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Application of RT-PCR to Detect Treated and Untreated *Staphylococcus aureus* Genes with Marine Algae

[1,2]Nagi A. AL-Haj, [2]Nurmas I. Mashan, [2,3]Mariana N. Shamsudin,
[4]Habsah Mohamad, [5]Charles S. Vairappan and [2]Zamberi Sekawi
[1]Department of Medical Microbiology,
Faculty of Medicine and Health Sciences, Sana'a University, Yemen
[2]Department of Microbiology and Parasitology, Faculty of Medicine and Health Sciences,
Putra Malaysia University, 43400 Serdang, Selangor, Malaysia
[3]Department Marine Science and Aquaculture, Institute of Bioscience,
Putra Malaysia University, 43400 Serdang, Selangor, Malaysia
[4]Department of Chemical Sciences, Faculty of Science and Technology,
Terengganu Malaysia University, 21030 Kuala Terengganu, Terengganu, Malaysia
[5]Laboratory of Natural Products Chemistry, Institute of Tropical Biology and Conservation,
Sabah Malaysia University, 88999 Kota Kinabalu, Sabah, Malaysia

Abstract: Methacillin 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 the study of DNA and RNA encoding genes of interest in MRSA and non-MRSA. The target of this study is to amplification of several untreated and treated *S. aureus* and *E. coli* genes that are potentially involved in the antibacterial activities through RT-PCR assay. *G. changii* and *E. denticulatum* extracts showed inhibitory activity against *S. aureus*, several genes in this pathogen were chosen to study the effect of both seaweed extracts on the genes through PCR and RT-PCR analysis. However, the predicted inhibitory mechanism of both seaweeds extracts on mecA gene was not fully elucidated in the study. The investigation could scientifically proof the natural products to be potentially potent antibacterial agents.

Key words: Reverse transcription-polymerase chain reaction, *Staphylococcus aureus*, marine algae, antimicrobial activities, extraction, sequencing, Malaysia

INTRODUCTION

Reverse Transcription-Polymerase Chain Reaction or RT-PCR is an *in vitro* method for enzymatically amplifying defined sequences of RNA (Rappolee *et al*., 1988) and permits the analysis of different samples from as little as one cell in the same experiment. It is the sensitive and the most flexible of the quantification methods (Wang and Brown, 1999) and can be used to compare the levels of mRNAs in different samples populations, to characterize patterns of mRNA expression, to discriminate between closely related mRNAs and to analyse RNA structure. RT-PCR can also circumvent time-consuming and technically demanding cloning steps and generate reagents, such as full-length complementary DNA (cDNA), inserts for cloning (Borson *et al*., 1992), or arbitrarily primed enhanced sequence tag cDNA libraries (Neto *et al*., 1997). RT-PCR is a complex assay and all physical and chemical components of the reaction are interdependent. They must be considered carefully when optimizing the specificity, sensitivity, reproducibility or fidelity of the reaction since these will be the most important qualitative characteristics for clinical diagnostic uses. RT-PCR techniques increase the sensitivity, but compromise the specificity of the reaction (Henke *et al*., 1997). The evolution of antibiotic-resistant pathogenic bacteria has stimulated the search for alternative antimicrobial agents from alternative sources including sources from the ocean. Seaweeds or algae are a eukaryotic organism (Michael *et al*., 2005) that lives in salty water in the ocean and is recognized as a potential source of bioactive natural products. They contain compounds ranging from sterols, terpenoids to brominated phenolic, which show bioactivity against

Corresponding Author: Nagi A. AL-Haj, Department of Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Putra Malaysia University, 43400 Serdang, Selangor, Malaysia

microorganisms (Wong *et al*., 1994). 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. Efficacy of red algae as antibacterial agent is few in mention especially, the local red algae found around Malaysian coasters. Therefore, this study is in search for the antibacterial properties in red algae in order to find a new antibacterial agent that can inhibit or reduce the growth of bacteria in human body. Red algae that are used in this study are *Gracilaria changii*, which grow mild in Pantai Morib, Selangor and *Euchema denticulatum*, which grow mild and cultivated in Pantai Sabah. The advent in the biotechnology field with cutting edge technologies enable scientists to explore new approaches such as the genotypic together with phenotypic approaches, which will speed up discovery of new findings. Since drug discovery programs are at the frontier line in combating antimicrobial resistance phenomenon, the genomic approach together with the combinatorial chemistry could shorten the lag period in designing new alternative antimicrobials, whereby through genomic, genes or gene expressions affected by antimicrobial agents could be predicted as sites of drug targets. In the present study, the advantages of molecular tools through genomic analysis could be used to prove that both seaweed extracts will work very well as a new antibacterial agent in a new decade not only just by prescreening test, but also through genomic analysis for understanding the mechanism of inhibition. The recent advances in gene amplification methods have greatly facilitated the accumulation of sequencing data for this study. The method used in this study Reverse-Transcriptase PCR (RT-PCR), allow amplification of specific gene of interest.

MATERIALS AND METHODS

Propagation of untreated bacterial culture: A single pure colony of *Staphylococcus aureus* and *Escherichia coli* isolates were inoculated to a 250 mL capacity Enlermeyer flask containing an adequate volume of sterilized Luria Bertani broth (Invitrogen Inc.). The broth was then incubated overnight at 37°C with constant shaking on the shaker. This was followed by the procedure of the total RNA extraction using MasterPure™ complete DNA and RNA Purification Kit from Epicentre.

DNA extraction: Five isolates of *S. aureus* including MRSA and non-MRSA strains were extracted using the GeniSpin™ Bacterial DNA Kit (BSTTechlab). One *E. coli* strain, which serve as negative control, was extracted by using the same method. The culture was aliquot into 1.5 mL microcentrifuged tube and pelleted, until an adequate quantity of bacteria was obtained. Cells then were resuspended in 100 μL TE buffer and the bacterial cell wall was removed by lysozyme (10 mg mL^{-1}) digestion and followed by buffer BTL and proteinase K (15 mg mL^{-1}) digestion. RNaseA (25 mg mL^{-1}) was added to remove the RNA, which normally co-purifies with genomic DNA. Following lysis, binding condition was adjusted and the sample was applied to an I-Spin™ column after adding the Buffer BDL and absolute ethanol. Two rapid wash steps using Wash Buffer will removed trace salt and protein contaminants and finally DNA was eluted in water or low ionic strength buffer. This DNA can be directly used in downstream applications without the need for further purification. The eluted DNA was then run onto 0.8% agarose gel electrophoresis to check the present of genomic DNA and stained with ethidium bromide to visualize under transilluminator. Lastly, the DNA was stored at -20°C in a refrigerato.

Propagation of bacterial culture treated with gracilaria changii and euchema denticulatum extract: A single pure colony of *S. aureus* and *E. coli* isolates was inoculated to a 250 mL capacity Enlermeyer flask containing an adequate volume of sterilized Luria Bertani broth (Invitrogen Inc.) containing either 50 mg mL^{-1} *G. changii* or 40 mg mL^{-1} *E. denticulatum* extract. The broth was then incubated 4-5 h at 37°C with constant shaking on the shaker. This was followed by the procedure of the total RNA extraction using the MasterPure™ Complete DNA and RNA Purification Kit from Epicentre.

Total RNA isolation: Five isolates of *S. aureus* including MRSA and non-MRSA strains were extracted using the MasterPure™ Complete DNA and RNA Purification Kit from Epicentre. The *E. coli* strain, which serve as negative control, was extracted using the same method. The culture was aliquot into 1.5 mL microcentrifuged tube and pelleted, until an adequate quantity of bacteria was obtained by centrifugation at 10000× g for 10 min at 4°C. Three hundred microliter of tissue and cell lysis solution containing 1 μL of 50 μg μL^{-1} proteinase K were added to bacteria cells and the homogenate was kept at 65°C for 15 min. After the adding of 150 μL of MPC Protein Precipitation Reagent to 300 μL of lysed sample, the debris from the homogenate was removed by centrifugation at 10,000 g for 10 min at 4°C. The supernatant then was transferred to a new tube and 500 μL of isopropanol was added. The tube was inverted for 30-40 times before centrifugation at 10,000 g for 10 min at 4°C. The supernatant was removed and 75% ethanol

was used to wash the RNA pellet. The homogenate was spin down again and the ethanol was pour off without dislodging the RNA pellet. Finally, the RNA pellet was resuspended in 35 μL of DEPC-treated water and was incubated at 55°C for 5 min. In order to check the quality of RNA sample, electrophoresing was proceed through 1.0% agarose gel in 1X TBE buffer (pH 8.3) at 65V for an indicated period of time. High Range RNA Ladder ready to use (MBI Fermentas) was used as a standard. The gel was stained with ethidium bromated (1 mg mL^{-1}) for 10 min and visualized under UV transilluminator (α-Innotech 2200).

Reverse-transcriptase polymerase chain reaction: The Reverse-Transcriptase Polymerase Chain Reaction was preceding using Cloned AMV Reverse Transcriptase kit (Invitrogen Corp). Oligo (dT) was combined with RNA and dNTP in one tube as shown in the Table 1. The mixture then was denatured by incubating at 65°C for 5 min and immediately placed on ice. The master reaction mixture then was prepared as shown in the Table 2. About 8 μL of the master reaction was mix with each reaction tube. Using the Thermal Block Cycler (T-Personal) provided by Biometra, the reaction tube was preheated and incubated to the cDNA synthesis temperature at 50°C for 1 h. The reaction then was terminated by incubating at 85°C for 5 min and stored at -20°C. The cDNA then was used to amplify the gene for partial expressions of treated and untreated *S. aureus* and *E. coli*, where 2 μL of the cDNA are was subjected to PCR using all primers, conditions and parameter, which was described before in Table 2. There are only slightly change in amplification of ogt gene, where're the initial denaturation was started at 95°C and the cycle was reduced to 30 cycle.

Agarose gel electrophoresis of PCR and RT-PCR product: An aliquot of 10 μL of the PCR product mixture was electrophoreses through 1.2% agarose gel in 1X TBE buffer, pH 8.3 at 70 V for an indicated period of time. A DNA molecular weight marker 100 bp ladder (MBI Fermentas) was used as a standard. The gel was stained with ethidium bromated (1 mg mL^{-1}) for 15 min, visualized under UV transilluminator (α-Innotech 2200). The PCR product, which amplify the gene of interest, then was purified before sent for sequencing.

PCR and RT-PCR purification: This step was proceeding by using PCR purification kit (Queen). Five volumes of buffer PB was added to one volume of the sample. The sample then was applied to the QIA quick column and was centrifuged at 13000 rpm for 1 min. The sample was proceed to several washing steps with 750 μL buffer PE and finally was eluted with buffer EB.

Table 1: The mixture in the reaction tube for RT-PCR

Component	Amount (μL)
Oligo dT	1
10 mM dNTP Mix	2
RNA (10 pg-5 μL)	8
DEPC-treated water	1
Total	12

Table 2: The mixture in the master reaction tube for RT-PCR

Component	Amount (μL)
5× cDNA Synthesis Buffer	4
0.1 M DTT	1
RNAse OUT (40 U μL^{-1})	1
DEPC-treated water	1
Cloned AMV RT (15 units μL^{-1})	1
Total volume	8

Sequencing analysis: All the PCR and RT-PCR products using of untreated and treated *S. aureus* and *E. coli* were sent for commercial automated sequencing (Invitrogen Inc.) to confirm the gene and to detect the changes in nucleotides between untreated and treated *S. aureus* and *E. coli*. Analysis of sequencing for PCR and RT-PCR products was analyzed by using BLASTN at website http://www.ncbi.nlm.nih.gov. These analysis were done to find, whether the genes that already amplified were homologous to the published sequence in the Gene Bank. The second analysis was done at the Biology Workbench 3.2, at website http://workbench.sdsc.edu/CGI/bw.cgi, whereas the analysis by multiple alignment of the sequencing was done. These alignments were done between three parameters. There are sequence of the gene in the Gene Bank, sequence of the untreated gene and sequence of the treated gene.

RESULTS AND DISCUSSION

The total genomic DNA of high molecular weight were successfully extracted from 5 isolates of untreated and treated *S. aureus* (Fig. 1), including MRSA and non-MRSA strains and one isolate of *E. coli* (Fig. 2) using GeniSpin™ Bacterial DNA Kit (BST[Techlab]). The ratio of absorbance at 260-280 nm (A260:A280) ranged from 1.238-2.000. The DNA concentrations were between 40-560 μg mL^{-1}.

Reverse-transcription polymerase chain reaction: RNA of untreated and treated *Staphylococcus aureus* (Fig. 3) and *Escherichia coli* (Fig. 4) were successfully extracted. In the current study, the ratio of absorbance at 260-280 nm (A260:A280) ranged from 1.65-2.17, while the RNA concentrations were between 42-752 μg mL^{-1}. The RT-PCR performed on the cDNA samples following manufacturer's instructions was successful to produce strong RNA signals on 1.2% agarose gel. RT-PCR result using primers as PCR for respective genes of interest (Fig. 5-10) were successful in amplifying the respective

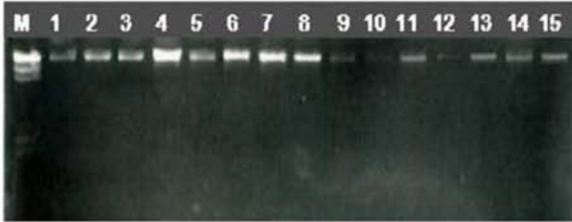

Fig. 1: The genomic DNA extracted from untreated and treatrd *Staphylococcus aureus*. Lane 1-5 are the non-isolated lanes, 6-10 are the isolates treated with *Gracilaria changii*, while lanes 11-15 are the isolates treated with *Euchema denticulatum* extracts. Lane M is the Lambda

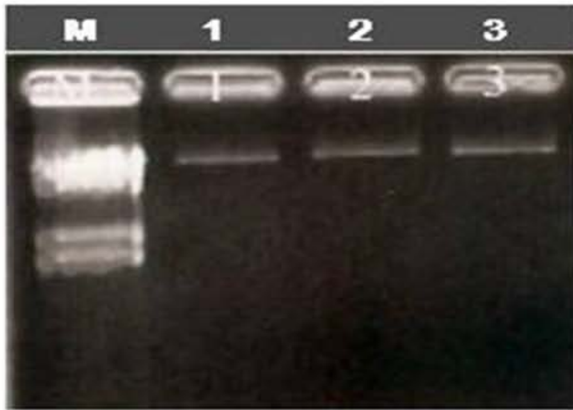

Fig. 2: The genomic DNA extracted from untreated and treated *Escherichia coli*. Lane 1 is the untreated isolate, lane 2 is the isolate treated with *Gracilaria changii* and lane 3 is the isolates treated with Euchema denticulatum extract. Lane M is the lambda hind 3 molecular weight marker

Fig. 3: Total RNA extracted from untreated and treated *Stophylococcus aureus*. Lane 1-8 are untreated RNA and lanes 9-16 are treated RNA. All the total RNA extracted was at position 1500 and 3000 kb. Lane M is the hight range RNA ladder (MBI Fermentas)

genes indicated by the PCR products with the bands positioned at the respective region. The amplification of adaB, sav1071, mecA, mecI, mecR1 and ogt genes showed

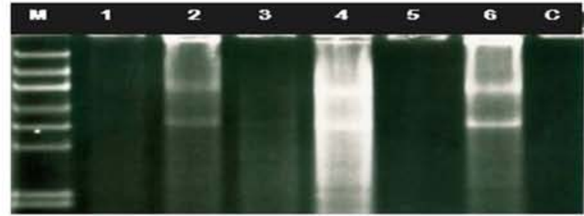

Fig. 4: Total RNA extracted from untreated and treated *Escherichia coli*. Lane1-2 are untreated RNA and lane 3-6 are treated RNA. All the bands were at position 1500 and 3000 kb. Lane M is the high range RNA ladder (MBI Fermentas)

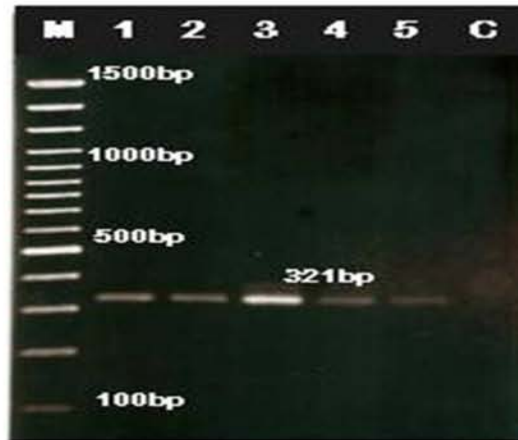

Fig. 5: Amplification of ada B gene by RT-PCR in untreated and treated *Staphylococcus aureus*. Lane M is the 100 bp molecular weight marker (MBI Fermentas). adaB gene positive isolates have a single band present in the region between the ladders of 300-400 bp. The actual band position is at 321 bp. Lane C is a negative control showing no band indicating that there was no contamination during the process

transcription of genes in the treated and untreated isolates. A single band was amplified from the RNA positive control and no band was amplified from the RNA negative control showing that the amplified products were specific for the respective genes.

Sequencing analysis: The respective adaB, sav1017, mecA, mecR1, mecI and ogt genes were amplified correctly using the corresponding primers as visualized in the agarose gel. The correct amplification is indicated by the appropriate sizes of bands for each gene corresponding to the respective region in the agarose. Amplified PCR products, which were confirmed by automated sequencing showed acceptable sequencing results with sharp peaks of the signals in the

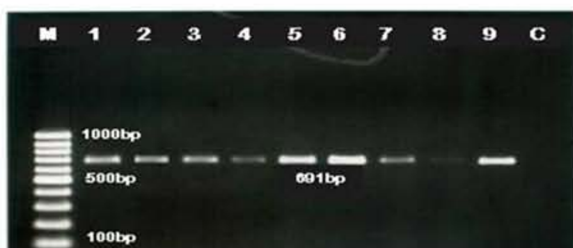

Fig. 6: Amplification of say1071 gene by RT-PCR in untreated and treated *Staphylococcus aureus*. Lane M is the 100 bp molecular weight marker (MBI fermentas). sav1071 gene positive isolates have a single band present in the region between the ladders of 600-700 bp. The actual band position is at 691 bp. Lane C is a negative control showing no band indicating that there was no contamination during the process

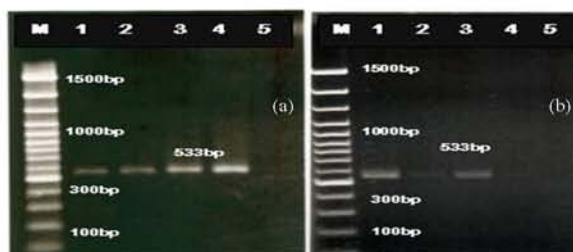

Fig. 7a-b: Amplification of mecA gene by in untreated and treated *Staphylococcus aureus*. Lane M is the 100 bp molecular weight marker (MBI fermentas). mecA gene positive isolates have a single band present in the region between the ladders of 500-600 bp. The actual band position is at 533 bp. Lane 5 for both figures are negative control showing no band indicating that there was no contamination during the process

Fig. 8: Amplification of mecR1 gene in untreated and treated *Staphylococcus aureus*. Lane M is the 100 bp molecular weight marker (MBI Fermentas). mecR1 gene positive isolates have a single band present in the region between the ladders of 300-400 bp. The actual band position is at 310 bp. Lane C is a negative control showing no band indicating that there was no contamination during the process

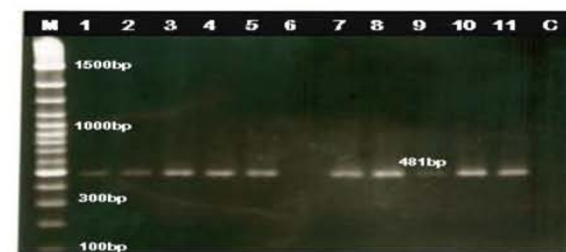

Fig. 9: Amplification of meci gene in untreated and treated *Staphylococcus aureus*. Lanes 1-5 are the untreated cDNA , while lanes 7-10 are the cDNA. Lane M is the 100 bp molecular weight marker (MBI Fermentas) meci gene positive isolates have a single band present in the region between the ladders of 400-500 bp. The actual band position is at 481 bp. Lane C is the negative control showing no band indicating that there was no contamination during the process

electrophorogram. Sequencing analysis of amplified products with published gene sequences. Since, the objective of sequence analysis study is to compare between the sequences of the untreated genes with the sequences of the genes treated with either *G. changii* or *E. dentiulatum* extracts, the software in the Biology Workbench 3.2 under alignment program (http://workbench.sdsc.edu) were used and results clearly illustrated degrees of similarities and differences between untreated isolates, treated isolates and the sequence from the Gene Bank database. Upon aligning of each gene, several mutations, which include changes, deletion or insertion of nucleotides were noted after the alignment using ClustalW program. The changes or mutation in nucleotides of the genes detected is correlated to the effect of the seaweed extracts and being assumed to predict inhibitory mechanisms of the extract. Figure 11 showed the type and number of nucleotide changes found in the sequences of the gene's sequences treated either with *G. changii* or *E. denticulatum* extract. These changes were grouped according to the type of mutation either by changes of purine and pyrimidine, insertion or deletion of base pairs. According to the results, only the sequences of mecI and ogt genes do not show any changes after treatment with either *G. changii* or *E. denticulatum* extract. adaB gene sequences showed obvious changes whereby all of the isolates tested showed sequence changes after amplification using PCR and 3 out of 5 isolates showed sequences changes after RT-PCR, when using DNA and cDNA treated with

G. changii extract as the template. The changes in nucleotide sequences are between 1.4-6.2% in amplification using RT-PCR assay. In this study, the pattern of nucleotide changes for adaB and sav1017 treated with *G. changii* extracts is similar to the pattern of nucleotide changes for both genes treated with *E. denticulatum* extracts whereby for adaB gene, the changes in nucleotide sequences are between 0.3-2.1% amplification using RT-PCR assay, while for sav1017 gene treated with *E. denticulatum* extract by RT-PCR assay showed the nucleotide changes in only one of the isolate tested with a slightly change of 0.5%. The effect of both seaweed extracts tested on antibiotic resistance genes of *S. aureus* isolates is presented in Fig. 11. Both seaweed extracts affected the sequence of nucleotides in mecA gene, while only 1 isolate treated with *E. denticulatum* extract showed changes in the nucleotide sequences of mecR1 gene by RT-PCR assays. The changes in nucleotide sequences of mecA gene in isolates treated with *G. changii* extract are in the range 0.8-1.6% for RT-PCR assay products. For isolates treated with *E. denticulatum* extract, the changes in nucleotide sequences of mecA gene ranged from 0.2-14.9% for PCR products and 1.0% for RT-PCR products.

Reverse Transcription-Polymerase Chain Reaction or RT-PCR is an *in vitro* method for enzymatically amplifying defined sequences of RNA. The technique consists of two parts: synthesis of cDNA from RNA by Reverse Transcription (RT) and followed by its exponential amplification of a specific cDNA in Polymerase Chain Reaction (PCR). Separation of the RT and PCR steps has the advantage of generating a stable cDNA pool that can be stored virtually indefinitely. The first consideration, when using RT-PCR for mRNA analysis is RNA isolation. The RNA should be high quality and free from genomic DNA contamination. However, since most RT-PCR methods amplify only a few hundred bases rather than the complete mRNA sequence, the sample RNA can be slightly degraded. Every RT-PCR begins with a reverse transcriptase reaction since RNA cannot serve as a template for PCR. Once the RNA has been isolated, the information was converted to DNA by using the enzyme reverse transcriptase. This enzyme requires a primer in order for it to begin working. In this study, a high molecular weight and good quality of DNA without RNA contamination was successfully extracted from all the treated and untreated *S. aureus* and *E. coli* isolates since the presence of RNA, which appears like a smear on the lower part of the gel instead of a band was not observed. Good quality of DNA was obtained as evidenced from the agarose gel analysis, which is a crucial technique in detecting the presence of genomic DNA. The purity and the concentration of the genomic DNA extracted were determined by reading the ratio of absorbance at A_{260} and A_{280}. According to Koch *et al.* (1993), the ratio in the range of 1.6-2.0 was sufficiently pure for PCR reaction. Inefficient DNA extraction might be caused by incomplete cell lysis or protein degradation due to insufficient

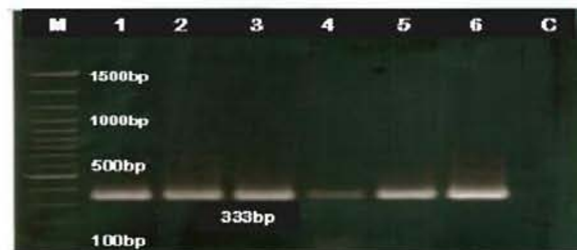

Fig. 10: Amplifacation of ogt gene by PCR and RT-PCR. Lane 1 is the band of gene of untreated genomic, lane 2 is the gene of genomic treated with *G. Changii*, lane 3 is the gene of genomic treated with *E. Denticulatum*, lane 4 is the band of gene of untreated cDNA treated with *E. Denticulatum*, while lane M is the 100 bp molecular weight marker. Ogt gene positive isolates have a single band present in the region between the ladders of 300-400 bp. The actual band position is at 333 bp. Lane C is a negative control showing no band indicating that there was no contamination during the process

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NCBI      ATACGTTGTTAGAGTCCCATAAGG-AACCTGTCTTAATTCATTCCAAACACACTGTTGAA
Untreated ATACGTTGTTAGAGTCCCATAAGG-AACCTGTCTTAATTCATTCCAAACACACTGTTGAA
treated   ATACGTTGTTAACGTCCCATAAGGGAATCTGTCTTAATTCATTCCAAACACACTGTTGAA

NCBI      AATGACTACCTGTTGGCTTTAAAGGTATT-GTGATTTCAGGATTGTCACCTTTAAAATAC
Untreated AATGACTACCTGTTGGCTTTAAAGGTATT-GTGATTTCAGGATTGTCACCTTTAAAATAC
treated   AACGA-TACCCGTTGGCTTTAAAGGTATTCGAGATTTCATGATTATCACCTTTAAAATAC

NCBI      GCGTCTAACCACTGTGTCGCCTCTCTAAATATCGCTAAAGACGTATTTTCTTCCCTAGTA
Untreated GCGTCTAACCACTGTGTCGCCTCTCTAAATATCGCTAAAGACGTATTTTCTTCCCTAGTA
treated   GCGTCTAACCACAGTGTCGCCTCTTTAAATATCACTAAAGACGTATTTTCTTCCCTAGTA
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Fig. 11: Type and number of nucleotide changes found in the sequences of the Genes after *Staphylococcus aureus* treated with seaweed extracts

incubation. To overcome this problem, the incubation time could be increased after the addition of the appropriate solution according to the manufacturer's instruction to ensure the cells are completely lysed and no visible pieces of cells remain. Meanwhile, if the ratio of A_{260}: A_{280} is >2.0, it might be caused by a high level of residual RNA contamination in the genomic sample (QIAGEN, Germany), whereby it can be overcome by adding the RNAse during the extraction. RNAse is an enzyme used frequently for the purification of DNA extracted from tissues and bacterial cultures. This enzyme specifically hydrolyzes RNA between the 3' phosphate of a pyrimidine ribonucleotide and the 5'hydroxyl of the adjacent nucleotide.

RNA plays important roles in the expression of genetic information in the cell (Michael *et al*., 2005). The total RNA was extracted rather than mRNA for preparing cDNA in this study because total RNA requires less handling and works better under most circumstances. Similar to DNA extraction, the purity and the concentration of the total RNA extracted can be determined from the absorbance readings at A_{260} and A_{280}. However, RNA quantitation is based on the formula 1 OD at A260 nm equals to 40 μg RNA mL^{-1}. In this study, the total RNA was diluted with 10 mM Tris, pH 7.5 to obtain a good reading, since the use of water as a dilution medium can affect the A260/A280 ratio reading. According to GeneChip Array by Affymetrix, A260/A280 absorbance reading for an acceptable RNA sample should be in the range of 1.9-2.1. In this study, RNA with ratios below 1.9 indicates protein contamination. Since most of the low A260/A280 absorbance readings were observed in the samples treated with either *G. changii* or *E. denticulatum* extract, the possibility of this protein contamination may come from both seaweeds extract, which may contain high protein content. Therefore, to overcome this problem, the treated samples were cleaned using an additional ethanol precipitation, since ethanol act as a denaturing solvent to remove protein. Meanwhile, ratios above 2.1 indicate presence of degraded RNA, truncated cRNA transcripts, excess free nucleotides and may be DNA contamination. In this condition, the samples were stored at -80°C prior to use in order to avoid RNA degradation, while the adding of DNAse can eliminate DNA contamination. It is also, important to analyze the extracted RNA in the agarose gel electrophoresis to detect the presence of RNA and to ensure that the RNA extracted was free from contamination whereby, the presence of DNA can be detected in agarose gel electrophoresis since it will appear like a band on the upper part of the gel instead of RNA band, while protein contamination can be detected like a smear on the lower part of the gel instead of a RNA band. Nevertheless, in this study, a few samples showed high quality RNA after running the RNA sample on a 0.8% agarose gel, exhibited by sharp rRNA bands, while low quality RNA showed ambiguous bands with thick smearing. After the total RNA extraction, the conversion of total RNA to the first strand cDNA is performed with oligo (dT) and reverse transcriptase according to manufacturer's protocol (Invitrogen, USA). The resulting single-stranded cDNA can than be directly amplified by the PCR (Holland *et al*., 1991; Saiki *et al*., 1988). In the present study on RT-PCR step, the target nucleic acid (RNA) is successfully converted to complementary double-stranded DNA (cDNA) in a reverse transcriptase step, followed by PCR amplification of the target cDNA sequences indicated by a level detectable by gel electrophoresis. Fundamental to the study of RT-PCR based gene detection assay, is the information of the nucleotide sequence of the genes of interest. Sequences of the adaB and sav1017 from *S. aureus* and ogt gene from *Escherichia coli* database were used to design the primers for those genes since no literature or previous study were found for those genes. Finally, the sequences of the adaB, sav1017, mecA, mecR1, mecI and ogt gene from this database also were used as a comparison between the sequence of untreated genes and the sequences of the gene treated with either methanol extract of *G. changii* or *E. denticulatum*. Comparison between the original gene sequence from database with the untreated and untreated gene sequence can confirm that every changes or mutation that occurred on the treated gene sequence are due to the activity of the seaweed extracts and not due to PCR amplification. Since the mode of action of either methanol extract of *G. changii* or *E. denticulatum* in inhibiting the growth of MRSA and non-MRSA isolates *in vitro* has not yet been fully elucidated by other scientist, the changes in the sequence of selected genes were used to predict the possible mechanisms of inhibitory activity according to each gene's function. Effect of both seaweeds extracts on the selected genes was considered important since gene is the element of information that specifies the sequence of amino acids of the protein, whereas proteins are the cells functional entities. Gene is composed of DNA and the genetic information in the gene is presented as the sequences of bases in the DNA. Information stored in the DNA specifies the sequence of protein only through RNA, which served as a true informational intermediate. RNA will translate the genetic information in DNA into protein. According to Michael *et al*. (2005), DNA carries the genetic blue print for the cell and RNA acts as an intermediary molecule to convert the blueprint into

defined amino acids sequences in proteins. Although, there is the partial expression of the antibiotic resistance genes indicated through the RT-PCR analysis, the translation of the functional protein encoded by the genes is not guaranteed.

The nucleotide sequences of amplified PCR and RT-PCR products can be used to clarify the inhibitory mechanism of extracts on MRSA isolates, when analyzed for any changes. The successful detection of antibiotic resistance genes in those isolates also, supported the antibiotic susceptibility testing, which showed that although, they were resistance to methicillin and penicillin, the changes in nucleotide sequence of the antibiotic resistance genes cDNA can indicate activity of extracts on resistant isolates.

REFERENCES

Borson, N.D., W.L. Salo and L.R. Drewes, 1992. A lock-docking oligo (dT) primer for 5' and 3' RACE PCR. PCR Methods Appl., 2: 144-148. PMID: 1477669.

Henke, W., M. Jung, K. Jung, M. Lein, H. Schlechte, C. Berndt, B. Rudolph, D. Schnorr and S.A. Loening, 1997. Increased analytical sensitivity of RT-PCR of PSA mRNA decreases diagnostic specificity of detection of prostatic cells in blood. Int. J. Cancer, 70: 52-56.

Holland, P.M., R.D. Abrahamson, R. Watson and D.H. Gelfand, 1991. Detection of specific polymerase chain reaction product by utilizing the 5-3' exonuclease activity of *Thermus aquaticus* DNA polymerase. National Acad. Sci. USA., 88: 7276-7280. PMCID: PMC52277.

Koch, W.H., W.L. Payne, B.A. Wentz and T.A. Cebula, 1993. Rapid polymerase chain reaction method for detection of *Vibrio cholera* in foods. Applied Environ. Microbiol., 59 (2): 556-560. PMCID: PMC20 2143.

Michael, T.M., M.M. John and P. Jack, 2005. Brock Microbiology of Microorganism. 11th Edn. New Jersey. ISBN: 13-978-0132192262.

Neto, E.D., R. Harrop, R. Correa-oliveira, R.A. Wilson, S.D. Pena and A.J. Simpson, 1997. Minilibraries constructed from cDNA generated by arbitrarily primed RT-PCR: An alternative to normalized libraries for the generation of EST's from nanogram quantities of mRNA. Gene, 186: 135-142. PMID: 9047356.

Rappolee, D.A., D. Mark, M.J. Banda and Z. Werb, 1988. Wound macrophages express TGF-Alpha and other growth factors *in vivo*: Analysis by mRNA phenotyping. Science, 241: 708-712. PMID: 3041594.

Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis and H.A. Ehlich, 1988. Primer directed enzymatic amplification with a thermostable DNA Polymerase. Science, 239: 487-491. PMID: 244 8875.

Wang, T. and M.J. Brown, 1999. mRNA quantification by real-time TaqMan polymerase chain reaction: Validation and comparison with RNase protection. J. Anal. Biochem., 269: 198-201. PMID: 10094795.

Wong, W.H., S.H. Goh and S.M. Phang, 1994. Antibacterial Properties of Malaysian Seaweeds. In: Phang, S.M., Y.K. Lee, M.A. Borowitzka and B.A. Whitton (Eds.). Algal Biotechnology in the Asia-Pacific Region. University of Malaya, Kuala Lumpur, pp: 75-81.