



Data in Brief

RNA-Seq reveals changes in the *Staphylococcus aureus* transcriptome following blue light illumination



Tamarah L. Adair*, Bayless E. Drum

Baylor University Department of Biology, One Bear Place 97388, Waco, TX 76798, United States

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ABSTRACT

In an effort to better understand the mechanism by which blue light inhibits the growth of *Staphylococcus aureus* in culture, a whole transcriptome analysis of *S. aureus* isolate BUSA2288 was performed using RNA-Seq to analyze the differential gene expression in response to blue light exposure. RNA was extracted from *S. aureus* cultures pooled from 24 1 ml well samples that were each illuminated with a dose of 250 J/cm² of 465 nm blue light and from control cultures grown in the dark. Complementary DNA libraries were generated from enriched mRNA samples and sequenced using the Illumina MiSeq Next Generation Sequencer. Here we report one type of analysis that identified 32 candidate genes for further investigation. Blue light has been shown to be bactericidal against *S. aureus* and is a potential alternative therapy for antibiotic resistant organisms. The mechanism for the inactivation of bacteria is hypothesized to involve reactive oxygen species. These RNA-Seq results provide data that may be used to test this hypothesis. The RNA-Seq data generated by these experiments is deposited in Gene Expression Omnibus (Gene accession GSE62055) and may be found at NCBI (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62055>).

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Specifications

Organism/cell line/tissue	<i>S. aureus</i> BUSA2288 (Nasal isolate)
Sex	NA
Sequencer or array type	MiSeq
Data format	Raw
Experimental factors	Blue light illumination vs no light
Experimental features	RNA-Seq was used to analyze the differential gene expression of <i>S. aureus</i> in culture in response to blue light exposure.
Consent	IRB Baylor University
Sample source location	NA

1. Direct link to deposited data [provide URL below]

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62055>

2. Experimental design, materials and methods

[complete description of the Experimental design and methods used to acquire the genomic data and where applicable, in the analysis. Include any relevant figures/tables.]

* Corresponding author.

2.1. Bacterial isolate

A methicillin resistant isolate of *S. aureus* was cultured from the nasal passage of a healthy Baylor University student in Waco, TX during the fall of 2009. This isolate is referred to as BUSA2288. Baylor University's Institutional Review Board for the protection of human subjects approved the consent form, collection procedures, and recording methods. The nasal passage sample was collected by swabbing each anterior nare and gently rolling the swab across the surface of a mannitol salt agar plate. Fermenting colonies were isolated and purified on tryptic soy agar (TSA) plates. Gram positive, catalase positive, coagulase positive, staphylococcal cultures were identified as *S. aureus* and stored in CRYOCARE beads (Key Scientific Products, Stamford, Texas) for future use. A Kirby Bauer disc diffusion assay was performed on *S. aureus* BUSA2288 and oxacillin resistance was confirmed using Etest (bioMérieux, Inc., Durham, NC) and positive PCR amplification of the *nuc* and *MecA* genes [4].

2.2. Growth conditions

BUSA2288 was grown overnight in 5 ml of Brain Heart Infusion (BHI) broth at 37 °C. The broth culture was inoculated from a single colony grown on a TSA plate. The contents of this overnight culture were added to 45 ml BHI broth resulting in a concentration of approximately

1×10^8 CFU/ml as measured by colony counts. 1 ml aliquots of this diluted overnight culture was transferred to each well of two BD Falcon™ non-treated 24-well plates. The control plate, labeled No Light (NL), was covered and protected from light, and the treatment plate, labeled Blue Light (BL), was illuminated. Both plates were incubated with shaking at 35 °C for 2 h.

2.3. Light source

The illumination box was designed and constructed in house. Twenty-four 1.5 mm Kingbright blue LED lights were attached to a 24-well plate lid. The lights were arranged so that when the modified lid was placed on a 24-well plate the lights were 0.5 mm above the broth of the individual wells as seen in Fig. 1. The LED lights are CIE 127 compliant with a dominant wavelength of 465 nm and a 2θ of 16° [1]. The lights were operated at a forward current of 20 mA for 2 h resulting in a total light dosage per well of 250 J/cm². The resistors were placed away from the light box so as to not increase the temperature inside of the incubator.

2.4. RNA extraction and mRNA enrichment

Total RNA was extracted from the NL control and BL treatment samples using a modified phenol chloroform extraction method as follows. The culture was removed from 24 wells and centrifuged. The pelleted cells were resuspended in RNase free water and incubated with an equal volume of 1:1 phenol/chloroform (~250 µl each). After a 30 min incubation at 70 °C, the phases were separated by centrifugation at 12,000 ×g for 10 min. The aqueous layer was removed (~200 µl) and 2x the volume of isopropanol was added followed by refrigerated centrifugation at 12,000 ×g for 10 min. The RNA pelleted was washed with 200 µl of cold 70% ethanol and centrifuged at 8000 ×g for 10 min. The pellet was dried in the inverted tube at room temperature for 10 min and resuspended in 95 °C Elution Buffer Solution (Ambion). Both samples were treated with DNase Inactivation Reagent (Ambion). The mRNA was enriched following the protocols provided with the MICROBExpress Bacterial mRNA Enrichment Kit (Ambion). This procedure uses a capture hybridization by magnetic beads to remove 16S and 23S ribosomal RNAs. The purity and concentration of the total and enriched RNA samples were analyzed by gel electrophoresis and by an Agilent Bioanalyzer. Complementary DNA libraries were built and sequenced using the Illumina MiSeq Next Generation Sequencer at the University of Oklahoma Health Science Center's lab for Molecular Biology and Cytometry Research.

2.5. RNA-seq data analysis

Two independent experiments were performed and sequenced. Whole genome sequencing has not been performed on BUSA2288, so in order to determine which reference genomes to use for alignment

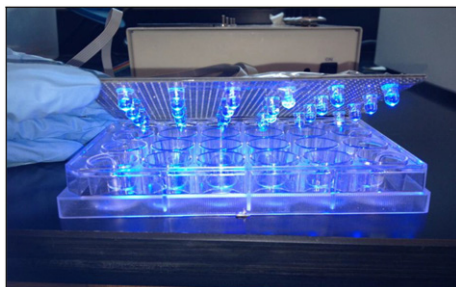


Fig. 1. Blue light box. The light box was built in house using Kingbright LED lights and an adjustable power supply [1].

we performed an alignment using BLAST and determined the two closest related reference genomes. High quality reads were then aligned to the genomes of MRSA252 (NC_002952) and N315 (NC_002745), in order to create a transcriptome map.

In one analysis, the combined results of both independent experiments were analyzed using the Pairwise Analysis tools in Gene Sifter® [3]. The genes were normalized by Mapped Reads using EdgeR statistics including a Benjamini and Hochberg false discovery rate correction. The Quality was set at a minimum number of 10 reads and the lower threshold for change was 5 fold, with a p-value of 0.05 or less. These criteria produced a list of 32 up or down regulated genes as shown in Table 1.

Table 1
Differentially regulated genes.

Functional category and gene	gene	MRSA252 Locus	n-fold change	P-value
1. Biosynthesis of amino acids				
aspartate semialdehyde dehydrogenase	<i>asd</i>	SAR1406	−5.54	4.41E-03
aspartate kinase	<i>lysC</i>	SAR1405	−5.3	7.70E-03
2. Cell envelope components				
sortase	<i>srtB</i>	SAR1108	5.03	9.45E-03
3. Cellular processes				
serine protease	<i>spIC</i>	SAR1906	7.45	1.49E-02
4. Central intermediary metabolism				
Nitrate reductase subunit alpha	<i>narG</i>	SAR2486	−5.88	5.96E-03
Nitrate reductase subunit beta	<i>narH</i>	SAR2485	−5.88	5.96E-03
Nitrate reductase gamma chain	<i>NarI</i>	SAR2483	−5.88	5.96E-03
Respiratory nitrate reductase delta chain	<i>narJ</i>	SAR2484	−5.88	5.96E-03
Small heat shock protein	<i>narK</i>	SAR2475	−9.24	1.25E-03
Nitrite transport protein	<i>narT</i>	SAR2476	−9.24	1.44E-03
Nitrite reductase large subunit	<i>nasD</i>	SAR2489	−5.46	2.50E-02
Assimilatory nitrite reductase small subunit	<i>nasE</i>	SAR2488	−6.15	1.20E-02
Tetrapyrrole (corrin/porphyrin) methylase	<i>nasF</i>	SAR2487	−6.15	1.20E-02
5. Energy metabolism				
Azoreductase	<i>acpD</i>	SAR0203	28.06	1.79E-02
Dioxygenase	<i>PcpA_N_like</i>	SAR2599	18.32	1.14E-02
6. Protein synthesis				
Aminoacyl-tRNA biosynthesis	<i>tRNA-Asp</i>	SARt023	−10.13	4.01E-02
7. Regulatory function				
Accessory gene regulator B	<i>agrB</i>	SAR2123	−8.03	5.29E-04
Nitrogen regulatory protein A	<i>nreB</i>	SAR2482	−5.56	1.38E-02
Response regulator	<i>nreC</i>	SAR2480	−5.68	3.52E-02
Dissimilatory nitrate/nitrite reduction) two-component regulatory system	<i>NreB-NreC</i>	SAR2481	−5.88	2.24E-02
RNAIII regulatory transcript/delta haemolysin	<i>RNAIII</i>	SARs022	−9.88	2.38E-04
8. conserved protein, unknown function	Pfam prediction			
Hypothetical protein	SepA	SAR2259	−8.38	3.47E-05
Hypothetical protein	YceI-like	SAR2769	11.74	3.47E-02
Hypothetical protein	CbiX, CbiK, DUF3928	SAR2490	−7.13	9.08E-03
Hypothetical protein	Pig-F GPI biosynthesis	SAR0742	−5.64	1.13E-02
Hypothetical protein	Trep-Strep	SAR1005	−5.76	3.68E-03
Hypothetical protein	DoxX, DoxX_2	SAR1010	−5.64	9.06E-03
Hypothetical protein	bPH_5	SA2264	−7.11	1.77E-03
Hypothetical protein	DUF5080, HRG	SAR0291	−6.24	3.48E-04
Hypothetical protein	DUF4293, RTA1, Serinc	SAR0292	−6.31	5.52E-03
Hypothetical protein	Ycf1	SAR2683	−5.7	5.96E-03
Hypothetical protein	Putative membrane protein	SAR0455	−5.51	4.12E-02

2.6. Conclusions

One hypothesis regarding the mechanism of blue light inhibition is that the interaction of blue light with intracellular or membrane bound molecules, results in production of reactive oxygen species and cell death [2]. This data indicates that there is a genetic response to the blue light involving the oxidative stress pathways. Whether this is a specific or general response is an important question to explore, since polymorphism exists at many of these alleles. This data provides a starting point for further exploration. It is possible that a small molecule acting to up or down regulate one or more of these pathways may provide a new antimicrobial. The raw data files for the RNA-seq experiment are deposited in the GEO, Gene accession GSE62055.

Transparency document

The [Transparency document](#) associated with this article can be found, in online version.

Acknowledgements

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References

- [1] Y. Lu, Preliminary Spec: T-1 3/4 (5 mm) SOLID STATE LAMP. <http://www.mouser.com/ds/2/216/wp7113pbc-a-308475.pdf>2009.
- [2] M. Maclean, S.J. Macgregor, J.G. Anderson, G.A. Woolsey, The role of oxygen in the visible-light inactivation of *Staphylococcus aureus*. *J. Photochem. Photobiol. B* 92 (2008) 180–184, <http://dx.doi.org/10.1016/j.jphotobiol.2008.06.006>.
- [3] PerkinElmer, Inc, Geospiza GeneSifter® for Genetic Analysis. <http://www.geospiza.com/Products/default.shtml>2016.
- [4] K. Zhang, J. Sparling, B.L. Chow, S. Elsayed, Z. Hussain, D.L. Church, D.B. Gregson, T. Louie, J.M. Conly, New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative *Staphylococci*. *J. Clin. Microbiol.* 42 (2004) 4947–4955, <http://dx.doi.org/10.1128/JCM.42.11.4947-4955.2004>.