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Evaluation of the Two-Component Regulatory System Genes in *Staphylococcus aureus* with Roles for Survival and Pathogenicity

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Abstract: The current research trend for a new drug development was expanded by using the proteomic approaches. However, the expression on those drug target must be well identified before taking part into proteomics study. One of the most commonly use protocol is the expression profiles on those selected genes during certain simulated conditions. *Staphylococcus aureus* has been blessed with several virulence factors which are essential for its pathogenicity. One of the most well studied systems is the Two-Component Regulatory Systems (TCRSs). The TCRSs play a vital role in *S. aureus* survival and it become a good candidate for the specific drug target. Prior to determining the role, this study was made to observe the expression pattern of two TCRS' regulatory genes during the treatment with antibiotics against two strains of *S. aureus*; the Methicillin Methicillin Susceptible *S. aureus* (MSSA) and Methicillin Resistant *S. aureus* (MRSA). The partial transcripted analysis of the Reverse Transcriptase PCR (RT-PCR) data shows that the rates of expression in selected *TCRS* genes were different in both strains. Knowing the importance to *S. aureus* growth and pathogenesis, the *TCRS* genes can be a good candidate for designing a novel specific drug target against *S. aureus*, especially the MRSA.

Key words: TCRS, regulatory gene, *Staphylococcus aureus*, MRSA, reserve transcriptase, antibiotics

INTRODUCTION

Staphylococcus aureus is responsible for many infections ranging from food-borne intoxications to severe endocarditis and septicemia (Toledo-Arana *et al*., 2005). The bacteria is a part of human normal flora on many skin surfaces, especially around the nose, mouth, genitals and rectum. The current issue is emergence of Community Acquired Methicillin Resistant *S. aureus* (CAMRSA) that has worsens and limits the choicee of available antibiotics for treatment (Millar *et al*., 2007, 2008; Fergie and Purcell, 2001). *S. aureus* produces several virulence factors which are essential for its pathogenicity.

Among these are extracellular toxins and enzymes and cell-wall-associated molecule (Luong and Lee, 2006). These virulence and survival genes are expressed according to their growth phase and growth conditions. Gene expression for survival and pathogenicity are the regulatory factor-base with specific and sensitive mechanisms, mostly act at the transcriptional level and drive specific interactions with target gene promoters. These factors are largely regulated by Two-Component Regulatory Systems (TCRSs) such as the agr, saeRS, srrAB, arlSR and lytRS systems (Bronner *et al*., 2004). These systems are sensitive to environmental changes and their surroundings. It consist of a sensor histidine kinase and a response regulator protein that act as a mediator to trigger on the expression of others essential survival genes (Cheung *et al*., 2002; Kato *et al*., 2010). The TCRSs play a vital role in *S. aureus* survival and is said to be good candidate for the specific drug target (Converse *et al*., 2009; Li *et al*., 2010; Gotoh *et al*., 2010a, b; Wuichet *et al*., 2010). Prior to developing the gene as drug target, this study was conducted to observe the expression pattern of 2 TCRS's regulatory genes through the simulation of antibiotics impregnated environment which mimic the conditions of drugs treatment *in vitro* against *S. aureus*.

MATERIALS AND METHODS

Bacterial strains: The study included two reference strains of *S. aureus* comprising of ATCC29247; Methicillin Susceptible *S. aureus* (MSSA) and ATCC700698;

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Methicillin Resistant *S. aureus* (MRSA). Those reference strains were recharacterized according to genotypic and phenotypic analysis (Bannerman, 2003; Kateete *et al*., 2010; McDonald and Chapin, 1995; Woo *et al*., 2001). The reference strains were obtained from the collection of Medical Microbiology Laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia.

Challenging the bacteria with antibiotics: The treatment was made in the conical flask with a total volume of 50 mL medium per treatment. Muller Hinton broth was used as the treatment medium and the Minimal Inhibitory Concentration (MIC) dosage of antibiotics used were 10 μg mL^{-1} (penicillin) and 0.2 μg mL^{-1} (vancomycin) with three replicates for each treatment. The pattern of the bacteria growth during treatment was determined by measuring the absorbance at 600 nm before treatment start and at every 1 h until the treatment finished for the total period of 6 h.

Extraction of RNA: The total of nucleic acids was extracted prior to the selected time frame by using MasterPure™ complete DNA and RNA purification kit (Epicentre biotechnologies) under the protocol for the total nucleic acid extraction of bacterial cells. The removal of contaminant DNA was done after a successful extraction. The RNA purity was observed on the 0.8% (w/v) agarose and was also quantified by using biophotometer (Eppendorf®, Germany) to ensure the remaining RNA is free from the DNA contaminant.

Further screening of contaminant DNA: Real-time PCR amplification with SYBR Green I was performed by using MasterCycler® Ep RealPlex (Eppendorf, Germany) with total volume of 20 μL per each reaction in clear 8 $strip^{-1}$ 0.2 mL thin wall tubes (Axygen, USA). The primers set of 16srRNA used in this study was described previously (Luong and Lee, 2006; Luong *et al*., 2006). The optimum annealing temperature (T_a) was readjusted by using Rychlik equation (Rychlik *et al*., 1990) and was automatically generated by using PrimerPremier software V 5.0 (PREMIER Biosoft, USA).

Conversion to cDNA: The remaining RNA was converted to cDNA by using MonsterScript™ 1st Strand cDNA synthesis kit (Epicentre biotechnologies). The converted cDNA was measured by using the Biophotometer (Eppendorf®, Germany) and the equal concentration of starting templates were used in the PCR proceeding with cDNA conversion.

Polymerase chain reaction: PCR were carried out in a total volume of 25 μL containing 1 μL of cDNA, 10 pmoL of each primer and 1.0 unit of Taq DNA polymerase, 1.5 mM $MgCl_2$ and 200 μM dNTP.

Twenty five cycles were performed as follows: denaturation at 94°C for 1 min, primer annealing at 48.1°C for AglR primers and 53.5°C for sigB primers for 30 sec; primer extension at 72°C for 30 sec and final extension 72°C for 3 min (Luong and Lee, 2006). The PCR product was run on prestained 1.2% agarose gel with GelRed™ (Biotium®) and was viewed under the UV light image analyzer (Huang *et al*., 2010).

RESULTS AND DISCUSSION

The growth curves of *S. aureus* during treatments were plotted (Fig. 1 and 2). As expected, the absorbance readings for the MSSA showed that the treatment with

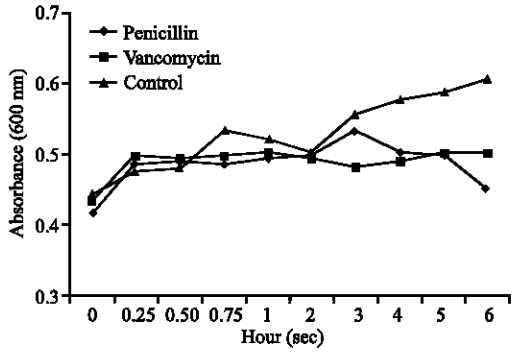

Fig. 1: Absorbance reading of the MSSA during the treatment with antibiotics. The declined growth pattern during the treatment with penicillin and vancomycin is expected

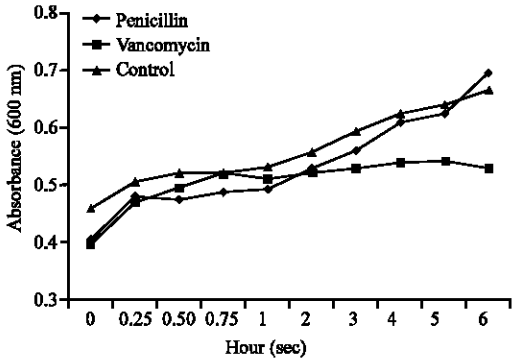

Fig. 2: Absorbance reading of the MRSA during the treatment with antibiotics. The further increment of cell number during penicillin treatment is expected in MRSA cell culture as they are resistant to this antibiotic

penicillin and vancomycin, respectively inhibited further increment of cell number in treatment flask in comparison to the control. For the MRSA, no further increment in cell number was observed in vancomycin treatment but increased in cell numbers were seen in control and penicillin treated cells.

In the molecular assay, two set of primer from the TCRS were used. The selected genes are *ArlR* and *SigB*. With the increment of treatment time, significant expression patterns of both genes were observed in both strains (Fig. 3 and 4).

Both strains expressed the selected *TCRS* genes but the rate of expression were slightly different. Both genes were amplified and expressed before the treatment started but intensity of signal for expressed bands between the two different strains were varied with time. The more solid banding patterns were observed with the increment of

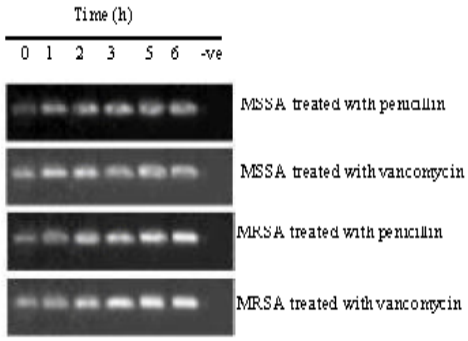

Fig. 3: Expression of *ArlR* gene of *S. aureus* during the drug treatment. It was observed that the expression of *ArlR* gene in MRSA will be increased with treatment time. This can be observed as a well intense banding pattern after 2 h of treatment

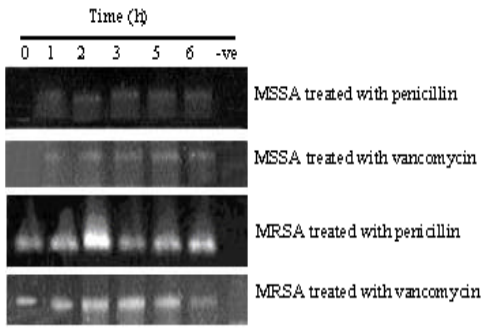

Fig. 4: Expression of *SigB* gene of *S. aureus* during the drug treatment. It was observed that the expression of *SigB* gene in MRSA will be increased with treatment time. This can be observed as a well intense banding pattern after 2 h of treatment

treatment time in MRSA strain and shows that the number of bacterial cell also increased. The resistant property of MRSA strains to antibiotics explained the observation. Further quantification through quantitative real-time PCR also showed a significant increment quantification of expressed genes with time.

CONCLUSION

In this study, the band intensity visualization is the indirect reflection on the rates of genes expression in selected *TCRS* genes as the intensity differ in drug sensitive and drug resistant strains.

Further study will be required to confirm the importance of *TCRS* gene to *S. aureus* growth and pathogenesis. The gene products expression effect findings of the present study indicated the potential of *TCRS* genes to be relevant candidates for designing a novel specific drug target against *S. aureus*, especially MRSA.

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