UNIVERSITI PUTRA MALAYSIA

ANTIBACTERIAL ACTIVITY OF SEAWEED EXTRACT AND ITS EFFECTS ON THE DNA SEQUENCE OF SELECTED ESSENTIAL GENES OF *Staphylococcus aureus*

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By

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March 2008

Chairman: Associate Professor Mariana Nor Shamsudin, PhD

Faculty : Medicine and Health Sciences

Mortality rate caused by bacteria infections is increasing to nearly 20 million deaths each year, world wide. One of the common causes contributing to the death is the increasing number of antibiotic resistance pathogens including Methicillin Resistant *Staphylococcus aureus* (MRSA), Extended Spectrum Beta Lactamase (ESBL) organisms, and Multiple Drug Resistant Organism (MDRO). Therefore, this study was designed to explore an alternative antibacterial product derived from seaweed extracts, *Gracilaria changii* and *Euchema denticulatum*, through several approaches including bioassays and molecular biology tools especially the study of DNA and RNA encoding genes of interest in MRSA and non-MRSA.

Bioassay studies revealed that *G. changii* and *E. denticulatum* extracts showed inhibitory activity only on gram positive organisms tested including *S. aureus* and *Streptococcus pyogenes* which were expressed in terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test. Thus,

gram negative pathogens tested including *Escherichia coli*, *Vibrio cholerae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed resistant phenotypic pattern to both extracts. Since *G. changii* and *E. denticulatum* extracts showed inhibitory activity against *S. aureus*, five genes in this pathogen were chosen to study the effect of both seaweed extracts on the genes through PCR and RT-PCR analysis. The results indicated genes for DNA repair, *adaB*; cell wall biogenesis gene, *sav1017*; and *mecA* gene yielding substantial effect by showing changes in the sequence of the genes. Based on the changes in the selected gene sequences of treated *S. aureus* isolates, the inhibitory activity for both seaweeds extracts on the respective genes is predicted according to the function of each gene. *G. changii* and *E. denticulatum* extracts were predicted to interfere with the function of *adaB* gene in producing the methyltransferase enzyme which was involved in the DNA repair in *S. aureus*. Both extracts were also predicted to interfere with the activity of *sav1017* gene in producing UDP-N-Acetylglucosamine transferase enzyme which is involved in the peptidoglycan synthesis in *S. aureus* since peptidoglycan is the major component in the cell wall of bacteria. However, the predicted inhibitory mechanism of both seaweeds extracts on *mecA* gene cannot be speculated based on the present research approach.

As a conclusion, *G. changii* and *E. denticulatum* extract can be categorized as a narrow spectrum antibacterial agent against *S. aureus* and *S. pyogenes in vitro*. The effectiveness of both seaweed extracts in affecting cell wall synthesis and DNA repair gene in *S. aureus* has significant conotation. The finding of antibacterial activity by both extracts against MRSA and non-MRSA strains is hoped to have

potential in producing alternative antibacterial agents from natural resources, against resistant *S. aureus* to reduce the infections and fatality.

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

AKTIVITI ANTIBAKTERIA EKSTRAK RUMPAI LAUT DAN KESAN KE ATAS TURUTAN DNA BAGI GEN YANG PENTING DALAM *Staphylococcus aureus*

Oleh

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Kadar kematian yang disebabkan oleh jangkitan bakteria semakin meningkat merangkumi 20 juta kematian setiap tahun di seluruh dunia. Penyebab utama yang menyumbang kepada kematian adalah peningkatan jumlah patogen yang rentan terhadap antibiotik termasuk *Staphylococcus aureus* rentan Methicillin (MRSA), patogen yang mempunyai spektrum luas untuk enzim Beta-lactamase (ESBL), dan patogen yang rentan terhadap pelbagai antibiotik (MRO). Oleh itu, kajian ini bertujuan untuk mencari produk antibakteria alternatif yang berasal daripada rumpai laut iaitu *Gracilaria changii* dan *Euchema denticulatum*, melalui beberapa kaedah termasuk bioesei dan aplikasi biologi molekul terutama kajian DNA dan RNA untuk gen-gen tertentu dalam kultur MRSA dan bukan MRSA.

Kajian bioesei mendedahkan bahawa ekstrak *G. changii* dan *E. denticulatum* menunjukkan aktiviti perencatan hanya terhadap bakteria gram positif yang diuji termasuk *S. aureus* dan *Streptococcus pyogenes* menerusi ujian kepekatan

minimum untuk merencat dan membunuh (MIC dan MBC). Sebaliknya bakteria gram negatif yang diuji termasuk *Escherichia coli*, *Vibrio Cholerae*, *Klebsiella pneumoniae* dan *Pseudomonas aeruginosa* menunjukkan sifat kerentanan terhadap kedua-dua jenis ekstrak. Memandangkan ekstrak *G. changii* dan *E. denticulatum* menunjukkan aktiviti perencatan terhadap *S. aureus*, lima gen di dalam bakteria ini dipilih untuk menguji aktiviti ekstrak *G. changii* dan *E. denticulatum* ke atas gen-gen tersebut melalui analisis PCR dan RT-PCR. Keputusan eksperimen menunjukkan gen untuk pemulihan DNA, *adaB*; gen untuk pembentukan dinding sel, *sav1017*; dan gen *mecA* menghasilkan keputusan yang memberansangkan dengan menunjukan perubahan dalam turutan nukleotida. Perubahan di dalam turutan nukleotida gen bagi kultur yang telah dirawat dengan ekstrak rumpai laut tersebut, digunakan untuk meramal aktiviti perencatan kedua-dua ekstrak rumpai laut tersebut terhadap kultur *S. aureus* berdasarkan kepada fungsi bagi setiap gen. Berdasarkan fungsi gen *adaB* dalam penghasilan enzim metiltransferase, kedua-dua ekstrak diramalkan menimbulkan gangguan dalam fungsi pemulihan DNA bagi kultur *S. aureus*. Kedua-dua ekstrak juga diramalkan mengganggu fungsi gen *sav1017* dalam penghasilan enzim UDP-N-Acetylglucosamine transferase yang terlibat dalam proses sintesis peptidoglikan di dalam kultur *S. aureus* memandangkan peptidoglikan merupakan komponen terbesar di dalam dinding sel bakteria. Walaubagaimanapun, di dalam kajian ini mekanisma antibakteria yang diramalkan bagi ekstrak *G. changii* atau *E. denticulatum* terhadap gen *mecA*, masih tidak dapat difahami sepenuhnya berdasarkan pendekatan penyelidikan yang telah dijalankan setakat ini.

Kesimpulannya, ekstrak *G. changii* dan *E. denticulatum* merupakan agen antibakteria yang berspektrum kecil. Keberkesanan kedua-dua ekstrak adalah dengan merencat gen yang terlibat dalam sintesis dinding sel dan pemulihan DNA di dalam *S. aureus*. Penemuan aktiviti antibakteria di dalam ekstrak *G. changii* dan *E. denticulatum* terhadap kultur *S. aureus* termasuk kultur MRSA dan bukan MRSA diharapkan mempunyai potensi dalam penghasilan agen antibakteria alternatif yang berasal dari alam semulajadi untuk melawan bakteria *S. aureus* bagi mengurangkan kadar jangkitan dan kematian.

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I certify that an Examination Committee has met on 13 March 2008 to conduct the final examination of Nurmas Idayu binti Mashan on her Master of Science thesis entitled "Antibacterial Activity of Seaweed Extract and its Effects on the DNA Sequence of Selected Essential Genes of *Staphylococcus aureus*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master Science.
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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NURMAS IDAYU BINTI MASHAN

Date: 21 June 2007

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LIST OF ABBREVIATIONS

CLSI	Clinical Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
ESBL	Extended Spectrum Beta-Lactamase
MDRO	Multiple Drug Resistant Organism
KUSTEM	Kolej Universiti Sains dan Teknologi Marin Terengganu
MBC	Minimal Bactericidal Concentration Test
MIC	Minimal Inhibitory Concentration Test
MRSA	Methicillin Resistant *Staphylococcus aureus*
PBP	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chin Reaction
UMMC	Universiti Malaya Medical Center
UMS	Universiti Malaysia Sabah

CHAPTER 1

INTRODUCTION

1.1 Introduction

Microbial infectious diseases account for nearly 20 million deaths each year world wide, thus the control of infectious diseases is vital both from a societal and economic standpoints. Antimicrobial agent has been the most effective therapeutic agent to control infectious diseases outbreaks or epidemic. It has the ability to either inhibit the growth of microbes or the ability to kill microbes. In microbiology term, antibacterial agent is defined as any compound that is clinically useful in the treatment of bacterial infections which may derive from a natural source, synthetic or produced semi synthetically. The mechanisms of inhibition involve inhibiting cell wall synthesis through inhibition of peptidoglycan layer cross linking or inhibition of peptidoglycan synthesis, inhibiting nucleotide synthesis, inhibiting nucleic acid synthesis or inhibiting protein synthesis which includes inhibition of 30S and 50S ribosomal subunit. The activity of antibacterial agent can be either bacteriostatic which will only inhibit the growth of bacterial or bactericidal which significantly reduces the number of viable bacteria in the culture, and can also be either narrow spectrum or broad spectrum. Narrow spectrum antibacterial agent is preferentially active against either gram negative or gram-positive bacteria while broad-spectrum antibacterial agent is active against both types of bacteria. In order to introduce some compound as antibacterial agent, the compound must be able to penetrate into bacterial surface and reach the target

especially found on the infected tissues in its active forms, attach itself to the site of infection in adequate concentration, and remain there for a sufficiently long period of time such that the bacteria is inhibited from carrying out its normal life functions. However, relying heavily on antimicrobial agent lands the global community to the great setback of antimicrobial resistance whereby high levels of antimicrobial drugs used resulted in high levels of resistance. Inappropriate and extensive use of antimicrobial drugs is leading to the rapid development of drug-specific resistance in disease-causing microorganisms and increased the number of pathogenic microorganisms that display antibiotic resistance. As a result, many antimicrobial drugs have lost effectiveness and some are no longer useful for treating certain infections.

When antibiotics were first introduced in the 1940s and 1950s, it was expected that they would eradicate infectious disease cause by bacteria. However, it soon became evident that some bacteria are intrinsically resistant to certain classes of antibiotics. The introduction of penicillin in the early 1940s dramatically improved the prognosis of patients with *Staphylococcal* infection (Franklin, 2003). However, as early as 1942, penicillin-resistant *Staphylococci* were recognized, first in hospitals and subsequently in the community (Franklin, 2003). In the early 1960s, the appearance of penicilinase-producing *Staphylococci* marked the onset of acquired resistant to antibiotics. By the late 1960s, more than 80% of both community and hospital-acquired *Staphylococcal* isolates were resistant to penicillin. The same phenomenon was soon observed with gram-negative bacteria. The discovery and clinical use of many known antibiotics have been paralleled by the emergence of bacteria that resist the actions of the antibiotics. The increasing

prevalence of multi-drug resistant organisms with few or no treatment options such as methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE) and the extended spectrum beta-lactamase (ESBL) producing gram-negative bacilli both in hospitalized patients and, to a lesser extent, in the community are a serious cause for concern and have become a global problem.

A member of the *Staphylococci* group, the *S. aureus* is perhaps the pathogen of the greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life threatening infections and its capacity to adapt to different environmental conditions (Lowy, 1998). It can grow at a temperature range of 15°C to 45°C and produces the coagulase enzyme-like factor which is generally associated with pathogenicity. It is resides mainly in the nasopharynx, hair and skin of the human body and other mammals. On microscopic examination, *S. aureus* appears in pairs or bunched, grape-like clusters. Although it is a part of our natural microflora, however, some strains of *S. aureus* are capable of producing a highly heat-stable toxin that is the main cause of illness in humans (Easmon *et. al*., 1983; Washington *et. al*., 2006). It can multiply in food held at room temperature, and produced the enterotoxins which is resistant to heat, refrigeration and freezing (Schlievert, 1993) causing gastroenteritis or inflammation of the lining of the intestinal tract. It is also released pyrogenic exotoxins into the blood stream and causing toxic shock syndrome. *S. aureus* grows to higher numbers in pimples, sores and when a person is down with a cold and can causes variety of suppurative (pus forming) infections such as boils and furuncles, and deep-seated infections such as osteomyelitis and endocarditis pneumonia. Other infections are mastitis, phlebitis, meningitis and urinary tract infections.

The evolution of antibiotic-resistant pathogenic bacteria has stimulated the search for alternative antimicrobial agents from alternative sources including sources from the ocean. The oceans cover 71% of the surface of the earth and comprise approximately half of the total global biodiversity, for which estimates range between 3 and 500 x 10^6 species of marine organisms (De Vries and Hall, 1994). The powers of marine organisms have been realized for thousands of years and its potential as producers of pharmaceutical products have been reviewed (Thompson *et. al*., 1985; Baker, 1984). As a consequence of an increasing demand for biodiversity and the screening programs seeking therapeutic drugs from natural products, there is now a greater interest in marine organisms, especially algae or seaweeds that can be found in all oceans except tropical western coast of Africa and western central of America. Majority of the seaweeds grow by attaching to the hard surfaces like rocks and shells and can be found as far as 130 feet (40 meters). The rich tropical waters surrounding the coast and islands, harbor a variety of seaweeds such as red algae (Rhodopyta), brown algae (Phaeopyta), green algae (Chlorophyta) and blue green algae (Cyanophyta), representing a potential source of useful products (Phang, 1984). Red algae which are found at where the water is much calmer can be utilized as a source of superfood for centuries. It comes in a variety of colors which gives rise to their variety of uses. In China, Japan and the Indo-Pacific region, several dozen species of red algae are used. This therapeutic superfood provides the body with a full array of nutrients including complete protein, complex carbohydrates, essential fatty acids, fiber, vitamins, minerals, trace elements, enzymes, and sulfated polysaccharides. Red algae are capable of working on multiple levels to strengthen the body and solidify the body's primary defense system. Its medicinal properties are thought to enhance the immune