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Full Length Research Paper

Partial genes expression assessment of two-component regulation system *ArlR* and *SigB* towards *Staphylococcus aureus* survival *in vitro*

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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 2 TCRS' regulatory genes during the treatment with antibiotics against two strains of *S. aureus*; the 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: Two-component regulatory systems (TCRSs), regulatory gene, *Staphylococcus aureus,* methicillin resistant *S. aureus* (MRSA).

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 (Fergie and Purcell, 2001; Millar et al., 2007; 2008).

S. aureus produces several virulence factors which are essential for its pathogenicity. Among these are extra-cellular 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

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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; Gotoh et al., 2010a; 2010b; Li et al., 2010; 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 2 reference strains of *S. aureus* comprising of ATCC29247; Methicillin Susceptible *S. aureus* (MSSA) and ATCC700698; Methicillin Resistant *S. aureus* (MRSA). Those reference strains were re-characterized according to genotypic and phenotypic analysis (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 (Penicillin) and 0.2 µg/ml (Vancomycin), with 3 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-per strip 0.2 ml thin wall tubes (Axygen, USA). The primers set of 16 *srRNA* used in this study was described previously (Luong et al., 2006; Luong and Lee, 2006). The optimum annealing temperature (T_a) was re-adjusted by using Rychlik equation (Rychlik et al., 1990) and was automatically generated by using Primer Premier software v5.0 (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 *ArlR* primers and 53.5°C for *SigB* primers for 30 s; primer extension at 72 °C for 30 s and final extension 72 °C for 3 min (Luong and Lee, 2006). The PCR product was run on pre-stained 1.2% agarose gel with GelRed™ (Biotium®) and was viewed under the UV light image analyzer (Huang et al., 2010).

RESULTS

The growth curves of *S. aureus* during treatments were plotted (Figures 1 and 2). As expected, the absorbance readings for the MSSA showed that the treatment with 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, 2 set of primers 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 (Figures 3 and 4).

DISCUSSION

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 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

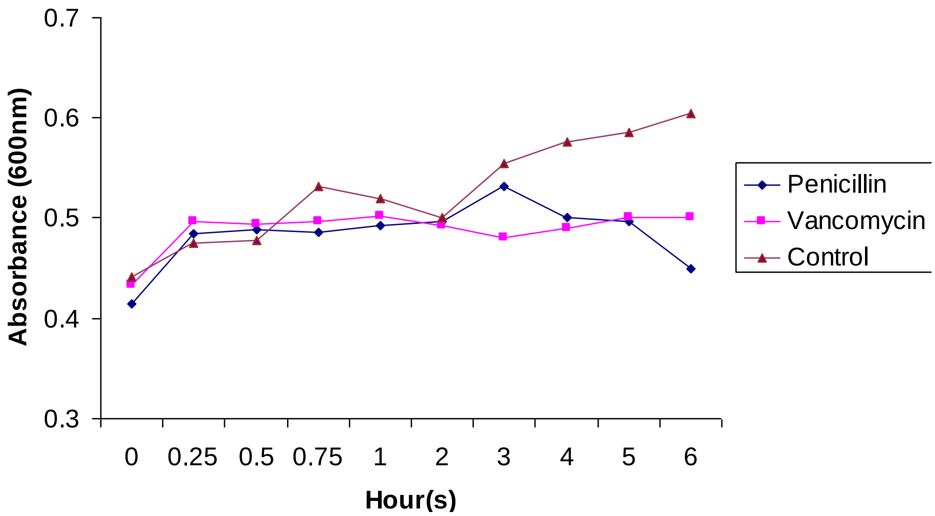

Figure 1. Absorbance reading of the MSSA during the treatment with antibiotics. The declined growth pattern during the treatment with Penicillin and Vancomycin is expected in methicillin sensitive *S. aureus* strain.

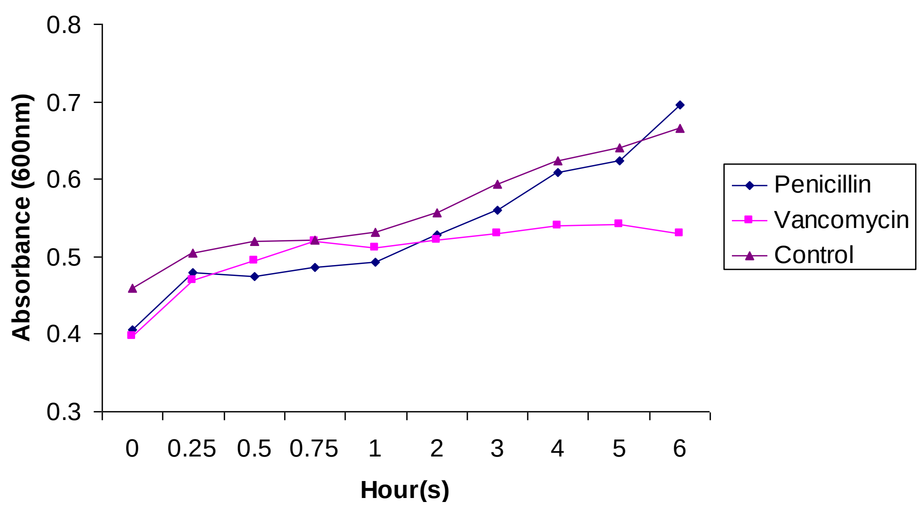

Figure 2. Absorbance reading of the MRSA during the treatment with antibiotics. The further increment of cell number during Penicillin treatment is expected in MRSA cell culture as they are resistant to this antibiotic.

increment quantification of expressed genes with time (data not shown).

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. The *sigB* gene in *S. aureus* was well documented to increase their expression profiles in antibiotic impregnated TSB medium (Kusch et al., 2011; Rogasch et al., 2006), and was tested in several *S. aureus* strains. However, the involvement of *Arl* systems in the virulence expression still inconclusive as the mutated strains on this genes are still enable to survive in the presence of other TCRS in *S. aureus* system; typically the *sarA* and *agr* regulatory loci in modulating the virulence regulation network (Fournier et al., 2001; Kusch et al., 2011; Ueda et al., 2011). It was predicted that the TCRS genes in *S. aureus* works together in complicated regulation network either by upregulation or downregulation response, thus the multiple target on specific TCRS genes is essential in combating the MRSA.

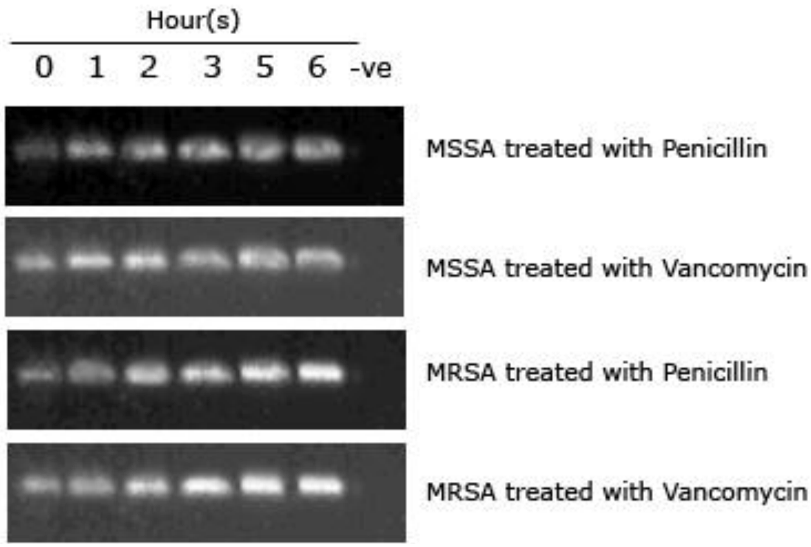

Figure 3. Expression of *ArlR* gene of *S. aureus* during the drug treatment. It was observed that the expression of *ArlR* gene in MRSA will be increased with treatment time. This can be observed as a well intense banding pattern after 2 h of treatment.

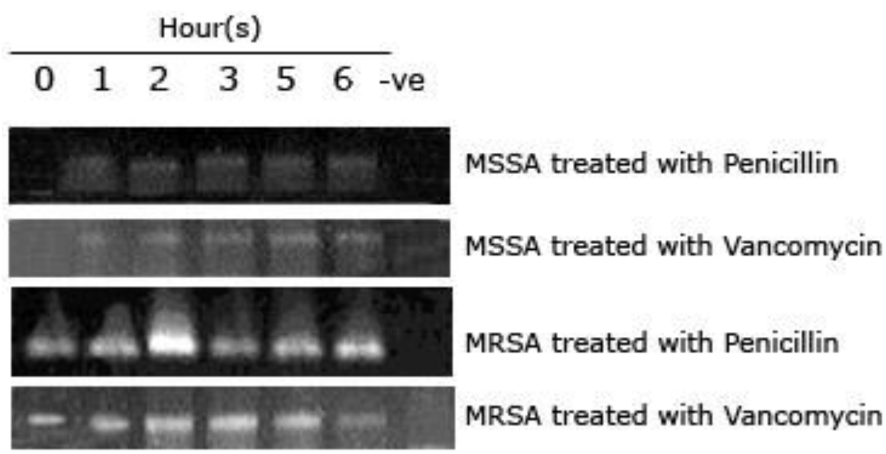

Figure 4. Expression of *SigB* gene of *S. aureus* during the drug treatment. It was observed that the expression of *SigB* gene in MRSA will be increased with treatment time. This can be observed as a well intense banding pattern after 2 hours of treatment.

Further work will be required to confirm the importance of TCRS gene to *S. aureus* growth and pathogenesis. Multiple gene knock-out model need to be developed since the correlation between TCRS system is needed to be evaluated in details. 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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REFERENCES

Bronner S, Monteil H, Prevost G (2004). Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications. FEMS Microbiol. Rev., 28: 183-200

Cheung AL, Projan SJ, Gresham H (2002). The Genomic Aspect of Virulence, Sepsis, and Resistance to Killing Mechanisms in *Staphylococcus aureus*. Curr. Infect. Dis. Rep., 4: 400-410

Converse PJ, Karakousis PC, Klinkenberg LG, Kesavan AK, Ly LH, Allen SS, Grosset JH, Jain SK, Lamichhane G, Manabe YC, McMurray DN, Nuermberger EL, Bishai WR (2009). Role of the dosR-dosS two-component regulatory system in Mycobacterium tuberculosis virulence in three animal models. Infect. Immun., 77: 1230-1237

Fergie JE, Purcell K (2001). Community-acquired methicillin-resistant *Staphylococcus aureus* infections in south Texas children. Pediatr. Infect. Dis. J., 20: 860-863

Fournier B, Klier A, Rapoport G (2001). The two-component system ArlS-ArlR is a regulator of virulence gene expression in *Staphylococcus aureus*. Mol. Microbiol., 41: 247-261

Gotoh Y, Doi A, Furuta E, Dubrac S, Ishizaki Y, Okada M, Igarashi M, Misawa N, Yoshikawa H, Okajima T, Msadek T, Utsumi R (2010a). Novel antibacterial compounds specifically targeting the essential WalR response regulator. J. Antibiot. (Tokyo), 63: 127-134

Gotoh Y, Eguchi Y, Watanabe T, Okamoto S, Doi A, Utsumi R (2010b) Two-component signal transduction as potential drug targets in pathogenic bacteria. Curr. Opin. Microbiol., 13: 232-239

Huang Q, Baum L, Fu WL (2010). Simple and practical staining of DNA with GelRed in agarose gel electrophoresis. Clin. Lab., 56: 149-152

Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, Joloba ML, Najjuka FC (2010). Identification of *Staphylococcus aureus:* DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Ann. Clin. Microbiol. Antimicrob., 9: 23

Kato Y, Suzuki T, Ida T, Maebashi K (2010). Genetic changes associated with glycopeptide resistance in *Staphylococcus aureus*: predominance of amino acid substitutions in YvqF/VraSR. J. Antimicrob. Chemother., 65: 37-45

Kusch K, Hanke K, Holtfreter S, Schmudde M, Kohler C, Erck C, Wehland J, Hecker M, Ohlsen K, Bröker B, Engelmann S (2011). The influence of SaeRS and [sigma]B on the expression of superantigens in different *Staphylococcus aureus* isolates. Int. J. Med. Microbiol., 301: 488-499

Li Y, Zeng J, Zhang H, He ZG (2010). The characterization of conserved binding motifs and potential target genes for M. tuberculosis MtrAB reveals a link between the two-component system and the drug resistance of *M. smegmatis*. BMC Microbiol., 10: 242

Luong TT, Dunman PM, Murphy E, Projan SJ, Lee CY (2006) Transcription Profiling of the mgrA Regulon in *Staphylococcus aureus*. J. Bacteriol., 188: 1899-1910

Luong TT, Lee CY (2006). The arl locus positively regulates *Staphylococcus aureus* type 5 capsule via an mgrA-dependent pathway. Microbiology, 152: 3123-3131

McDonald CL, Chapin K (1995). Rapid identification of *Staphylococcus aureus* from blood culture bottles by a classic 2-hour tube coagulase test. J. Clin. Microbiol., 33: 50-52

Millar BC, Loughrey A, Elborn JS, Moore JE (2007). Proposed definitions of community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA). J. Hosp. Infect., 67: 109-113

Millar BC, Prendergast BD, Moore JE (2008). Community-associated MRSA (CA-MRSA): an emerging pathogen in infective endocarditis. J. Antimicrob. Chemother., 61: 1-7

Rogasch K, Rühmling V, Pané-Farré J, Höper D, Weinberg C, Fuchs S, Schmudde M, Bröker BM, Wolz C, Hecker M, Engelmann S (2006). Influence of the two-component system SaeRS on global gene expression in two different *Staphylococcus aureus* strains. J. Bacteriol., 188: 7742-7758

Rychlik W, Spencer WJ, Rhoads RE (1990) Optimization of the annealing temperature for DNA amplification *in vitro*. Nucleic Acids Res., 18: 6409-6412

Toledo-Arana A, Merino N, Vergara-Irigaray M, Debarbouille M, Penades JR, Lasa I (2005) *Staphylococcus aureus* develops an alternative, ica-independent biofilm in the absence of the arlRS two-component system. J Bacteriol., 187: 5318-5329

Ueda T, Kaito C, Omae Y, Sekimizu K (2011). Sugar-responsive gene expression and the agr system are required for colony spreading in *Staphylococcus aureus*. Microb. Pathog., 51: 178-185

Woo PC, Leung AS, Leung KW, Yuen KY (2001). Identification of slide coagulase positive, tube coagulase negative *Staphylococcus aureus* by 16S ribosomal RNA gene sequencing. Mol. Pathol., 54: 244-247

Wuichet K, Cantwell BJ, Zhulin IB (2010). Evolution and phyletic distribution of two-component signal transduction systems. Curr. Opin. Microbiol., 13: 219-225