Natural Product Activity Against Methicillin-Resistant Staphylococcus aureus Genes

E. Amghalia, Nagi A. AL-Haj, Mariana N. Shamsudin,
Nurmas I. Mashan, V. Necla and Zamberi Sekawi

Department of Clinical Laboratory Sciences, Faculty of Medicine and Health Sciences,
Laboratory of Immunotherapeutic and Vaccine, Institute of Bioscience,
University Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Abstract: Methicillin Resistant Staphylococcus aureus (MRSA) implicated in many post-surgical and cancer treatment as well hospital and community fatalities need to be treated with an effective alternative antimicrobial agent. In the search for anti-MRSA agent, 2 types of natural products were investigated for inhibitory activity against MRSA. In addition to the bioassay, the activity of the anti-MRSA agent was elucidated based on the effect of both natural products on nucleotide changes of chromosome-encoded genes. In this study, the methanol extract of the red marine algae and the natural pure honey were studied for its antibacterial property based on disc diffusion test and Minimum Inhibitory Concentration (MIC). The effects of both natural products on selected gene sequences of S. aureus's were determined by RT-PCR analysis. The genes of interest, which have been chosen in this study, are genes that are involved in the antibacterial mechanism including inhibition of cell wall synthesis, protein synthesis and nucleic acid synthesis. Five genes of interest chosen in this study include mecA gene, mecR1 gene, mecI gene, adaB gene and sav1017 gene. The results for antibacterial property showed the methanol extract of a red seaweed and the pure honey, inhibited growth of S. aureus strain according to the inhibition zones around discs saturated with the seaweed extract and pure honey, respectively. The MIC test showed decrease in growth of MRSA isolates after growing in broth incorporated with the extract and honey, respectively. The effect of the inhibitory activity of the natural products on selected gene sequences showed that several nucleotide changes occurred in the sequences of certain genes of interest based on the gene sequences of the cDNA after RT-PCR was carried out on the mRNA of S. aureus treated with the natural products. This research underlined that the inhibition effect of the natural products may be chromosome mediated evidenced by the changes of chromosome-encoded genes. The significant findings on activities of the seaweed extract and pure honey may become very useful in the process to find a better treatment for S. aureus infection especially, for the multiple drug resistant isolates. In addition, it is also, a new finding for natural product discovery through gene-expression analysis.

Key words: Methicillin resistant Staphylococcus aureus, minimal inhibitory concentrations, minimal bactericidal concentrations, seaweed, honey, polymerase chain reaction, Malaysia

INTRODUCTION

The increasing prevalence of multi-resistant bacteria made the search of new antimicrobial agents an important strategy for the establishment of alternative therapies in difficult handling infections. Staphylococcus aureus is a major nosocomial pathogen (Calderon-Jaimet et al., 2002) that causes a range of diseases, including endocarditis, osteomyelitis (Deora and Misra, 1996), pneumonia, toxic-shock syndrome, food poisoning (Baldassarri et al., 2001), carbuncles and boils (Shopsin and Kreiswirth, 2001). S. aureus that are resistant to an antibiotic called methicillin are referred to as Methicillin-Resistant S. aureus (MRSA). Many commonly prescribed antibiotics are not effective against these bacteria. MRSA causes outbreaks in hospitals and can be epidemic. MRSA rarely, if ever, presents a danger to the public. It is no more dangerous or virulent than methicillin-sensitive S. aureus but it is more difficult to treat, which results in increased pain, discomfort, inconvenience, cost of hospitalization and can lead to life threatening illnesses and death. The evolution of MRSA has stimulated the search for alternative antimicrobial agents from alternative sources. Seaweeds and honey are recognized as a potential...
sources of bioactive natural products for discovering the development of new antibacterial drugs. The antimicrobial properties from honey were recognized more than a century ago and have been subsequently studied (Bogdanov, 1997). In recent studies, the susceptibility of wound pathogens (Willix et al., 1992) and bacteria isolated from infected wounds (Cooper and Molan, 1999, Cooper et al., 1999) to honey of known floral source and defined antibacterial activity has been reported. However, the inhibition of antibiotic-resistant bacteria by honey has not been fully explored. By using red marine seaweed and natural honey, this study aims to investigate, the inhibitory activity of both natural products to MRSA strain which, focused on the effect of both natural products on nucleotide changes of chromosome-encoded genes.

MATERIALS AND METHODS

Culture of bacteria and seaweed extract: Clinical isolates of S. aureus collected from patients in Hospital Miri and Pusat Perubatan Universiti Malaya, Kuala Lumpur. The stock of each isolates were grown on Mannitol-salt agar and cultured into Luria-Bertani (LB) broth (Oxoid, UK) (16-18 h). Methanol extract of the red seaweed is provided by KUSTEM.

Antibacterial activity testing: Diluted suspension of S. aureus was spread onto the Mueller Hinton agar to produce a lawn of bacteria and a sterilized punctured Whatman filter paper No. 1 was placed on the bacterial lawn. The methanol extract of seaweed was pipetted out onto the filter paper and the plate was incubated at 37°C. The zone of inhibition (if any) was observed after 24-48 h. Minimal inhibitory and bactericidal concentrations of seaweed extract and natural honey were determined by using microdilution method in the microtitre plate. Different concentrations of seaweed extract and honey were incubated with a suspension of an actively growing culture of bacteria diluted at a starting concentration equal to 0.5 McFarland standard. After overnight incubation, each of the treated bacterial suspension was plated out on Mueller Hinton agar to determine the cell counts in different concentrations of seaweed extract and honey, respectively.

RNA molecular work: The RNA of S. aureus were extracted from cultures grown to log phase and was harvested by centrifugation. RNA extraction protocols were according to the manufacturer’s instruction using the TRI Reagent kit (Molecular Research Centre, USA). The RNA was electrophoresed through 1.0% agarose at 70V and visualized under UV transilluminator (Alpha Innotech 2200). Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) was performed according to the Clone AMV-RT kit (Invitrogen I.N.C) manufacturer’s instruction. The amplification reaction mixture of sav1017 and adaB genes in a total volume of 25 µL reaction mixture contained 1.2 mM MgCl2, 0.4 µL of dNTP mix, 2.5 µL Buffer (MBI Fermentas), 0.1 µL each forward and reverse primers, 100 ng DNA template and 0.2 µL of Taq DNA polymerase. Amplification was carried out according to the following thermal cycling profile: initial denaturation at 96°C for 5 min, extension denaturation at 95°C for 1 sec, annealing reactions at 59°C for 30 sec, elongation at 55°C for 30 sec for 35 cycles, respectively, followed by the final extension reaction at 55°C for 30 sec. RT-PCR products were sent for commercial automated sequencing to confirm the genes and to detect the changes in nucleotides between untreated and treated S. aureus.

RESULTS AND DISCUSSION

Natural product compounds are the source of numerous therapeutic agents. Recent progress to discover drugs from natural product sources has resulted in compounds that are being developed to treat cancer, resistant bacteria and viruses and immunosuppressive disorders. A number of target areas are under investigation, including fungal mRNA splicing and bacterial DNA synthesis. A major part of the endeavor is in the historically productive area of natural product screening. To make the best use of natural product resources for the discovery of novel antibiotics, a balance is struck between screening for inhibitors of rationally chosen targets for which, clinically useful inhibitors are not yet available and screening more broadly to ensure that rare activities of unanticipated mode-of-action are not missed. The inhibition assay of methanol extract showed activity only in MRSA and non-MRSA isolates but not in gram negative isolates. The nucleotide sequences changes were seen in four genes of treated MRSA isolates, which were the mecA, mecRI, mecI and the adaB. The nucleotide changes in non-MRSA and MRSA were only seen in the adaB gene but not the sav1017. The BLASTN analysis Fig. 1 showed variation in nucleotide changes in all genes involved in MRSA phenomenon.

Disc diffusion test of the MRSA isolate showed a clear inhibition zone on the disc containing seaweed extract as shown in Table 1. The sequencing results
Fig. 1: Sequencing results of adaB gene, which involved in DNA repair showed changes in the nucleotide on the sequence treated with medicinal extract.

Table 1: Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MEC) of seaweed

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC of seaweed (µg ml⁻¹)</th>
<th>MEC of seaweed (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1R5 (MRESA)</td>
<td>25.0</td>
<td>50</td>
</tr>
<tr>
<td>S1R9 (MRESA)</td>
<td>25.0</td>
<td>50</td>
</tr>
<tr>
<td>N8 (MRESA)</td>
<td>6.25</td>
<td>40</td>
</tr>
<tr>
<td>20 (MRESA)</td>
<td>6.25</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 2: Number and percentage of MRSA inhibited on with honey at different concentrations

<table>
<thead>
<tr>
<th>Concentration of honey on Mueller Hinton agar</th>
<th>10%</th>
<th>1%</th>
<th>0.1%</th>
<th>0.01%</th>
<th>0.001%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number and percentage of strains inhibited</td>
<td>0</td>
<td>9</td>
<td>6 (60%)</td>
<td>9 (90%)</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

showed several changes occurred in the nucleotide of adaB gene. The novel aspect of this study indicates that the inhibition effect of seaweed may be chromosome mediated evidenced through the changes in chromosome based on nucleotides from the cDNA changes of adaB gene, which involve in DNA repair mechanism determined through RT-PCR assay. adaB gene encode for cysteine-5-methyltransferase. The DNA changes are predicted to affect the functional property of the adaB gene possibly through changes in the methytransferase function of the bacteria. The MIC values obtained in this study demonstrate that the honey (Table 2) at medium levels of potency were significantly effective in inhibiting MRSA in in vitro tests. Honey at least 10 times dilute prevented growth and achieved equivalent inhibitory effects at concentrations 6 and 3 times more dilute. The mode of action of honey has not yet been fully elucidated, but osmolarity, acidity, hydrogen peroxide generation and phytochemical components are considered important. In undiluted honey, the osmolarity and acidity undoubtedly limit bacterial growth. When honey is diluted, a bee-derived enzyme (glucose oxidase) present in the honey is activated and catalyses the slow generation of hydrogen peroxide, which inhibits bacterial. Generally, in vitro tests provide only an indication of the dilution capacity of an antimicrobial agent and do not assure that such potency will persist in vivo. In this study, we are looking forward to get some of answer and to understand the essential role that natural product compounds play in the understanding of the basic science and development of novel therapeutics.

CONCLUSION

These preliminary results utilizing genomics for study on nucleotide sequences of pathogen can aim at utilizing the biomolecules from natural products to target the affected nucleotides so as to inhibit growth of the organism. The research activity has the potential of speeding up drug discovery programme and the nucleotide changes in several genes treated with the extract indicates the potential target sites of the extracts. The inhibitory effect of extract on the nucleotide of some genes remained unchanged in the PCR and RT-PCR assay, indicating selective effect of extracts on the genes and the extracts has the potential to be applied as antibiotic agents. Implication of the findings is directed towards discovery of antibiotic drug target sites, which warrants further study.

ACKNOWLEDGEMENT

Thanks to the Ministry of Science and Technology for the Microbial Genome Grant and to Universiti Malaya Medical Centre, Hospital Tengku Chuan Zain, Kuantan and Hospital Min, Sarawak for providing the S. aureus isolates.

REFERENCES


