potential antimicrobial activity of the fermented plant extracts which may find its application to produce environmental friendly products that act as a surfactant in order to reduce cross contamination in laboratory to occur.

ABSTRAK

Ekstrak tumbuhan yang diperolehi daripada proses penapaian daripada 4 tumbuhan iaitu *Murraya koenigii*, *Etlingera elatior*, *Cymbopogon citrate* dan *Citrullus lanatus* telah diuji untuk aktiviti antibakteria terhadap *Escherichia coli* dan *Bacillus subtilis* dengan menggunakan teknik cakera resapan. Ekstrak tumbuhan yang ditapai daripada sisa organik adalah produk yang dihasilkan daripada proses penapaian sisa pepejal organik dan ia boleh digunakan sebagai bahan permukaan, baja cecair, agen antimikrob, rawatan air sisa domestik, rawatan kumbahan perbandaran dan perindustrian. Di antara semua ekstrak tumbuhan yang ditapai, apabila diuji, semua menunjukkan tindak balas positif kepada aktiviti anti-bakteria dengan menunjukkan zon perencatan selepas tempoh pengesanan terhadap bakteria yang diuji. Kecuali *E. elatior* yang tidak menunjukkan aktiviti kepada *B. subtilis*. Hasil yang diperolehi oleh aktiviti antimikrob daripada ekstrak tumbuhan yang ditapai boleh dikaji akan datang untuk menghasilkan produk mesra alam yang berfungsi sebagai surfactant untuk mengurangkan pencemaran silang di makmal untuk berlaku

CHAPTER 1

INTRODUCTION

At the point when working in a microbiology research facility, aseptic procedure is one of the key to the achievement of the experiment, and safety of an experiment. These aseptic systems are techniques that were performed by researchers under sterile conditions to guarantee that microbial contaminants do not hurt partners and are not brought into sterile arrangements, supplies, or other experimental culture.

Prevention of cross contamination by other microorganism can result in the success of propagation of any microbial strain. There are many sources of contamination such as non-sterile supplies, media, reagents, unclean work surfaces, airborne particles and also unclean gloves. Microscopic organisms are discovered all over the place. While most species are beneficial, some are destructive and even pathogenic. Chemical and physical agents might be utilized to control the bacterial growth and development.

There are a lot of ways that commonly been used in order to keep the laboratory from any infection of microorganism such as sterilization, disinfectant, decontamination, antiseptis and sanitization. Most of the common ways to kill the organism in laboratory are using chemical. Nowadays people are searching for more environmental friendly products that will contribute to less pollution to the earth. One of the potential environmental friendly products that can be further investigate is by using fermented plant extract prepared from organic waste products.

Fruit, vegetable markets and food processing industries create decomposable waste such as vegetables and fruit peels in a very high quantity. There is a need to manage this organic waste properly since it has become one of the big issues throughout the world.

Greenhouse gases like methane and nitrous oxide can be the result of disposal of the kitchen waste. In order to solve this problem the decomposable waste can be turned into value added product which can reduce production of greenhouse gas. Dr. Rosukon from Thailand introduced the use of organic solid waste in 2006 and named it as garbage enzyme. The process of fermentation of waste fruits and vegetables or peels, together with brown sugar and water can produce complex organic enzyme (Arun, 2014). This organic enzyme could degrade molecules in a short period of time and could be used as antimicrobial agent.

1.2 Problem statement

Cross contamination in the laboratory often occurs and can disrupt the on-going experiments. The common used control measure is by using chemicals. In order to reduce the harmful effect to the environment, an organic product such as fermented plant extracts can be used as substitute of chemical treatment.

1.3 Objectives of the research

To examine antibacterial activity of fermented plant enzyme common bacteria used for teaching class which are isolates of *Escherichia coli* and *Bacillus subtilis*.

REFERENCES

- Ajuru, M. G., & Okoli, B. E. (2013). The morphological characterization of the melon species in the family cucurbitaceae juss., and their utilization in Nigeria. *International Journal of Modern Botany*, 3(2), 15-19.
- Al Harbi, H., Irfan, U. M., & Ali, S. The antibacterial effect of curry leaves (*Murraya Koenigii*).
- Alexander, M. (1978). Introduction to soil microbiology. *Soil Science*, 125(5), 331.
- Allan, E. J., Amijee, F., Tyson, R. H., Strang, J. A., Innes, C. M., & Paton, A. M. (1993). Growth and physiological characteristics of *Bacillus subtilis* L-forms. *The Journal of applied bacteriology*, 74(5), 588-594.
- Arun, C., & Sivashanmugam, P. (2015). Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge. *Process Safety and Environmental Protection*, 94, 471-478.
- Asaolu, M. F., Oyeyemi, O. A., & Olanlokun, J. O. (2009). Chemical compositions, phytochemical constituents and in vitro biological activity of various extracts of *Cymbopogon citratus*. *Pakistan Journal of Nutrition*, 8(12), 1920-1922.
- Bais, H. P., Fall, R., & Vivanco, J. M. (2004). Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant physiology*, 134(1), 307-319.
- Balakrishnan, B., Paramasivam, S., & Arulkumar, A. (2014). Evaluation of the lemongrass plant (*Cymbopogon citratus*) extracted in different solvents for antioxidant and antibacterial activity against human pathogens. *Asian Pacific Journal of Tropical Disease*, 4, S134-S139.

- Balouiri, M., Sadiki, M., & Ibsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71-79.
- Chan, E. W. C., Lim, Y. Y., and Omar, M. (2007). Antioxidant and antibacterial activity of leaves of *Etilingera* species (Zingiberaceae) in Peninsular Malaysia. *Food Chemistry*, 104(4), 1586-1593.
- Chiang, E. C. W., Yan, L. Y., and Ali, N. A. M. (2010). Composition and antibacterial activity of essential oils from leaves of *Etilingera* species (Zingiberaceae). *Int. J. Adv. Sci. Art*, 1(2), 1-12.
- Choon, S. Y., Ding, P., Mahmud, T. M. M., & Shaari, K. (2016). Phenological Growth Stages of Torch Ginger (*Etilingera elatior*) Inflorescence. *Pertanika Journal of Tropical Agricultural Science*, 39(1).
- Choudhary, A. P. Study of the nutritional value and antimicrobial activity of juice extracted from watermelon waste. *IJCBS Research Paper*. 1(1): 24.
- Dombek, K. M., & Ingram, L. O. (1984). Effects of ethanol on the *Escherichia coli* plasma membrane. *Journal of bacteriology*, 157(1), 233-239.
- Earl, A. M., Losick, R., & Kolter, R. (2008). Ecology and genomics of *Bacillus subtilis*. *Trends in microbiology*, 16(6), 269-275.
- El Zawawy, N. A. (2015). Antioxidant, antitumor, antimicrobial studies and quantitative phytochemical estimation of ethanolic extracts of selected fruit peels. *Int. J. Curr. Microbiol. App. Sci*, 4(5), 298-309.
- Errington, J. (2003). Regulation of endospore formation in *Bacillus subtilis*. *Nature Reviews Microbiology*, 1(2), 117-126.

- Facey, P. C., Pascoe, K. O., Porter, R. B., & Jones, A. D. (1999). Investigation of Plants used in Jamaican Folk Medicine for Anti-bacterial Activity. *Journal of Pharmacy and Pharmacology*, 51(12), 1455-1460.
- Fotadar, U., Zaveloff, P., & Terracio, L. (2005). Growth of *Escherichia coli* at elevated temperatures. *Journal of basic microbiology*, 45(5), 403-404.
- Gillen, A. and Oliver. (2010). The Genesis of Pathogenic *E. coli*. <https://answersingenesis.org/biology/microbiology/the-genesis-of-pathogenic-e-coli/>. (Accessed : November 22th, 2016).
- Green, D. H., Wakeley, P. R., Page, A., Barnes, A., Baccigalupi, L., Ricca, E., & Cutting, S. M. (1999). Characterization of Two *Bacillus* Probiotics. *Applied and environmental microbiology*, 65(9), 4288-4291.
- Hannah, A., & Shanmugasundaram, K. (2015). Qualitative phtochemistry profile of watermelon (*Citrullus vulgaris schrad*) rind extracts with different solvents. *Asian Journal of Pharmaceutical and Clinical Research*, 8(4), 62-65.
- Harikumar, P. S., & Manjusha, C. M. (2013). Study on the antibacterial activity of selected natural herbs and their application in water treatment. *Drinking Water Engineering and Science Discussions*, 6(2), 199-231.
- Hegde, A., Bhat, G. K. and Mallya, S. (2008). Effect of exposure to hydrogen peroxide on the virulence of *Escherichia coli*. *Indian Journal of Medical Microbiologists* 26(1): 25-28.
- Hendriksen, R. S. (2003). MIC susceptibility testing of *Salmonella* and *Campylobacter*. A global *Salmonella* surveillance and laboratory support project of the World Health Organization.

- Hindumathy, C. K. (2011). In vitro study of antibacterial activity of *Cymbopogon citratus*. *Biotechnology and Bioengineering*, 26, 13551.
- Hong, H. A., & Cutting, S. M. (2005). The use of bacterial spore formers as probiotics. *FEMS microbiology reviews*, 29(4), 813-835.
- Hu, A. (2002). Looks can be deceiving: the case of *Escherichia coli*. *Journal of Young Investigators*.
- Hufnagel, D. A., Depas, W. H., & Chapman, M. R. (2015). The Biology of the *Escherichia coli* Extracellular Matrix. *Microbiology spectrum*, 3(3).
- Ingledeu, W. J., & Poole, R. K. (1984). The respiratory chains of *Escherichia coli*. *Microbiological reviews*, 48(3), 222.
- Irobi ON1, Daramola SO (1994). Bactericidal properties of crude extracts of *Racarpu villosus*. *Journal of Enthopharmacology*.
- Ishii, S., Ksoll, W. B., Hicks, R. E., & Sadowsky, M. J. (2006). Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Applied and environmental microbiology*, 72(1), 612-621.
- Jang, H. H., Ann, S. H., Kim, M. D., & Kim, C. W. (2008). Use of hydrogen peroxide as an effective disinfectant to *Actinobacillus ureae* (vol 43, pg 225, 2008). *Process biochemistry*, 43(9), 1018-1018.
- Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic *Escherichia coli*. *Nature Reviews Microbiology*, 2(2), 123-140.
- Larsen, K., Ibrahim, H., Khaw, S. H., & Saw, L. G. (1999). *Gingers of peninsular Malaysia and Singapore*. Natural History Publications (Borneo).

- Lalitha, M. K. (2004). Manual on antimicrobial susceptibility testing. Performance standards for antimicrobial testing: Twelfth Informational Supplement, 56238, 454-456.
- Lambert, P. A. (2002). Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. *Journal of applied microbiology*, 92(s1).
- Lim, C. K. (2001). Taxonomic notes on *Etilingera Giseke* (Zingiberaceae) in Peninsular Malaysia: the "Achasma" taxa, and supplementary notes on the "Nicolaia" taxa. *Folia Malaysiana*, 2(3), 141-178.
- Lin, C. S. K., Pfaltzgraff, L. A., Herrero-Davila, L., Mubofu, E. B., Abderrahim, S., Clark, J. H., & Thankappan, S. (2013). Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. *Energy & Environmental Science*, 6(2), 426-464.
- Maheswani, N. U., & Cholarani, N. (2013). Pharmacognostic effect of leaves extracts of *Murraya koenigii* Linn. *Journal of chemical and pharmaceutical Research*, 5(4), 120-123.
- Mainil, J. (2013). *Escherichia coli* virulence factors. *Veterinary Immunology and Immunopathology*, 152(1), 2-12.
- Martin, J. G. P., Porto, E., Corrêa, C. B., Alencar, S. M., Gloria, E. M., Cabral, I. S. R., & Aquino, L. M. (2012). Antimicrobial potential and chemical composition of agro-industrial wastes. *Journal of Natural Products*, 5(2).
- Naik, M. I., Fomda, B. A., Jaykumar, E., & Bhat, J. A. (2010). Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacterias. *Asian Pacific Journal of Tropical Medicine*, 3(7), 535-538.

- Negrelle, R. R. B., & Gomes, E. C. (2007). *Cymbopogon citratus* (DC.) Stapf: chemical composition and biological activities. *Rev Bras Pl Med*, 9(1), 80-92.
- Nithya, T. G. and Aminu I. M. (2015). Antibacterial activity of *Murraya koenigi* leaves against Urinary Tract Infection causative pathogens. *International Journal of PharmTech Research* 8(8): 112-117.
- Ochiai, A., Itoh, T., Kawamata, A., Hashimoto, W and Murata, K. (2007). Plant cell wall degradation by saprophytic *Bacillus subtilis* strains: gene clusters responsible for rhamnogalacturonan depolymerization. *Journal of Applied Environmental Microbiology* 73(12): 3803–3813.
- Parul, S., Javed, A., Neha, B., Honey, J., and Anuj, B. (2012). Curry Leaves – A Medicinal Herb. *Asia pharmaceutical press* 2(2): 51-53.
- Peeters, J. E., Charlier, G. J., & Raeymaekers, R. (1985). Scanning and transmission electron microscopy of attaching effacing *Escherichia coli* in weanling rabbits. *Veterinary Pathology Online*, 22(1), 54-59.
- Perez, A. R., Abanes-De Mello, A., & Pogliano, K. (2000). SpoIIB localizes to active sites of septal biogenesis and spatially regulates septal thinning during engulfment in *Bacillus subtilis*. *Journal of bacteriology*, 182(4), 1096-1108.
- Pike, R. M. (1976). Laboratory-associated infections: summary and analysis of 3921 cases. *Health Lab Sci* 13(2):105-14.
- Pleissner, D., & Lin, C. S. K. (2013). Valorisation of food waste in biotechnological processes. *Sustainable chemical processes*. 1(1).

- Priyadarshini, S. S., Vadivu, R., & Jayshree, N. (2010). Hypolipidaemic and Renoprotective study on the Ethanolic & Aqueous extracts of leaves of *Ravenala madagascariensis* Sonn. on alloxan induced diabetic rats. *International J Pharm Sci*, 2, 44-50.
- Rahman, B. (2013). *Phytochemical investigation of Citrullus lanatus (Watermelon) rind* (Doctoral dissertation, East West University).
- Randall S. Edson and Terrell, C. L. (1999). The Aminoglycosides. *Mayo Clinic Proceedings* 74(5): 519–528
- Reller, L. B., Weinstein, M., Jorgensen, J. H., & Ferraro, M. J. (2009). Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clinical infectious diseases*, 49(11), 1749-1755.
- Savageau, M. A. (1983). *Escherichia coli* Habitats, Cell Types, and Molecular Mechanisms of Gene Control. *The American Naturalist*.
- Sawyer, E. (1982). Traditional medicine in Sierra Leone - a critical appraisal. *Nigerian Journal of Pharmacy* 13: 28-33.
- Schaechter, M., Ingraham, J. L. and Neidhardt, F. C. (2006). *Microbe* 1st Edition. Washington: ASM Press.
- Sewell, D. L. (2006). Laboratory-Acquired Infections: Are Microbiologists at Risk? *Clinical Microbiologist Newsletter* 28(1): 1–6.
- Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. (2011). Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass). *Journal of advanced pharmaceutical technology & research*, 2(1), 3.

- Simon, M. I., Emerson, S. U., Shaper, J. H., Bernard, P. D., & Glazer, A. N. (1977). Classification of *Bacillus subtilis* flagellins. *Journal of bacteriology*, 130(1), 200-204.
- Singh, S., Omreb, P. K. and Mohan, S. M. (2014). Curry Leaves (*Murraya koenigii* Linn. Sprengal)- A Miracle Plant. *Indian Journal Science* 4(1): 46-52.
- Sinha, R. K.(2009). Human waste – a potential resource converting trash into treasure by embracing the 5R's philosophy for safe and sustainable waste management. *Environmental Resource Journal* 3(3): 143: 204.
- Skerman, V.B.D., McGowan, V. and Sneath PHA(1980). Approved Lists of Bacterial Names . *International Journal of Systematic and Evolutionary Microbiology* 30: 225-420.
- Subramanian Jo Thy Lachumy, Sasidharan, S. B., Sumathy, V. A. and Zuraini, Z. (2010). Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etilingera elatior* (torch ginger) flowers. *Asian Pacific Journal of Tropical Medicine* 3(10): 769-774.
- Tam, N. K., Uyen, N. Q., Hong, H. A., Duc, L. H., Hoa, T. T., Serra, C. R., ... & Cutting, S. M. (2006). The intestinal life cycle of *Bacillus subtilis* and close relatives. *Journal of bacteriology*, 188(7), 2692-2700.
- Tirumalasetty. J., Basavaraju, A. and Praveena (2014). Antimicrobial activity of methanolic extracts of *Azadirachta indica*, *Rosmarinus officinalis* and *Lagenaria siceraria* leaves on some important pathogenic organisms . *Journal of Chemical and Pharmaceutical Research*. 6(4):766-770.
- Todar, K. (2012). The Good, the Bad, and the Deadly. *Science Magazine* Vol 304: 1421.
- Tortora, G. J., Funke, B. R. and Case, C. L. *Microbiology, An Introduction* (11th edition).

Wanke, C. A. (2001). To Know *Escherichia coli* Is to Know Bacterial. *Clinical Infectious Diseases* 32 (12): 1710-1712.

Wang, J. D., & Levin, P. A. (2009). Metabolism, cell growth and the bacterial cell cycle. *Nature Reviews Microbiology*, 7(11), 822-827.

Weinstein, R. A., & Hota, B. (2004). Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection?. *Clinical infectious diseases*, 39(8), 1182-1189.

Welman, M. (2014, October). *Citrullus lanatus* (watermelon). <http://www.plantzafrica.com/plantcd/citrullanat.html>. (Accessed : November 27th, 2016).

Zuber, Z. and Nakano, N. M. (1998). Anaerobic growth of a *Bacillus subtilis*. *Annual Review of Microbiology* 52: 165-19.