Whole genome sequencing and comparative analysis of novel pathogen elizabethkingia anophelis against oxidative stress

Li, Yingying

2017

Li, Y. (2017). Whole genome sequencing and comparative analysis of novel pathogen elizabethkingia anophelis against oxidative stress. Doctoral thesis, Nanyang Technological University, Singapore.

<http://hdl.handle.net/10356/70627>

<https://doi.org/10.32657/10356/70627>

NANYANG
TECHNOLOGICAL
UNIVERSITY

**WHOLE GENOME SEQUENCING AND
COMPARATIVE ANALYSIS OF NOVEL
PATHOGEN *ELIZABETHKINGIA ANOPHELIS*
AGAINST OXIDATIVE STRESS**

LI YINGYING

School of Biological Sciences

2017

**WHOLE GENOME SEQUENCING AND
COMPARATIVE ANALYSIS OF NOVEL
PATHOGEN *ELIZABETHKINGIA ANOPHELIS*
AGAINST OXIDATIVE STRESS**

LI YINGYING

School of Biological Sciences

A thesis submitted to the Nanyang Technological University in fulfillment
of the requirement for the degree of
Doctor of Philosophy

2017

ACKNOWLEDGEMENT

I would like to take this opportunity to express my sincere gratitude to my supervisor Prof Yang Liang and Prof Michael Givskov for giving me an opportunity to work in NTU and for their mentorship and guidance throughout my Ph.D study. I am very grateful that they always gave me the freedom to explore concepts and hypotheses during my study. Their encouragement and enthusiasms for my work have inspired me to work towards the various goals of my project.

I would also like to thank Dr. Jeanette Teo for offering me the strain *Elizabethkingia anophelis* to study with; Dr. Liu Yang and Dr. Martin Tay for their help and guidance in transcriptomics works and sequencing analysis; Ms. Chen Yicai for her guidance in lab skills and data analysis; Mr. Ding Yichen for helping me in data analysis; Dr. Chew Su Chuen and Mr. Yam Kuok Hoong Joey for helping me in imaging study and data analysis; all other group members and all my colleagues in Singapore Centre for Environmental Life Sciences Engineering (SCELSE) for their assistance and help throughout the years.

Heartful thanks to my family members especially my dad and mum for always being there giving me support and encouragement. It would not have been possible for me to make it this far in my education without their encouragement and support. Special thanks to my husband Mr. Mao Xianwei, for his patient accompany and consolation at all the difficult times.

Last but not least, I would like to acknowledge the financial support from National Research Foundation and Ministry of Education Singapore under its Research Centre of Excellence Program, and Nanyang research scholarship for the year 2013-2016.

TABLE OF CONTENTS

ACKNOWLEDGEMENT	I
TABLE OF CONTENTS	II
LIST OF FIGURES.....	IV
LIST OF TABLES.....	VII
LIST OF ABBREVIATIONS.....	VIII
LIST OF PUBLICATIONS.....	X
ABSTRACT	XI
CHAPTER 1: Introduction	1
1.1 BACKGROUND	1
1.2 KNOWLEDGE GAP AND CHALLENGES	2
1.3 OBJECTIVE	3
CHAPTER 2 Literature Review	4
2.1 THE GENUS <i>ELIZABETHKINGIA</i>	4
2.1.1 Identification	4
2.1.2 Characteristics of the genus <i>Elizabethkingia</i>	5
2.1.3 <i>Elizabethkingia</i> spp. as pathogens.....	6
2.2 BIOFILM	7
2.3 ANTIMICROBIAL RESISTANCE	11
2.4 OXIDATIVE RESISTANCE IN BACTERIUM	16
2.5 IRON AND HEME UTILIZATION BY BACTERIUM	22
CHAPTER 3 Genome Sequencing and Comparative Genomic Analysis of <i>E. anophelis</i> NUHP1	24
3.1 INTRODUCTION	24
3.2 MATERIALS AND METHODS.....	25
3.2.1 Bacterial strains	25
3.2.2 Growth medium and conditions	25
3.2.3 MIC of different antibiotics to <i>E. anophelis</i> NUHP1	25
3.2.4 Sample preparation for complete genome sequencing.....	26
3.2.5 Comparative genomic analysis	26
3.3 RESULTS AND DISCUSSION.....	27
3.3.1 Genome characterization.....	27
3.3.2 Antibiotic resistance	30
3.3.3 Virulence mechanisms revealed by genome analysis	32
3.3.4 Comparative genomic analysis	36
<i>Genome comparison between NUHP1 and E. anophelis assembly from mosquito</i>	36

Core genome-based phylogenetic structure of 16 <i>E. anophelis</i> isolates from NUH.....	37
CHAPTER 4 Biofilm Formation of <i>E. anophelis</i> NUHP1 and Transcriptomic Analysis of Stress Response of NUHP1 to Hydrogen Peroxide	39
4.1 INTRODUCTION	39
4.2 MATERIALS AND METHODS.....	40
4.2.1 Bacterial strains	40
4.2.2 Growth medium and conditions	40
4.2.2 Biofilm formation assay	41
4.2.2.1 Static biofilm.....	41
4.2.2.2 Flow cell biofilm.....	41
4.2.4 CAS Liquid Assay.....	43
4.2 RESULTS AND DISCUSSION	43
4.2.3 Biofilm formation.....	43
<i>Biofilm formation under static conditions</i>	43
<i>Biofilm formation in flow cell system</i>	45
4.2.1 Transcriptomic analysis of stress response of <i>E. anophelis</i> to hydrogen peroxide.....	48
4.2.2 RT-PCR analysis of stress response of <i>E. anophelis</i> to hydrogen peroxide.....	50
4.2.3 Siderophore production is enhanced by oxidative stress in <i>E. anophelis</i> ...	51
CHAPTER 5 Transcriptomic Analysis of <i>E. anophelis</i> in Response to Blood and Comparative Transcriptome Analysis of <i>E. anophelis</i> in Response to Blood and Hydrogen Peroxide.....	54
5.1 INTRODUCTION	54
5.2 MATERIALS AND METHODS.....	55
5.2.1 Sample harvest, RNA extraction and transcriptomic analysis of <i>E. anophelis</i> in response to mouse blood	55
5.2.2 Time-Kill assay	56
5.2.3 Biofilm assay.....	56
5.3 RESULTS AND DISCUSSION	57
5.3.1 Transcriptomic analysis of <i>E. anophelis</i> in response to mouse blood	57
5.3.2 Comparison of <i>E. anophelis</i> transcriptome in response to mouse blood and to H ₂ O ₂	61
5.3.3 Hemoglobin utilization enhances growth, hydrogen peroxide tolerance and biofilm formation of <i>E. anophelis</i>	66
CHAPTER 6 Conclusion and Future Plan	69
6.1. Conclusion	69
6.2. Future Plan.....	70
References	72
Appendix	83

LIST OF FIGURES

Figure 2.1 “Diagram showing the wide-ranging impacts of biofilms on human health and industries.” Image credit: Phil Stewart / Peg Dirckx (1996), Montana State University Center for Biofilm Engineering	10
Figure 2.2 Different classes of antibiotics.	12
Figure 2.3 “Intrinsic mechanisms of resistance.” The figure shows an overview of intrinsic resistance mechanisms. PBP: penicillin-binding protein. Antibiotic A enters the cell via a membrane-spanning porin protein and bind to the target PBP to inhibit peptidoglycan biosynthesis. For antibiotic B, although it also enters the cell via a porin, the efflux pump eliminate it efficiently. Antibiotic C cannot even enter the cell and so is unable to bind the target. <i>The figure is reprinted from (Blair J M A, Webber M A, Baylay A J, et al. Nature Reviews Microbiology, 2015, 13(1): 42-51.) with permission from Nature Publishing Group.</i>	12
Figure 2.4 “Direct interactions with antibiotics.” a: An antibiotic bind to the target efficiently. b: The host destroys the antibiotic to prevent binding to the target. c: The host modifies the structure of the antibiotic to prevent binding to the target. <i>The figure is reprinted from (Blair J M A, Webber M A, Baylay A J, et al. Nature Reviews Microbiology, 2015, 13(1): 42-51.) with permission from Nature Publishing Group.</i>	14
Figure 2.5 “Three hypotheses for mechanisms of antibiotic resistance in biofilms.” The bottom is attachment surface and the top is the aqueous phase which contains the antibiotic. The three hypothesis for biofilm resisting to antibiotics includes slow penetration, resistant phenotype and alters microenvironment as shown in the figure. <i>The figure is reprinted from (Stewart P S, Costerton J W. The lancet, 2001, 358(9276): 135-138.) with permission from Elsevier.</i>	16
Figure 2.6 “The generation of reactive oxygen species and the enzymes used for scavenging.” a: The potent oxidants H_2O_2 , O_2^- and $HO\bullet$. b: The enzymes that involve in ROS degradation in <i>Escherichia coli</i> . SOD target s O_2^- and catalases as well as Ahp target H_2O_2 . <i>The figure is reprinted from (Imlay J A. Nature Reviews Microbiology, 2013, 11(7): 443-454.) with permission from Nature Publishing Group.</i>	17
Figure 2.7 “Overview of damage caused by reactive oxygen species in <i>Escherichia coli</i> .” The formation of hydrogen peroxide and superoxide damages Fe–S cluster proteins and mononuclear iron enzymes. H_2O_2 could also react with free Fe^{2+} directly, leading to the damage of biomolecules such as DNA. In <i>E. coli</i> , the accumulation of H_2O_2 and O_2^- could be prevented by catalases peroxidases and superoxide dismutases. <i>The figure is reprinted from (Imlay J A. Nature Reviews Microbiology, 2013, 11(7): 443-454.) with permission from Nature Publishing Group.</i>	19

Figure 2.8 “Simplified schematic overview of important determinants involved in staphylococcal response to oxidative stress affecting whole cell physiology.” Image from Gaupp (Gaupp, Ledala <i>et al.</i> 2012).....	21
Figure 2.9 “Mechanisms of hemoglobin utilization by pathogens.” (Pishchany and Skaar 2012).....	23
Figure 3.1 Circular representation of sequence conservation between <i>E. anophelis</i> NUHP1 and 11 <i>Elizabethkingia spp.</i> strains for identifying genome regions with high flexibility. Circles are numbered from 1 (outermost circle) to 14 (innermost circle). Circle 1: <i>E. meningoseptica</i> ATCC13253 (OSU). Circle 2: <i>E. meningoseptica</i> ATCC13253 (NITE). Circle 3: <i>E. meningoseptica</i> 502. Circle 4: <i>E. anophelis</i> R26. Circle 5: <i>E. anophelis</i> Ag1. Circle 6: <i>E. anophelis</i> NUH11. Circle 7: <i>E. anophelis</i> NUH6. Circle 8: <i>E. anophelis</i> NUH4. Circle 9: <i>E. anophelis</i> NUH1. Circle 10: <i>E. anophelis</i> NUHP3. Circle 11: <i>E. anophelis</i> NUHP2. Circle 12: GC skew (positive GC skew, green; negative GC skew, violet). Circle 13: GC content. Circle 14: Scale of NUHP1 genome.	28
Figure 3.2 Positions of GIs as predicted by IslandViewer program. Blue: GIs predicted by IslandPath-DIMOB approach. Orange: GIs predicted by SIGI-HMM approach. Red: Integrated GIs predicted by both approaches.	29
Figure 3.3 <i>E. anophelis</i> NUHP1 genome contains a siderophore biosynthesis operon (2421051 - 2432865 nt) similar to the siderophore biosynthesis operon of <i>Yersinia pestis</i> revealed by the antiSMASH server (http://antismash.secondarymetabolites.org/).	32
Figure 3.4 Circular representation of sequence conservation between <i>E. anophelis</i> NUHP1 and <i>E. anophelis</i> assembly from mosquitoes for identifying genome regions with high flexibility. Circles are numbered from 1 (outermost circle) to 6 (innermost circle). Circle 1: Genomic islands. Circle 2: <i>E. anophelis</i> isolated from mosquitoes. Circle 3: <i>E. anophelis</i> NUH1. Circle 4: GC skew (positive GC skew, green; negative GC skew, violet). Circle 5: GC content. Circle 6: Scale of NUHP1 genome.	37
Figure 3.5 Phylogenetic tree based on multiple genome alignment of 2,622 core genes showing relationship of <i>Elizabethkingia spp.</i> . The open reading frames of all 16 genomes of the NUH isolates were predicted using Prokka v1.11 (Seemann, 2014), and the alignment of 2,622 core genes from all the genomes were performed using Roary v3.6.8 (Page, Cummins <i>et al.</i> 2015). The phylogenetic tree was constructed by RAxML-VI-HPC v8.2.9 (Stamatakis 2006) based on the core gene alignment, with ATCC13253 as an outgroup. Bootstrap supporting values were calculated based on 1,000 replicates. The CSID_3015183678 and FMS-007 are reference strains.	38
Figure 4.1 Static biofilm of <i>E. anophelis</i> NUHP1, <i>P. aeruginosa</i> PAO1 and <i>E. coli</i> DH5 α in different medium at different temperature.....	45
Figure 4.2 Static biofilm of <i>E. anophelis</i> in different medium.	46

Figure 4.3 Live/Dead stain of flow cell biofilm. A: <i>E. anophelis</i> NUHP1 B: <i>E. coli</i> DH5α C: <i>E. meningoseptica</i> ATCC 13253 (NITE) D: <i>P. aeruginosa</i> PAO1. Scale bars, 20 μm. Green are live cells that stained with Cyto 9 and dead cells that have a compromised/breached membrane will be stained in red by propidium iodide.	47
Figure 4. 4 Effect of DNase on <i>E. anophelis</i> biofilm biomass.	48
Figure 4.5 Heat map of 142 genes whose mRNA level significantly changed. The differentially expressed genes (fold-change > 4, adjusted P-value < 0.01) between H ₂ O ₂ -treated and non-treated NUHP1 cells were identified by performing a negative binomial test using the DESeq package of R/Bioconductor.	49
Figure 4.6 Standard curve for the determination of siderophore (deferoxamine) concentration using a CAS solution. (A). Siderophore production by <i>E. anophelis</i> NUHP1 cultivated with and without the presence of H ₂ O ₂ (B). Means and standard deviation (s.d.) from triplicate experiments are shown. *P < 0.05, Student's t-test.	52
Figure 5.1 Venn diagram showing overlaps of data sets A–D that were derived from transcriptome analysis with RNA sequencing of the genes up regulated in blood treated NUHP1 (A), genes up regulated in H ₂ O ₂ - treated NUHP1 (B), genes down regulated in blood treated NUHP1 (C), and genes down regulated in H ₂ O ₂ treated NUHP1 (D). Genes of all data sets are listed in Tables S4-S7.....	62
Figure 5.2 Dose-dependent growth enhancement by FeCl ₃ (A) and hemoglobin (Hb) (B) to <i>E. anophelis</i> NUHP1. The results were the average of duplicate measurements. Molar concentration is for the Fe element only.	66
Figure 5. 3 Time-kill curves of <i>E. anophelis</i> NUHP1 by 20 mM H ₂ O ₂ in ABTGC medium with and without supplementation of 10 mM Hb.	67
Figure 5.4 Confocal images of 7,228.4 μm ² substratum area of 24 h <i>E. anophelis</i> NUHP1 and <i>E. meningoseptica</i> ATCC13253 biofilms grown in iron free ABTGC medium and ABTGC medium supplemented with different iron sources. Representative confocal images from triplicate experiments are shown for each condition. Scale bars: 20 μm.	68
Figure S1 Distribution of stress response genes revealed by the RAST Server	83
Figure S2 Mauve alignment of genomes of <i>E. anophelis</i> NUHP1 and <i>E. anophelis</i> assembly from mosquitoes.....	83

LIST OF TABLES

Table 2.1 “Functions of extracellular polymeric substances in bacterial biofilms.” <i>The table is reprinted from (Flemming H C, Wingender J. Nature Reviews Microbiology, 2010, 8(9): 623-633.) with permission from Nature Publishing Group.</i>	8
Table 2.2 Characteristics of different elements involved in resistance gene spread. Table from Salyers (Salyers and AmabileCuevas 1997)	15
Table 3.1 Proteins involved in antibiotic resistance encoded from <i>E. anophelis</i> NUHP1 identified by BLAST search against the Comprehensive Antibiotic Resistance Database.	30
Table 3.2 MICs of <i>E. anophelis</i> NUHP1 against common antibiotics.	31
Table 3.3 Proteins involved in virulence encoded from <i>E. anophelis</i> NUHP1 identified by BLAST search against the Virulence Factors of Pathogenic bacteria Database (VFDB).	33
Table 4.1 Effects of treatment with H ₂ O ₂ on mRNA levels	48
Table 4.2 Top induced and reduced genes determined by RNA-seq and by qRT-PCR in H ₂ O ₂ -treated cells.	50
Table 5.1 Effects of treatment with mouse blood on mRNA levels	57
Table 5.2 Top upregulated and downregulated genes determined by RNA-seq and by qRT-PCR in mouse blood-treated cells.	60
Table 5.3 Overlaps of induced genes between mouse blood-treated and H ₂ O ₂ -treated NUHP1	63
Table 5.4 Overlaps of reduced genes between mouse blood-treated and H ₂ O ₂ -treated NUHP1	65
Table S1 List of GIs as predicted by IslandViewer program	84
Table S2 Stress response genes	100
Table S3 Primers for RT-PCR.....	104
Table S4 Genes up regulated at least 2-fold in H ₂ O ₂ treated NUHP1 cells. ^a	106
Table S5 Genes down regulated at least 2-fold in H ₂ O ₂ treated NUHP1 cells. ^a	115
Table S6 Genes up regulated at least 4-fold in mouse blood treated NUHP1 cells. ^a	122
Table S7 Genes downregulated at least 4-fold in mouse blood treated NUHP1 cells. ^a	135

LIST OF ABBREVIATIONS

Terms	Abbreviation
Brain Heart Infusion broth	BHI
Casitone-yeast extract	CYE
Colony forming unit	CFU
Crystal violet	CV
Degree celcius	°C
Deoxyribonucleic acid	DNA
Extracellular deoxyribonucleic Acid	eDNA
Extracellular polymeric substances	EPS
Gram	g
Hemoglobin	Hb
intensive care unit	ICU
Litre	L
Luria Bertani medium	LB
M9 minimal medium	M9
Microgram	µg
Microliter	µL
Micrometre	µm
Micromolar concentration	µM
Milimole	mmoles
Milligram	mg
Millilitre	mL
Millimetre	mm
Millimolar concentration	mM
Modified M1 medium	M1
Molar concentration	M
Nanometer	nm
National Center for Biotechnology Information	NCBI
National University Hospital	NUH
Nutrient Broth	NB
Optical density	OD
Polymerase chain reaction	PCR

Quantitative polymerase chain reaction	q-PCR
Reactive oxygen species	ROS
Revolutions per minute	rpm
Ribonucleic Acid	RNA
Ribosomal ribonucleic acids	rRNA
RNA sequencing	RNA-seq
Superoxide dismutases	SOD
Tryptic Soy Broth	TSB
Virulence Factors of Pathogenic bacteria Database	VFDB

LIST OF PUBLICATIONS

Journal publication

- Teo, J., Tan, S. Y. Y., Liu, Y., Tay, M., Ding, Y., **Li, Y.**, ... & Yang, L. (2014). Comparative genomic analysis of malaria mosquito vector-associated novel pathogen *Elizabethkingia anophelis*. *Genome biology and evolution*, 6(5), 1158-1165.
- Tan, S. Y. Y., Liu, Y., Chua, S. L., Vejborg, R. M., Jakobsen, T. H., Chew, S. C., **Li, Y.**, ... & Givskov, M. (2014). Comparative systems biology analysis to study the mode of action of the isothiocyanate compound Iberin on *Pseudomonas aeruginosa*. *Antimicrobial agents and chemotherapy*, 58(11), 6648-6659.
- **Li, Y.**, Liu, Y., Chew, S. C., Tay, M., Salido, M. M. S., Teo, J., ... & Yang, L. (2015). Complete genome sequence and transcriptomic analysis of the novel pathogen *Elizabethkingia anophelis* in response to oxidative stress. *Genome biology and evolution*, 7(6), 1676-1685.

Conference Papers and Presentations

- Poster presentation Biofilms 7, Porto, Portugal, June 2016

A novel matrix-based regulation mechanisms of bacterial biofilm formation

ABSTRACT

Elizabethkingia anophelis is an emerging pathogen that causes life-threatening infections in neonates, severely immunocompromised and postoperative patients with extremely high mortality. Multi-drug resistance, biofilm formation and oxidative stress resistance imply its pathogenesis. However, the lack of genomic information on *E. anophelis* hinders our understanding of its mechanisms of pathogenesis.

In this study, we report the complete genome sequence of a clinically isolated *E. anophelis* NUHP1 strain, which is the first complete genome of the *Elizabethkingia* genus. *E. anophelis* NUHP1 has a circular genome of 4,369,828 base pairs and 4,141 predicted coding sequences. Sequence analysis and the genome comparison between *E. anophelis* NUHP1 and assemblies of *E. anophelis* isolated from mosquito gut indicates that *E. anophelis* has well-developed systems for scavenging iron and stress response. Many putative virulence factors and antibiotic resistance genes were identified, underscoring the potential host–pathogen interactions and antibiotic resistance.

RNA-sequencing-based transcriptome profiling indicates that expressions of genes involved in synthesis of a yersiniabactin-like iron siderophore and heme utilization are highly induced as a protective mechanism toward oxidative stress caused by hydrogen peroxide treatment. Chromeazurol sulfonate assay verified that siderophore production of *E. anophelis* is increased in the presence of oxidative stress.

Moreover, the transcriptome profiling comparison between *E. anophelis* NUHP1 treated with hydrogen peroxide and with mouse blood further showed that *E. anophelis* NUHP1 displayed the similar protective mechanism associated with iron siderophore and heme utilization when contact with mouse blood, indicating that oxidative stress is the major stress for *E. anophelis* NUHP1 when infect hosts and the strong resistance to oxidative stress enables *E. anophelis* to be dominant in the blood meal feeding mosquitoes.

Furthermore, we also showed that hemoglobin but not ferric iron greatly facilitates the growth, hydrogen peroxide tolerance and biofilm formation of *E. anophelis* NUHP1. Our study suggests that siderophore production and heme uptake pathways might play essential roles in stress response and virulence of the emerging pathogen *E. anophelis*.

1.1 BACKGROUND

The genus *Elizabethkingia* is a gram-negative rod-shaped bacterium that is obligate aerobic, non-glucose-fermenting, non-motile, non-fastidious and non-spore-forming. *Elizabethkingia* was reported to be widely distributed in nature; from plants, soil, to aquatic environment (Edward Hadas, 2004; Kämpfer, Glaeser, Busse, & McInroy, 2015). The genus *Elizabethkingia* is comprised of four species — *Elizabethkingia meningoseptica*, *Elizabethkingia miricola*, *Elizabethkingia anophelis* and *Elizabethkingia endophytica*. Among the four species, *E. meningoseptica* was well studied since it was identified in 1959.

As an opportunistic pathogen, *E. meningoseptica* usually causes infections such as meningitis and sepsis in newborns and immunocompromised patients with high mortality rate ranging from 23% to more than 50% (Chang Chien, Chiu, Li, & Huang, 2000; M.-S. Hsu *et al.*, 2011; Lin *et al.*, 2009). *E. anophelis* was first isolated from the midgut of malaria vector mosquito *Anopheles gambiae* (Kämpfer *et al.*, 2011). Shortly after it was discovered, *E. anophelis* was also reported to cause nosocomial infections with high mortality rate in newborns and immunocompromised patients worldwide, including the Central African Republic, Singapore and Hong Kong (Thierry Frank *et al.*, 2013; Lau *et al.*, 2015; Teo *et al.*, 2013). Since the first report of *E. anophelis* infection, increasing incidence of nosocomial *E. anophelis* colonization or infections are reported (da Silva & Pereira, 2013; Pereira, Garcia Dde, Abboud, Barbosa, & Silva, 2013; Teo *et al.*, 2013). Recently, a serious *E. anophelis* outbreak was reported in Wisconsin with 63 patients infected by *E. anophelis*. In this case, 18 patients died of the blood stream infection caused by *E. anophelis* (Elbadawi, 2016; Johnson, 2016; Wahlberg). Other than meningitis and sepsis, *Elizabethkingia spp.* was also responsible for brain abscess, ocular infections and other severe symptoms including cellulitis, endocarditis and pneumonia (Adachi *et al.*, 2004; Bloch, Nadarajah, & Jacobs, 1997; Cartwright *et al.*, 2010; Kirby, Sader, Walsh, & Jones, 2004).

Microbial genome sequencing is a high-resolution technique which has been widely applied in diagnosis of infections caused by bacteria (Fournier, Drancourt, & Raoult, 2007; Loman *et al.*, 2012; Shah *et al.*, 2014; Snyder *et al.*, 2013; Walker *et al.*, 2013). This approach has also been used to reveal the notable features of *Elizabethkingia spp.* that may be responsible for its pathogenesis (Kukutla *et al.*, 2014; Lau *et al.*, 2015; Jeanette Teo *et*

al., 2014). However, the knowledge of the genomic information on *E. anophelis* is still inadequate, which hinders our understanding of its epidemiology.

In both natural and clinical environment, bacteria favored the biofilm form of growth as the extracellular polymeric substances (EPS) in biofilm matrix could protect bacteria from environmental changes and hazardous substances (Rickard, Gilbert, High, Kolenbrander, & Handley, 2003). *E. meningosepticum* was found to form biofilms in either natural or clinical environment which may explain their potential pathogenicity (Balm *et al.*, 2013).

During the course of infection, bacteria always need to defense oxidative stress from the host (J. A. Imlay, 2013; O'Rourke *et al.*, 2003; Thomas, Lehrer, & Rest, 1988). The transcriptomic analysis has been used to reveal the oxidative stress response of many bacteria to human blood. A lot of genes were reported to involved in stress response, including the genes encoding for superoxide dismutase and catalase, as well as genes associated with DNA protection and iron storage (Hedman, Li, Langford, & Kroll, 2012; Vebø, Snipen, Nes, & Brede, 2009).

1.2 KNOWLEDGE GAP AND CHALLENGES

In contrast to *E. meningoseptica*, the epidemiology and pathogenicity of *E. anophelis* are poorly understood. The lack of genomic information on *E. anophelis* hinders our understanding of its mechanisms of pathogenesis. Moreover, the biological distribution and clinical importance of *E. anophelis* have been underestimated for a long time. This species is always mistaken as *Elizabethkingia meningoseptica* due to the use of 16S rRNA sequences with low resolution to distinguish *E. anophelis* from other *Elizabethkingia* species. In 2016, Lau *et al.* investigated a series of cases from Hong Kong and they found that *E. anophelis* rather than *E. meningoseptica* causes majority *Elizabethkingia* bacteremia. (Lau *et al.*, 2016).

As an emerging pathogen, *E. anophelis* causes infections with high mortality rate in neonates and immunocompromised patients. For instance, in the *E. anophelis* outbreak in Wisconsin in 2016, 29% patients died of the blood stream infection caused by *E. anophelis*. (Elbadawi, 2016; Johnson, 2016; Wahlberg). The intrinsic multidrug resistance of *Elizabethkingia* genus to most antimicrobial agents that are commonly used to treat gram-negative bacterial infections could partially explain why it is hard to eliminate the infections caused by *E. anopheles*. However, the mechanisms of antibiotic resistance and virulence of *E. anopheles* are still obscure.

Moreover, *Elizabethkingia spp.* was reported to be dominant in the midgut of blood meal feeding mosquitoes that are commonly vectors for malaria transmission (Ngwa *et al.*, 2013; Ying Wang, Gilbreath III, Kukutla, Yan, & Xu, 2011). The colonization in the mosquito guts suggest that *Elizabethkingia spp.* may be capable to protect itself from the oxidative stress resulting from the catabolism of blood meal. Another evidence that suggests the striking capacity of oxidative stress resistance is that *E. anophelis* NUHP1 was identified from the sink of the hospital that is routinely cleaned by hydrogen peroxide (J. Teo *et al.*, 2014). Nonetheless, no such studies have really focused on the oxidative stress resistance of *E. anophelis*, or upon contact with blood.

1.3 OBJECTIVE

Here, to figure out the key questions mentioned above, the first objective of this study was to have a comprehensive understanding of the genome characters of clinical isolate *E. anophelis* NUHP1, as well as the mechanisms regarding antibiotic resistance and virulence. Hence, we analysed the genome of *E. anophelis* NUHP1 and compared the genome of NUHP1 to other *Elizabethkingia* isolates.

As bacteria in biofilm mode is able to defend themselves from environmental changes and hazardous agents and *Elizabethkingia spp.* is reported to form biofilms in nosocomial environments, we also tested the biofilm formation ability of *E. anophelis* NUHP1 under various conditions using either microtiter plates or flow cell system.

RNA-sequencing based transcriptome profiling was also applied to understand the response of NUHP1 to either hydrogen peroxide or exposure to blood. As *Elizabethkingia spp.* is dominant in the blood meal-feeding mosquitoes, we were also curious about the relationship between exposure to blood and oxidative response of *E. anophelis* NUHP1. Therefore, a comparison of the transcriptome of *E. anophelis* NUHP1 either in response to exposure to blood or to hydrogen peroxide was performed.

2.1 THE GENUS *ELIZABETHKINGIA*

2.1.1 Identification

The first identified species of the *Elizabethkingia* genus was *Elizabethkingia meningoseptica* by Elizabeth O. King, when she was studying unclassified bacteria which caused meningitis in newborns in 1959 at CDC Atlanta. At that time, the bacteria was classified as *Flavobacterium*, and later renamed as *Chryseobacterium* based on DNA-rRNA hybridization approach (Bernardet, 1996; P. Vandamme, J.-F. Bernardet, P. Segers, K. Kersters, B. Holmes, 1994). In 2003, polyphasic taxonomic approach was used by Li *et al.* to identify another species *Chryseobacterium miricola* that was isolated from condensation water in the space station Mir (Li *et al.*, 2003).

In 2005, both *C. meningosepticum* and *C. miricola* were reported to represent a separate lineage from other *Chryseobacterium* species on the basis of 16S rRNA sequencing, together with phenotypic studies. Therefore, they were split from *Chryseobacterium*–*Bergeyella*–*Riemerella* branch and relocated to a new genus, *Elizabethkingia* gen. nov., with the names *Elizabethkingia meningoseptica* and *Elizabethkingia miricola* (Kim, Kim, Lim, Park, & Lee, 2005). The epithet was based on the two names of the first person that identified the bacterium, since other bacteria and various eukaryotes were already given the more simple epithets derived from the family name of her (King, 1959).

Later in 2011, another species named *Elizabethkingia anophelis* was identified from the midgut of 40 adults and two pupae of mosquito *Anopheles gambiae* (Kampfer *et al.*, 2011). *E. anophelis* shares 98.6 % similarity with *E. meningoseptica* reference strain ATCC13253 based on 16SrRNA sequencing (Kampfer *et al.*, 2011).

E. endophytica is the latest species that was isolated from stem tissue of the healthy sweet corn in Alabama USA based on 16S rRNA sequencing, DNA–DNA hybridization, as well as some differentiating biochemical properties (Kämpfer *et al.*, 2015). 16S rRNA gene sequence similarity to the type strains of *E. anophelis* was 99.1%.

2.1.2 Characteristics of the genus *Elizabethkingia*

The genus *Elizabethkingia* is gram-negative rod-shaped (around 1 µm wide and 2 µm long) bacterium that is obligate aerobic, non- glucose-fermenting, non-motile, non-fastidious and non-spore-forming (P. Vandamme, J.-F. Bernardet, P. Segers, K. Kersters, B. Holmes, 1994). This organism also displayed identical characteristic of oxidase-positive, catalase-positive, indole-positive, and formed visible, white yellow, semi-translucent, circular and glistening colonies while the characteristic of growth on MacConkey agar, citrate utilization, urea hydrolysis and fermentation of cellobiose and melibiose was strain-dependent (Kämpfer *et al.*, 2015; Lau *et al.*, 2016).

The optimal growth temperature for this genus is 30–31 °C and 37 °C, so the bacterium was widely distributed in natural environments from soil, plants, food to dairy products. Meanwhile, since they are tolerant to NaCl, *Elizabethkingia* species could both exist in freshwater and seawater. *E. meningoseptica* was also reported to be parasitic in many other organisms (A. Jacobs & H. Y. Chenia, 2011; Kukutla *et al.*, 2013; Xie *et al.*, 2009). More specifically, *E. meningoseptica*, together with other endosymbiotic bacteria were found to be present in around half of the free-living amoebae with potential pathogenicity isolated from a lake in Poland (Edward Hadas, 2004). It was also reported that *E. meningoseptica* was frequently isolated from tiger frog with cataract disease (Xie *et al.*, 2009), as well as lab-reared *An. stephensi* and *An. gambiae* (Boissiere *et al.*, 2012; Lindh, Borg-Karlson, & Faye, 2008; Rani, Sharma, Rajagopal, Adak, & Bhatnagar, 2009). *E. anophelis* was also found to be the predominant species in the midgut of the lab-reared mosquitoes *Anopheles gambiae* (Kämpfer *et al.*, 2011). A dynamic analysis on the microbial community of mosquitoes revealed that there are more *Elizabethkingia spp.* in the mosquitoes compared with water where larvae were reared.

In addition, they were also found in clinical environments such as tap and sinks within ICUs, even after adequate disinfection.(Schreckenberger PC, 2007; P. Vandamme, Bernardet, Segers, Kersters, & Holmes, 1994), as well as medical devices such as implanted devices including long-term indwelling intravascular catheters and prosthetic valves (du Moulin, 1979; S. N. Hoque, J. Graham, M. E. Kaufmann, & S. Tabaqchali, 2001; Nulens, Bussels, Bols, Gordts, & Van Landuyt, 2001). It has been reported that there was an increasing incidence of *E. meningoseptica* infections due to misuse of the contaminated water sources (Balm *et al.*, 2013). Researchers in Singapore also isolated *E. anophelis* from sinks in *E. anophelis* outbreak ICU wards (Teo *et al.*, 2013). Taken together, these

evidences suggested that sink is a potential reservoir for nosocomial *Elizabethkingia* infections.

2.1.3 *Elizabethkingia* spp. as pathogens

Except the newly identified species *E. endophytica*, the other three species of *Elizabethkingia* were reported to be opportunistic pathogens. *E. meningoseptica* is the best known species among the *Elizabethkingia* genus. As an opportunistic pathogen, *E. meningoseptica* usually causes infections such as meningitis and sepsis in newborns and immunocompromised patients (Chang Chien *et al.*, 2000). Between 2004 and 2016, numerous of infections caused by *E. meningoseptica* were reported worldwide from Taiwan to Saudi Arabia, most of which were related to neonatal meningitis that causes more than 50% fatal rate in premature infants (Güngör, Özen, Akinci, & Durmaz, 2004; Lin *et al.*, 2009; Malaka Z. Amer, 2011; Mohammad I. Issack, 2011; Pereira *et al.*, 2013). Although the most common form of infections caused by *E. meningoseptica* are neonatal meningitis, it is also responsible for brain abscess, abdominal abscess, wound infections, ocular infections and other severe symptoms including bacteremia following burns, cellulitis, pneumonia, endocarditis, sinusitis, bronchitis, epididymitis, dialysis-associated peritonitis, and tissue-allograft- associated infections. (Adachi *et al.*, 2004; Bloch, Nadarajah, & Jacobs, 1997; Cartwright *et al.*, 2010; Kirby, Sader, Walsh, & Jones, 2004)

On the other hand, *E. miricola* and *E. anophelis* were less studied compared to *E. meningoseptica* in terms of epidemiology and pathogenicity. The first clinical case of *E. miricola* infection was reported in 2008, a lymphoma patient who had undergone allogeneic stem cell transplant. The patient initially suffered from a pneumonia, and later from a blood infection. Since a positive *E. miricola* culture was identified three days earlier in the lung than in the blood, the initial infection site was probably the respiratory tract (Green, Murray, & Gea-Banacloche, 2008).

The first case of infection caused by *Elizabethkingia anophelis* was reported by Thierry Frank *et al.* in the Central African Republic. In this case, an 8-day-old baby with a history of intubation and mechanical ventilation in hospital died one month later with symptoms of long lasting fever, delayed weight gain, and anorexia caused by *E. anophelis* infection. (T. Frank *et al.*, 2013), There was an increasing incidence of nosocomial *E. anophelis* colonization or infection than preceding years since the first *E. anophelis* infection has been reported. In 2013, an *Elizabethkingia* outbreak at two intensive care units was also

reported in NUH, Singapore (Teo *et al.*, 2013). Moreover, it was reported that many infections caused by *E. anophelis* were mistakenly attributed to *E. meningoseptica* with significant morbidity and mortality in previous studies (Bobossi-Serengbe G, 2006; Nicholson *et al.*, 2016). For example, in 2016, Lau *et al.* rescreened the clinical and molecular epidemiology of 45 episodes infections caused by *Elizabethkingia*-like species in Hong Kong from 2004-2013 and they concluded that *E. anophelis* accounted for the majority of the cases of bacteremia, which is opposite to the previous belief that *E. meningoseptica* was the major species responsible for *Elizabethkingia* bacteremia (Lau *et al.*, 2016). In addition, in previous studies, only neonates and immunocompromised adults were reported to be susceptible to *Elizabethkingia* species with nosocomial infections (da Silva & Pereira, 2013; Pereira *et al.*, 2013; Teo *et al.*, 2013). However, community-acquired infections outbreak caused by *Elizabethkingia anophelis* was reported in Wisconsin, USA in 2016 with obscure route of transmission and infection source (Bobossi-Serengbe G, 2006; Nicholson *et al.*, 2016). This outbreak has caused blood stream infection in 63 people and 18 of them dead. Taken together, the morbidity and mortality of *E. anophelis* has been probably underestimated for a long time and we should pay more attention to it.

2.2 BIOFILM

In most natural and clinical environment, bacteria exist in the biofilm mode of growth (J. W. Costerton, Lewandowski, Z., Caldwell, D.E., Korber, D.R., Lappin-Scott, H.M., 1995). Biofilms are structured communities of bacterial cells that aggregate and are enclosed in a self-produced polymeric matrix and adherent to an inert or living surface (J. W. Costerton, Stewart, & Greenberg, 1999). The extracellular material in which the biofilm is enclosed is mostly produced by the microorganisms themselves, which accounts for over 90% of the dry mass in most biofilms. It is composed of different types of extracellular polymeric substances (EPS), including proteins, lipids, and polysaccharides form a hydrated barrier between the cells and their external environment. Among all the EPS, polysaccharides are the major component (Frølund, Palmgren, Keiding, & Nielsen, 1996; Wingender, Strathmann, Rode, Leis, & Flemming, 2001). EPS can serve various purposes, including nutrient source, and are responsible for surfaces adhesion and biofilm cohesion. They can also protect organisms against ultraviolet radiation, pollutants and toxins such as antibiotics, as well as determine the biofilm living condition by affecting porosity, sorption properties, hydrophobicity, and mechanical stability (Hans-Curt Flemming, Neu, & Wozniak, 2007;

Sutherland, 2001; Zhang & Bishop, 2003). Table 2.1 shows the EPS involved in bacterial biofilms and their functions.

ExtracellularDNA (eDNA) is another important component of biofilm matrix that is ubiquitous in environments. eDNA has been observed in biofilms of several bacteria such as *Pseudomonas*, *Streptococcus*, and *Staphylococcus*. eDNA influences the initial attachment and/or biofilm structure of these organisms. Autolysis of cells and active secretion are common mechanisms associated with eDNA release. eDNA also serves various functions, including a role as a structural component, an energy and nutrition source. In *P. aeruginosa*, eDNA plays a role as the intercellular connector (Yang *et al.*, 2007) and in *Bacillus cereus*, eDNA function as an adhesion (Vilain, Pretorius, Theron, & Brözel, 2009). It also represents an important mechanism for horizontal gene transfer in naturally competent bacteria (Rasmussen *et al.*, 2005).

Table 2. 1 “Functions of extracellular polymeric substances in bacterial biofilms.” *The table is reprinted from (Flemming H C, Wingender J. Nature Reviews Microbiology, 2010, 8(9): 623-633.) with permission from Nature Publishing Group.*

Function	Relevance for biofilms	EPS components involved
Adhesion	Allows the initial steps in the colonization of abiotic and biotic surfaces by planktonic cells, and the long-term attachment of whole biofilms to surfaces	Polysaccharides, proteins and DNA
Aggregation of bacterial cells	Forms a hydrated polymer network (the biofilm matrix), mediating the mechanical stability of biofilms (often in conjunction with multivalent cations) and, through the EPS structure (capsule, slime or sheath), determining biofilm architecture, as well as allowing cell–cell communication	Neutral and charged polysaccharides, proteins (such as amyloids and lectins), and DNA
Cohesion of biofilms	Forms a hydrated polymer network (the biofilm matrix), mediating the mechanical stability of biofilms (often in conjunction with multivalent cations) and, through the EPS structure (capsule, slime or sheath), determining biofilm architecture, as well as allowing cell–cell communication	Neutral and charged polysaccharides, proteins (such as amyloids and lectins), and DNA
Retention of water	Maintains a highly hydrated microenvironment around biofilm organisms, leading to their tolerance of dessication in water-deficient environments	Hydrophilic polysaccharides and, possibly, proteins
Protective barrier	Confers resistance to nonspecific and specific host defences during infection, and confers tolerance to various antimicrobial agents (for example, disinfectants and antibiotics), as well as protecting cyanobacterial	Polysaccharides and proteins

	nitrogenase from the harmful effects of oxygen and protecting against some grazing protozoa	
Sorption of organic compounds	Allows the accumulation of nutrients from the environment and the sorption of xenobiotics (thus contributing to environmental detoxification)	Charged or hydrophobic polysaccharides and proteins
Sorption of inorganic ions	Promotes polysaccharide gel formation, ion exchange, mineral formation and the accumulation of toxic metal ions (thus contributing to environmental detoxification)	Charged polysaccharides and proteins, including inorganic substituents such as phosphate and sulphate
Enzymatic activity	Enables the digestion of exogenous macromolecules for nutrient acquisition and the degradation of structural EPS, allowing the release of cells from biofilms	Proteins
Nutrient source	Provides a source of carbon-, nitrogen- and phosphorus-containing compounds for utilization by the biofilm community	Potentially all EPS components
Exchange of genetic information	Faciliates horizontal gene transfer between biofilm cells	DNA
Electron donor or acceptor	Permits redox activity in the biofilm matrix	Proteins (for example, those forming pili and nanowires) and, possibly, humic substances
Export of cell components	Releases cellular material as a result of metabolic turnover	Membrane vesicles containing nucleic acids, enzymes, lipopolysaccharides and phospholipids
Sink for excess energy	Stores excess carbon under unbalanced carbon to nitrogen ratios	Polysaccharides
Binding of enzymes	Results in the accumulation, retention and stabilization of enzymes through their interaction with polysaccharides	Polysaccharides and enzymes

Biofilms are ubiquitous in both natural and clinical environment from teeth as dental plaque, to water treatment systems and medical implants and catheters. Biofilms can be both beneficial and detrimental to our lives (Figure 2.1). Biofilms is considered to be associated with various chronic nosocomial infections some of which are due to the misuse of various contaminated medical devices such as catheters and intravascular devices. Biofilms allow bacteria to develop resistance to immune system and antimicrobial treatment. These important characteristics have been found to protect the microbial

community from environmental stresses (Ahimou, Semmens, Haugstad, & Novak, 2007; H-C Flemming & Wingender, 2001).

Figure 2.1 “Diagram showing the wide-ranging impacts of biofilms on human health and industries.” Image credit: Phil Stewart / Peg Dirckx (1996), Montana State University Center for Biofilm Engineering

The biofilm formation and adherent ability of *E. meningosepticum* have been studied previously. It was demonstrated that *E. meningosepticum* is a strong biofilm-forming organism in nutrient-rich medium, a feature that increase resistance to antibiotic such as ciprofloxacin, and that contribute to adherence to abiotic surfaces due to their hydrophobic nature (Anelet Jacobs & Hafizah Y Chenia, 2011). Furthermore, protease- and heat-sensitive adhesins are predicted to be localized on the cell surface of *E. meningoseptica* (Anelet Jacobs, 2011). Biofilm formation was also reported to be responsible for the pathogenesis of *E. meningoseptica* (Kodama *et al.*, 2013; P.-Y. Lin, H.-L. Chen, C.-T. Huang, L.-H. Su, & C.-H. Chiu, 2010). But beyond that, little is known about biofilm of the members of *Elizabethkingia*. It will be significative to investigate the biofilm-forming potential of *E. anophelis*.

Elizabethkingia spp. isolates have also been shown to exist in clinical biofilm communities (S. Hoque, J. Graham, M. Kaufmann, & S. Tabaqchali, 2001). The strong biofilm formation capacity of *E. meningosepticum* suggests that biofilm may play a role in its potential spreading in hospital settings where *E. meningosepticum* outbreaks are often linked to taps and sinks contamination in hospitals (Balm *et al.*, 2013). In clinical settings,

inappropriate antimicrobial treatment and the use of medical implants and catheters affected the outcome of patients infected with *E. meningosepticum* (P. Y. Lin, H. L. Chen, C. T. Huang, L. H. Su, & C. H. Chiu, 2010a). Although the biofilm formation ability of *E. meningoseptica* has been proved and biofilm communities have been found in *Elizabethkingia* spp outbreak clinical environment, the factors related to biofilm formation by this nonmotile bacterium are still not clear.

Overall, pathogens developed various strategies such as multidrug resistance and the ability to form biofilm to evade eradication from environment as well as in hosts. The former enables bacteria to survive during antimicrobial treatment and the latter enables bacteria to persist in tanks or wounds. Hence, to investigate the abilities of *Elizabethkingia* to resist antibiotics treatment and adhere to surfaces by forming biofilms would be of great significance to understand its pathogenicity, as well as to develop effective treatment strategies to treat infections caused by *Elizabethkingia*.

2.3 ANTIMICROBIAL RESISTANCE

Since *Elizabethkingia* is extremely resistant towards conventional antibiotics, it is difficult to eliminate infections caused by *Elizabethkingia*. High mortality rates of *Elizabethkingia* infections are partially due to their multidrug resistance (Ceyhan and Celik 2011, Issack and Neetoo 2011). The *Elizabethkingia* genus showed intrinsic multidrug resistance to most antimicrobial agents which are commonly used to treat Gram-negative bacterial infections, such as aminoglycoside, β -lactams, chloramphenicol and carbapenems. It was reported that its resistance to β -lactams is most likely due to the intrinsic production of two types of chromosomal metallo- β -lactamases (MBLs), *blaB* and *gOB* which belong to subclass B1 and B3, respectively (Bellais, Aubert *et al.* 2000, Steinberg and Burd 2005, Yum, Lee *et al.* 2010). It was noted that class A extended-spectrum β -lactamases also contribute to the resistance of some *E. meningoseptica* isolates (Lin, Xu *et al.* 2012).

However, *Elizabethkingia* spp are susceptible to some agents used for gram-positive bacterial infections, which include rifampicin, ciprofloxacin, vancomycin and piperacillin-tazobactam (Fraser & Jorgensen, 1997; Lau *et al.*, 2016; Spangler, Visalli, Jacobs, & Appelbaum, 1996). In clinical practice, combined antibiotic regimens were frequently applied such as ciprofloxacin or rifampicin with piperacillin-tazobactam or vancomycin (Malaka Z. Amer, 2011). Figure 2.2 demonstrates antibiotics commonly used in molecular biology.

Figure 2.2 Different classes of antibiotics.

The mechanism of resistance to antibiotics could be either intrinsic or acquired from mutations or horizontal gene transfer. There are various examples of intrinsic resistance (Figure 2.3) such as the susceptible target replaced by an insensitive allele of a specific antimicrobial or an intrinsic difference in the composition of the membrane so that the antibiotic cannot cross the membrane to access the target (Randall, Mariner, Chopra, & O'Neill, 2013; Tsuchido & Takano, 1988; Zhu, Lin, Ma, Cronan, & Wang, 2010).

Figure 2.3 “Intrinsic mechanisms of resistance.” The figure shows an overview of intrinsic resistance mechanisms. PBP: penicillin-binding protein. Antibiotic A enters the cell via a

membrane-spanning porin protein and bind to the target PBP to inhibit peptidoglycan biosynthesis. For antibiotic B, although it also enters the cell via a porin, the efflux pump eliminate it efficiently. Antibiotic C cannot even enter the cell and so is unable to bind the target. *The figure is reprinted from (Blair J M A, Webber M A, Baylay A J, et al. Nature Reviews Microbiology, 2015, 13(1): 42-51.) with permission from Nature Publishing Group.*

Bacteria not only have intrinsic resistance to antibiotic but could also acquire resistance via mainly three different mechanisms (Blair, Webber, Baylay, Ogbolu, & Piddock, 2015). Firstly, bacteria could prevent the penetration of antibiotics to minimize the intracellular concentrations of the drug by drug-specific efflux pumps or decreased outer membrane permeability to the drug (Floyd, Smith, Kumar, Floyd, & Varela, 2010; Kojima & Nikaido, 2013; Vargiu & Nikaido, 2012). Secondly, bacteria could modify the antibiotic target genetic mutation or post-translational modification (Billal, Feng, Leprohon, Légaré, & Ouellette, 2011; Hidalgo *et al.*, 2013). Thirdly, some bacteria could direct hydrolyse or inactivate the antibiotic by various modification enzymes (Norris & Serpersu, 2013; Tzouvelekis, Markogiannakis, Psychogiou, Tassios, & Daikos, 2012). Figure 2.4 demonstrates the direct interactions between the host and antibiotics.

Figure 2.4 “Direct interactions with antibiotics.” a: An antibiotic bind to the target efficiently. b: The host destroys the antibiotic to prevent binding to the target. c: The host modifies the structure of the antibiotic to prevent binding to the target. *The figure is reprinted from (Blair J M A, Webber M A, Baylay A J, et al. Nature Reviews Microbiology, 2015, 13(1): 42-51.) with permission from Nature Publishing Group.*

The spreading of antibiotic resistance amongst various bacterial species is considered to be related to horizontal gene transfer. Antimicrobial resistance genes can be transferred within bacteria of the same population as well as the same genera. Resistance gene recombined with chromosomal gene encoded the sensitive target molecule which resulted in novel alleles that encode resistance and spread by transformation, conjugation or transduction (Hakenbeck, 1995; Spratt, 1994). In clinical practice, multidrug-resistant organisms are emerging at an alarming rate, partially because antimicrobials are intensively used in the ICUs and for immunocompromised patients (Lucet *et al.*, 1999; Wiener *et al.*, 1999). Table 2.2 summarizes the characteristics of different elements involved in resistance gene spread.

Table 2. 2 Characteristics of different elements involved in resistance gene spread. Table from Salyers (Salyers & AmabileCuevas, 1997)

Element	Characteristics	Role in spread of resistance genes
Self-transmissible plasmid	Circular, autonomously replicating element; carries genes needed for conjugal DNA transfer	Transfer of resistance genes; mobilization of other elements that carry resistance genes
Conjugative transposon	Integrated elements that can excise to form a nonreplicating circular transfer intermediate; carries genes needed for conjugal DNA transfer	Same as self-transmissible plasmid
Mobilizable plasmid	Circular, autonomously replicating element; carries gene that allows it to use conjugal apparatus provided by a self-transmissible plasmid	Transfer of resistance genes
NBU*	Integrated elements that cannot excise and transfer themselves; can be triggered to excise and transfer by conjugative transposons; transfer intermediate is a nonreplicating circle carrying a gene that allows the NBU to take advantage of the conjugal transfer apparatus of a conjugative transposon	Transfer of resistance genes
Transposon	Can move from one DNA segment to another within the same cell	Can carry resistance genes from chromosome to plasmid or vice versa
Gene cassette	Circular, nonreplicating DNA segments containing only open reading frames; integrates into integrons	Carry resistance genes
Integron	Integrated DNA segment that contains an integrase, a promoter, and an integration site for gene cassettes	Forms clusters of resistance genes, all under the control of the integron promoter

* NBU, nonreplicating Bacteroides unit.

The mechanisms of antibiotic resistance of bacteria in a biofilm is quite different from the conventional mechanisms discussed above. On one hand, conventional mechanisms of antibiotic resistance such as mutations in antibiotic target, modifying enzymes, and upregulated efflux pumps also play a role in resistance in biofilms. For example, the increased mutation rate and horizontal gene transfer were found to be conducive to the resistance of biofilms to antibiotics such as fluoroquinolones, β -lactam and aminoglycosides (Driffield, Miller, Bostock, O'Neill, & Chopra, 2008; Molin & Tolker-Nielsen, 2003). In addition to the conventional resistance mechanisms, biofilms could protect susceptible bacteria that do not harbor antibiotic resistance mechanisms in other possible ways, including slow penetration and altered microenvironment (Anderl, Franklin, & Stewart, 2000; Stewart & Costerton, 2001). Evidence showed that aminoglycoside activity could be enhanced after DNase and alginate were lysed in biofilm, indicating that the biofilm matrix such as eDNA and alginate may also be part of the resistance mechanism to antibiotics with the biofilm (Alipour, Suntres, & Omri, 2009; Wood, Leech, & Ohman, 2006)

Figure 2.5 “Three hypotheses for mechanisms of antibiotic resistance in biofilms.” The bottom is attachment surface and the top is the aqueous phase which contains the antibiotic. The three hypothesis for biofilm resisting to antibiotics includes slow penetration, resistant phenotype and alters microenvironment as shown in the figure. *The figure is reprinted from (Stewart P S, Costerton J W. The lancet, 2001, 358(9276): 135-138.) with permission from Elsevier.*

2.4 OXIDATIVE RESISTANCE IN BACTERIUM

Reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot\text{OH}$) as well as the superoxide anion radical (O_2^-) are generated as the byproduct of respiration during cellular activities (Elisa Cabiscol, Tamarit, & Ros, 2010). On the other hand, cells also suffered the exogenous oxidative stress from the environment once the level of active oxygen generated by ionization or near-UV radiation exceed the cell's defense capacity. Moreover, some immune cells exploit oxidative stress to defend themselves against pathogenic bacteria invasion. The ubiquitous ROS created a paradoxical situation: ROS are reactive chemicals, and organisms had to elaborate scavenging strategies to protect themselves from ROS (Figure 2.6). However, cells could not scavenge the intracellular O_2 quickly enough to the level that cells could sustain as the small molecule O_2 penetrate biological membranes very quickly (Ligeza, Tikhonov, Hyde, & Subczynski, 1998).

Figure 2.6 “The generation of reactive oxygen species and the enzymes used for scavenging.”

a: The potent oxidants H_2O_2 , O_2^- and $\text{HO}^\bullet$. b: The enzymes that involve in ROS degradation in *Escherichia coli*. SOD targets O_2^- and catalases as well as Ahp target H_2O_2 . The figure is reprinted from (Imley J A. *Nature Reviews Microbiology*, 2013, 11(7): 443-454.) with permission from Nature Publishing Group.

ROS targets DNA, RNA, proteins and lipids in cells, leading to dysfunction of membranes and proteins, and block DNA replication or cause mutations (Figure 2.7). Most of the damage are caused by the generation of hydroxyl radicals by H_2O_2 during Fenton reaction, which requires divalent metal ion — usually iron and reducing equivalents to regenerate the metal.

In eukaryotic systems the major targets of oxidative stress are lipids. Membrane properties alters due to lipid peroxidation caused decreased fluidity. However, most bacteria cannot undergo peroxidation (Bielski, Arudi, & Sutherland, 1983) for the reason that most bacteria only harbor monounsaturated and saturated fatty acids (Nichols & McMeekin, 2002) instead of polyunsaturated fatty acids which are necessary for the initiation of lipid peroxidation.

Another main target of oxidative stress is DNA even though H_2O_2 and O_2^- cannot damage DNA directly, both the base and the ribose moieties could be oxidized by active species $\text{HO}^\bullet$, leading to a various lesions (Dizdaroglu, Rao, Halliwell, & Gajewski, 1991; Hutchinson, 1985) including cross-links to other molecules, backbone broken with single- or double-strand, as well as obstructed polymerase progression (Sies, 1993; Sies & Menck, 1992). Among all the four bases, guanine is more vulnerable to oxidative stress which leads to high mutagenesis because of its low reduction potential (Candeias & Steenken, 1993; Hogg, Wallace, & Doublié, 2005). On the contrary, bacteria will die rather than mutate as the consequence of blocked polymerase progression during thymine oxidation (Dempfle, Johnson, & Fung, 1986).

In 1906, Dakin reported the Fenton reaction to explain the oxidation of amino acids, initiating the study of oxidation of proteins mediated by free radicals (Dakin, 1906). A variety of damages are caused by protein oxidation, such as generation of disulfide bonds, inactivation of Fe–S-dependent dehydratases and mononuclear iron proteins, leading to the dysfunction of proteins which is harmful to the cell (J. A. Imlay, 2013). The oxidation of protein could be either reversible or irreversible. The disulfide bonds generated during oxidative stress is reversible when the transcription factors SoxRS and OxyR were activated to eliminate the hazardous oxidant. Ionizing radiation and oxidation reactions catalyzed by metal ion are the two major mechanisms of irreversible oxidation of protein (E Cabiscol, Tamarit, & Ros, 2000).

O_2^- obstructs the dehydration reaction of Fe–S cluster-cofactored dehydratases by destroying the catalytic Fe–S cluster of the dehydratases such as dihydroxy-acid dehydratase (DHAD), aconitase A and B, and fumarase A and B (D. H. Flint, Tuminello, & Emptage, 1993; Kuo, Mashino, & Fridovich, 1987). A single electron was deprived from the Fe–S cluster by the strong radical oxidant O_2^- and the iron atom is detached from the unstable oxidized cluster, giving rise to the inactivation of the enzyme (D. Flint, Smyk-Randall, Tuminello, Draczynska-Lusiak, & Brown, 1993). As a consequence, the key pathway which is required for energy production and biosynthesis is blocked. The activity of an enzyme group that only contains single iron atom could also be destroyed by O_2^- or H_2O_2 (Anjem & Imlay, 2012; Sobota & Imlay, 2011), including deaminases, dehydrogenases, epimerases, and deformylases. As little as 0.5 μ M intracellular H_2O_2 could inactivate the enzymes through Fenton reaction, releasing the Fe^{3+} from the enzymes. Mononuclear enzymes with single iron atom could also be disabled by O_2^- and disassociated from the iron (Gu & Imlay, 2013). The enzyme function is declined rather than totally destroyed when the enzyme is mismetalated with catalytically inefficient zinc after O_2^- oxidation as this reaction is not strong enough to damage the polypeptide (Anjem & Imlay, 2012; Sobota & Imlay, 2011).

Figure 2.7 “Overview of damage caused by reactive oxygen species in *Escherichia coli*.” The formation of hydrogen peroxide and superoxide damages Fe–S cluster proteins and mononuclear iron enzymes. H_2O_2 could also react with free Fe^{2+} directly, leading to the damage of biomolecules such as DNA. In *E. coli*, the accumulation of H_2O_2 and O_2^- could be prevented by catalases peroxidases and superoxide dismutases. *The figure is reprinted from (Imlay J A. Nature Reviews Microbiology, 2013, 11(7): 443-454.) with permission from Nature Publishing Group.*

Bacteria have evolved mechanisms to defend the damage caused by ROS. They either keep the concentration of the ROS at tolerable levels by detoxifying enzymes and freeing radical-scavenging substrates or repairing oxidative damages with DNA and protein repair systems (Figure 2.8).

Bacteria employ either specific enzymes or nonenzymatic antioxidants to maintain the intracellular concentration of ROS. NADPH, glutathione (GSH), α -tocopherol, vitamin C as well as other molecules with high reduction potential are the examples of the nonenzymatic antioxidants which could maintain the reducing environment of the cell.

Moreover, specific scavenging enzymes are employed by cells to decrease the concentration of ROS. The model organism *Escherichia coli* produces superoxide dismutases (SOD) to break down O_2^- to H_2O_2 and O_2 . The coordinately regulated cytoplasmic SODs Fe SOD and Mn SOD are the predominant SODs that maintain the O_2^- at tolerable level and ensure the vigorous growth of cell. The regulation of these two enzymes are in the control of Fur in response to the iron concentration in the cell. When iron is deficient in cell, the iron Fur is inactive thereby stimulating the synthesis of Mn

SOD and blocking the Fe SOD synthesis; when there is adequate iron in the cell, the activation of Fur triggers the degradation of Mn SOD (Massé & Gottesman, 2002; Tardat & Touati, 1991). A third SOD is the Cu–Zn SOD that was found in the *E. coli* periplasm. However, Cu–Zn SOD mutants of *E. coli* has not been reported to show any phenotype (Ligeza *et al.*, 1998).

E. coli expresses mainly three scavengers, two catalases— catalase G (KatG) and catalase E (KatE) plus the alkyl hydroperoxide reductase (Ahp) to convert H_2O_2 to H_2O and O_2 (Seaver & Imlay, 2001). Cells employ catalases to maintain the reducing environment when H_2O_2 levels are high; when H_2O_2 levels are low, cells will rely on Ahp alternatively. To be specific, catalases form a potent oxidant ferryl/radical which grab electrons from the surrounding polypeptide when H_2O_2 concentrations are low (Díaz, Loewen, Fita, & Carpena, 2012; Putnam, Arvai, Bourne, & Tainer, 2000), while Ahp does not form an oxidizing intermediate and is therefore more effective compared with catalases under low-level H_2O_2 threat. However, Ahp reaches saturation easily once the intracellular H_2O_2 increases. On the contrary, catalases could response much more quickly under high level of H_2O_2 .

The mechanisms of scavenging discussed above are at the basal level which only defend the endogenous ROS that generated from the autooxidation processes in aerobic cells. However, ROS are also formed by chemical oxidation or competing organisms from the environment, under which conditions the above scavenging systems are insufficient to protect the cells. As the small and uncharged H_2O_2 enters biological membrane as quickly as water, oxidative stress takes place when bacteria encounter H_2O_2 in the environments. As for the case of O_2^- , its charged feature prevents it from entering membranes, meaning that O_2^- must be generated inside the cytoplasm to impair the cell. In spite of that, the formation of O_2^- in the cytoplasm will be greatly induced by the redox-active compounds such as phenazines and quinones secreted from the surrounding environments (J. Imlay & Fridovich, 1992) because these molecules can penetrate the interior of the cell passively (Hassan & Fridovich, 1979; Hassett, Charniga, Bean, Ohman, & Cohen, 1992), deprive electrons from low-potential compounds and then provide O_2 with electrons to produce O_2^- .

Figure 2.8 “Simplified schematic overview of important determinants involved in staphylococcal response to oxidative stress affecting whole cell physiology.” Image from Gaupp (Gaupp, Ledala, & Somerville, 2012)

In the model organism *E. coli*, there are two systems—the OxyR system and the SoxR system to protect themselves from the oxidative stress from the exterior. The H₂O₂ threat is responded by the OxyR system while the O₂^{•−} threat is sensed by the SoxR system. The positive transcription factor OxyR is activated when a disulphide bond is generated in response to high level of H₂O₂. As a consequence, the synthesis of KatG and Ahp which are regulated by OxyR will be induced and therefore, the H₂O₂ level will decrease to harmless levels (Choi *et al.*, 2001). Alternatively, instead of OxyR some bacteria such as *Bacillus subtilis* uses PerR repressor as H₂O₂ sensor (Herbig & Helmann, 2001; Lee & Helmann, 2006). The homodimeric regulator SoxR is activated when its [2Fe–2S] cluster is oxidized by O₂^{•−} (Greenberg, Monach, Chou, Josephy, & Demple, 1990). The secondary transcription factor SoxS is activated by the oxidized SoxR thereby inducing the expression of many other genes, including *acrAB* and *sodA* (Pomposiello, Bennik, & Demple, 2001).

DNA repair is the second defense system apart from the scavenging system after DNA damage occurs. MutM and Fpg, endonuclease III and IV are the DNA repair enzymes that repair DNA by excising the oxidized bases and restore the 3' termini for repair (Demple *et*

al., 1986). Post-replication recombination will back up the repair enzymes if lesions cannot be recognized by the excision enzymes. Whole-genome sequencing approach has been used to investigate an *Elizabethkingia* outbreak in two intensive care units (ICUs) at the National University Hospital (NUH), Singapore (Teo *et al.*, 2013). It was found that the outbreak agent was *Elizabethkingia anophelis* and three *E. anophelis* isolates from patients shared close genomic content with one ICU sink isolate (J. Teo *et al.*, 2014). As the ICU sinks of the NUH are routinely cleaned by using virex, acidified bleach and hydrogen peroxide, it is highly possible that *E. anophelis* has acquired resistance or stress response mechanisms towards these disinfectants. However, the mechanism of oxidative response of *Elizabethkingia* is still unclear.

2.5 IRON AND HEME UTILIZATION BY BACTERIUM

It is essential for a pathogen to scavenge essential nutrients from the host to initiate and to establish infection. Iron is one of these essential nutrients for the pathogen as well as for the host, as both require this metal as a cofactor or a prosthetic group for enzymes that are essential for many basic cellular functions and metabolic pathways including the respiratory pathways of both the host and the microorganism. Furthermore, sufficient iron acquisition has been shown to associate with the virulence of many bacterial pathogens such as *Neisseria* and *Pseudomonas aeruginosa* (Schryvers & Stojiljkovic, 1999; Vasil & Ochsner, 1999). On the other hand, as ferrous iron converts the less reactive hydrogen peroxide to the more reactive oxygen species via the Fenton reaction, it potentiates oxygen toxicity to cells, resulting in protein denaturation, DNA break and lipid peroxidation. Therefore, in the host, the majority of iron is incorporated into specific proteins, such as transferrin (TF), lactoferrin (LF) and ferritin, or when it is complexed to heme proteins such as hemoglobin to prevent an excess of free intracellular iron that could lead to oxidative stress.

As a consequence, bacterial pathogens must liberate intracellular iron and compete with host iron-sequestering proteins under iron-limiting environment. Many bacterial species produce various siderophores which are small iron chelators with strong Fe^{3+} -binding affinity to scavenge ferric iron from iron proteins (Crosa & Walsh, 2002; Ferguson, Hofmann, Coulton, Diederichs, & Welte, 1998). Bacteria release the iron-free forms of chelators to extracellular environment and then the ferricsiderophore complexes will be transported back into the cell according to the intracellular iron concentration (Lamont, Beare, Ochsner, Vasil, & Vasil, 2002).

Moreover, since heme proteins are abundant in the host, they are also valuable sources for bacterial pathogens to acquire iron (Smith & Wilks, 2012; Wilks & O'Neill, 2014). Gram-negative bacteria have developed two systems to bind hemoglobin. The best-described system is that bacteria directly bind free heme or heme protein to their surface receptors. After that, heme is passed to transmembrane systems — ATP binding cassette (ABC) transporters (Rice, Park, & Pinkett, 2014). The energy used by outer membrane heme transport systems is generated within the inner membrane by the TonB complex. An additional system involves a secreted heme binding protein that functions to capture and shuttle heme to the specific outer membrane receptors (Figure 2.9). Several heme uptake systems have been described, such as *phu* and *has* in *Pseudomonas aeruginosa* and a TonB-dependent hemoglobin receptor, *HmuR* in *Porphyromonas gingivalis* (Ochsner, Johnson, & Vasil, 2000; Simpson, Olczak, & Genco, 2000).

Figure 2.9 “Mechanisms of hemoglobin utilization by pathogens.” (Pishchany & Skaar, 2012)

Genome Sequencing and Comparative Genomic Analysis of *E. anophelis* NUHP1

3.1 INTRODUCTION

Elizabethkingia anophelis is an emerging opportunistic pathogen that can cause life-threatening infections such as meningitis and sepsis in neonates, severely immunocompromised and post-operative patients. Especially, an increasing incidents of *E. anophelis* outbreaks have been reported from 2013, including an outbreak in an ICU in Singapore and a large outbreak in Wisconsin, USA (da Silva & Pereira, 2013; Pereira *et al.*, 2013; Teo *et al.*, 2013). It is reported that *E. anophelis* isolates showed intrinsic multidrug resistance to most antimicrobial agents which might explain the high mortality of infections *E. anophelis* caused.

On the other hand, some interesting genomic features have been revealed by the genomic studies on *E. anophelis* strains from mosquitoes, which might be responsible for the adaptation of *E. anophelis* to mosquitoes (Kukutla *et al.*, 2014). Moreover, the predominant resident of *E. anophelis* in the malaria vector *Anopheles gambiae* at various development stages has drawn our attention and implied that mosquitoes might play a role in the pathogenesis of *E. anophelis*.

However, *E. anophelis* is underestimated for a long time in terms of the biological distribution and clinical importance. This species is always mistaken as *Elizabethkingia meningoseptica* due to the use of 16S rRNA sequences with low resolution to distinguish *E. anophelis* from other *Elizabethkingia* species. On the contrary to the impression that *E. meningoseptica* is responsible for most *Elizabethkingia*-like infections, in 2016, Lau *et al.* investigated the 45 cases of *Elizabethkingia*-like infections from hospitals in Hong Kong and claimed that *E. anophelis* rather than *E. meningoseptica* causes majority *Elizabethkingia* bacteremia (Lau *et al.*, 2016).

On the other hand, microbial genome sequencing has been widely applied in diagnosis and control of infections caused by *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, mycobacteria and so on (Fournier *et al.*, 2007; Loman *et al.*, 2012; Shah *et al.*, 2014; Snyder *et al.*, 2013; Walker *et al.*, 2013). This high-resolution technique

enables the studies of *E. anophelis* strains and has revealed notable features of this bacterium that may be responsible for its pathogenesis and that may contribute to the adaptation to mosquito (Kukutla *et al.*, 2014; Lau *et al.*, 2015; Jeanette Teo *et al.*, 2014). However, the knowledge of the genomic information on *E. anophelis* is still inadequate for us to understand the mechanisms of its pathogenesis.

Here, we reported the first complete genome sequence of *E. anophelis* NUHP1 which is isolated from the sputum of the patient in cardiothoracic ICU of NUH, Singapore; determined its genomic features and compared it with genome of other *Elizabethkingia* isolates from other sources including the assembly from mosquito guts.

3.2 MATERIALS AND METHODS

3.2.1 Bacterial strains

Elizabethkingia anophelis NUHP1 as well as other *Elizabethkingia anophelis* isolates NUH1, NUH4, NUH6, NUH11, NUHP2, NUHP3, CTICU2, CTICU3, CTICU 5, CTICU7, CTICU8, CTICU9, CTICU10, CTICU12, and CTICU 17 were provided by Dr. Jeanette Teo from NUH Singapore which were either isolated from the sputum of the patient or the sink in cardiothoracic ICU (Teo *et al.*, 2013; J. Teo *et al.*, 2014).

3.2.2 Growth medium and conditions

The bacteria strains were streaked on Luria-Bertani (LB) agar and incubated for 16 h at 37°. Single colonies were picked from the LB agar plate and inoculated into Luria-Bertani (LB) medium, and incubated at 37°C, 200 rpm, for 16 h.

LB media: 1.0% tryptone, 0.5% yeast extract and 1.0% NaCl adjusted to pH 7.0.

3.2.3 MIC of different antibiotics to *E. anophelis* NUHP1

The MIC for different antibiotics was tested following Wiegand's protocol (Wiegand, Hilpert, & Hancock, 2008). Triplicate of overnight *Elizabethkingia anophelis* NUHP1 culture were prepared as mentioned above for 16h at 37°C. Ten two-fold serial dilutions of each antibiotic were made with 100 µL LB broth in the 96-well plate. 100 µL diluted culture was also transferred to each well to obtain a solution that will contain 5×10^5 cells. The plate was incubated at 37 °C for 18-24 hours and the growth curve was read by Tecan Infinite® 200 PRO. Take a 10 ml aliquot from the wells that did not show growth after incubation and dilute it 10-fold and 100-fold respectively with sterile saline. Plate the

dilutions on LB agar plates and incubate at 37 °C for 16–20 h. The MIC was defined as the lowest concentration of antibiotic that prevented bacterial growth after plating on the LB agar. All of the antibiotics used in this study were purchased from Sigma-Aldrich.

3.2.4 Sample preparation for complete genome sequencing

Elizabethkingia anophelis strain NUHP1 was grown as described above for 16h at 37°C. DNA was recovered from harvested cell pellets using the QIAGEN QIAamp DNA Mini Blood Mini kit following manufacturer's instruction. The quality and concentration of DNA were measured by Qubit® 2.0 Fluorometer (Invitrogen) and NanoDrop 2000 Spectrophotometer (Thermo Scientific).

Illumina HiSeq 2000 sequencing platform was used for complete genome sequencing. 604 Mb data was produced for Tube of the 500 bp library, and 303 Mb data for the 2,000 bp library, 301 Mb data for the 6,000 bp library. The paired reads were *de novo* assembled with SOAPdenovo v. 1.03 software (BGI) (<http://soap.genomics.org.cn/soapdenovo.html>), and the reads were assembled into 8 large scaffolds. Gaps between contigs were closed by custom primer walks or by long-distance PCR amplification followed by DNA sequencing. Genome annotation was performed by using the RAST Server (<http://rast.nmpdr.org>). Metabolite synthesis operon was predicted by using the antiSMASH server (<http://antismash.secondarymetabolites.org/>).

The genomic DNA of 16 *Elizabethkingia anophelis* isolates from NUH were extracted by the QIAGEN Blood & Cell Culture DNA Midi kit according to the manufacturer's instruction. The quality and concentration of DNA were measured by Qubit® 2.0 Fluorometer (Invitrogen) and NanoDrop 2000 Spectrophotometer (Thermo Scientific). Pacbio sequencing platform with standard sample QC & SMRTbell library preparation and SMRTcell sequencing run was used to close the genomes of 16 *Elizabethkingia anophelis* isolates.

3.2.5 Comparative genomic analysis

The genomic sequence comparisons between the reference genome of *E. anophelis* NUHP1 and other 10 available draft genomes of *Elizabethkingia spp.* (accession numbers AVCQ000000000, AHHG000000000, ASAN000000000, ASYH010000000, ASYI010000000, ASYJ010000000, ASYK010000000, ASYF010000000, ASYG010000000, ANIW010000000) was performed as follows. Initially, the scaffolds from the draft sequences were oriented and joined by aligning them to the reference genome of *E. anophelis* NUHP1 using the

custom perl script scaffolding.pl (available at https://github.com/flauro/3tck_comparative (Lauro *et al.*, 2014)). The junctions between each adjacent scaffold were filled with the 6-frame stop-codon spacer 'NNNNCACACACTTAATTAATTAAGTGTGTGNNNN' resulting in contiguous pseudomolecules. Each pseudomolecule was then compared by BLAST searches against the reference genome of *E. anophelis* NUHP1 and visualized using the BLAST Ring Image Generator v0.95 (Alikhan, Petty, Ben Zakour, & Beatson, 2011).

The *E. anophelis* NUHP1 genome was BLAST searched against the Comprehensive Antibiotic Resistance Database (McArthur *et al.*, 2013) and Virulence Factors of Pathogenic bacteria Database (VFDB) (Chen, Xiong, Sun, Yang, & Jin, 2012) to identify antibiotic resistant genes and virulence genes by using Bio-Edit (Ibis Biosciences, Carlsbad, CA, USA, <http://www.mbio.ncsu.edu/bioedit/bioedit.html>) (minimum 30% identity with E-value less than $1e^{-5}$). Stress response genes were predicted from the *E. anophelis* NUHP1 genome by using the RAST server (Aziz *et al.*, 2008b). Genomic islands (GIs) were predicted and visualized from the NUHP1 genome by using the IslandViewer server (Langille & Brinkman, 2009).

Full genomes of *E. anophelis* NUHP1 were also aligned with *E. anophelis* assemblies isolated from mosquitoes using BRIG based on blast search. GIs were predicted using islandviewer 3. Positions of GIs in the *E. anophelis* NUHP1 genome is annotated based on islandviewer 3 prediction results using brig.

The open reading frames of all 16 genomes of the NUH isolates were predicted using Prokka v1.11 (Seemann, 2014), and the alignment of 2,622 core genes from all the genomes were performed using Roary v3.6.8 (Page *et al.*, 2015). Finally, the phylogenetic tree was constructed by RAxML-VI-HPC v8.2.9 (Stamatakis, 2006) based on the core gene alignment, with ATCC13253 as an outgroup. Bootstrap supporting values were calculated based on 1,000 replicates.

3.3 RESULTS AND DISCUSSION

3.3.1 Genome characterization

The genome of *E. anophelis* strain NUHP1 consists of a circular chromosome with a size of 4,369,828 base pairs (bp) and an average GC content of 35.62% (Figure 3.1). For the complete genome, 4,141 genes were predicted, 4,074 of which are protein-coding genes

and 67 of which are RNA-coding genes (52 tRNAs, 10 rRNAs, and 5 other RNAs). 2489 of the protein coding genes were assigned to a putative function with the remaining annotated as hypothetical proteins.

Figure 3.1 Circular representation of sequence conservation between *E. anophelis* NUHP1 and 11 *Elizabethkingia* spp. strains for identifying genome regions with high flexibility. Circles are numbered from 1 (outermost circle) to 14 (innermost circle). Circle 1: *E. meningoseptica* ATCC13253 (OSU). Circle 2: *E. meningoseptica* ATCC13253 (NITE). Circle 3: *E. meningoseptica* 502. Circle 4: *E. anophelis* R26. Circle 5: *E. anophelis* Ag1. Circle 6: *E. anophelis* NUH11. Circle 7: *E. anophelis* NUH6. Circle 8: *E. anophelis* NUH4. Circle 9: *E. anophelis* NUH1. Circle 10: *E. anophelis* NUHP3. Circle 11: *E. anophelis* NUHP2. Circle 12: GC skew (positive GC skew, green; negative GC skew, violet). Circle 13: GC content. Circle 14: Scale of NUHP1 genome.

Alignment of the draft genomes of available *Elizabethkingia* spp. (J. Teo *et al.*, 2014) with the NUHP1 complete genome showed that there are several serovar-specific genomic regions with low sequence identities (Figure 3.1). To identify the cause of these serovar-specific genomic regions, we predicted the genomic islands (GIs) and visualized their distribution in the NUHP1 genome by using the IslandViewer server (Langille & Brinkman,

2009). A total of fourteen GIs were identified by either the SIGI-HMM or the IslandPath-DIMOB methods used by the IslandViewer server (Figure 3.2). The distribution of GIs colocalized well with the serovar-specific genomic regions among the different genomes of *Elizabethkingia spp.* (Figure 3.1). The functional annotation of genes carried by these predicted GIs was listed in Table S1.

A large number of genes from the GIs encode products involved in transposon, virulence, efflux pumps and capsule polysaccharides (Table S1), which emphasizes the importance of these GIs on the survival of *Elizabethkingia spp.* under stressful conditions. A striking feature of the GIs is the existence of two large size conjugative DNA-transfer (Tra) regions at two different GIs (close to 0.5 M and 4M of the genome) (Figure 3.2, Table S1), indicating the importance of this mobile genetic element in modifying the genome content of *E. anophelis* NUHP1. The 0.5 M region GI contains a large number of genes encoding heavy metal resistance related proteins such as the cobalt-zinc-cadmium resistance protein CzcA, probable Co/Zn/Cd efflux system membrane fusion protein, lead, cadmium, zinc, and mercury transporting ATPase and nickel resistance protein (Table S1). The high level of heavy metal resistance of *E. anophelis* might enable it to play an important role in metal bioremediation (Rajendran, Muthukrishnan, & Gunasekaran, 2003) in the gut of *Anopheles* malaria vector species as the *Anopheles* malaria vector species are able to tolerate aquatic habitats with metal pollutants (Mireji *et al.*, 2010; Mireji *et al.*, 2008).

Figure 3.2 Positions of GIs as predicted by IslandViewer program. Blue: GIs predicted by IslandPath-DIMOB approach. Orange: GIs predicted by SIGI-HMM approach. Red: Integrated GIs predicted by both approaches.

3.3.2 Antibiotic resistance

One major reason for the failure of treatment of infections caused by *Elizabethkingia* spp. is the lack of proper treatment regimen (M. S. Hsu *et al.*, 2011). The NUHP1 genome was searched against the Comprehensive Antibiotic Resistance Database (P. Y. Lin, H. L. Chen, C. T. Huang, L. H. Su, & C. H. Chiu, 2010b) to identify antibiotic resistance genes. Forty-percent identity was used as a threshold when performing the BLASTP searches since the genome of NUHP1 is very new and highly likely not been included in any of these databases before. 14 antibiotic resistance genes were identified from the NUHP1, including genes conferring resistance to aminoglycosides, beta-lactamases, macrolides, chloramphenicol, tetracyclines, and trimethoprim (Table 3.1). The presence of these genes correlates well with the antibiotic resistant profiles of NUHP1 obtained by MIC assay (Table 3.2). Among all tested antibiotics, only ciprofloxacin showed a relatively low MIC (15.6 µg/ml), which is still much higher than the MIC of ciprofloxacin against other nosocomial Gram-negative pathogens such as *Pseudomonas aeruginosa* (0.25 µg/ml) and *Escherichia coli* (0.0625 µg/ml). These antibiotic resistance genes explained the difficulty in treatment of nosocomial infections caused by *E. anophelis* (Dooley, Nims, Lipp, Beard, & Delaney, 1980; Teo *et al.*, 2013).

Table 3.1 Proteins involved in antibiotic resistance encoded from *E. anophelis* NUHP1 identified by BLAST search against the Comprehensive Antibiotic Resistance Database.

Database ID	% Identity	Antibiotic Resistance	Annotation
(AGly)3Aac6-Iad:AB119105:1-435:435	42.42	aminoglycosides	aminoglycoside 6'-N-acetyltransferase
(Bla)B-1:AF189298:1-750:750	99.6	beta-lactams	class B carbapenemase BlaB-1
(Bla)CME-1:AJ006275:72-959:888	99.64	beta-lactams	CME-1 protein
(Bla)GOB-4:AF189293:1-756:756	99.6	beta-lactams	class B carbapenemase GOB-4
(Bla)TLA-1:GU441460:3220-4125:906	44.65	beta-lactams	beta-lactamase blaTLA-1
(MLS)CarA:M80346:411-2066:1656	41.54	macrolide	carA, carbomycin resistance protein
(MLS)MsrE:JF769133:7246-8721:1476	46.81	macrolide	MsrE, macrolide efflux protein
(MLS)OleB:L36601:1421-3130:1710	48.89	macrolide	OleB, ATP-binding protein. Confers

(MLS)TlrC:NC_016113:803268-384890:1623	46.05	macrolide	oleandomycin resistance and secretion TlrC, Tylosin resistance ATP-binding VAT B. involved in
(MLS)VatB:U19459:67-705:639	51.02	macrolide	resistance to virginiamycin A-like antibiotics
(Phe)CatB4:EU935739:59054-59602:549	76.87	chloramphenicol	CatB4, chloramphenicol acetyltransferase
(Tet)otrA:X53401:349-2341:1992	43.66	tetracycline	OtrA. Confers oxytetracycline resistance
(Tet)TetX:M37699:586-1752:1167	59.36	tetracycline	tetracycline resistance protein
(Tmt)DfrA20:AJ605332:1304-1813:510	40.96	trimethoprim	dihydrofolate reductase

Table 3.2 MICs of *E. anophelis* NUHP1 against common antibiotics.

Antibiotics	MIC
Tetracycline	62.5 µg/ml
Chloramphenicol	62.5 µg/ml
Ciprofloxacin	15.6 µg/ml
Erythromycin	62.5 µg/ml
Gentamycin	>500 µg/ml
Streptomycin	>500 µg/ml
Ceftazidime	>500 µg/ml
Tobramycin	>500 µg/ml

3.3.3 Virulence mechanisms revealed by genome analysis

As *Elizabethkingia* spp. colonized the gut of *Anopheles* malaria vector species together with a range of opportunistic pathogens (Boissiere *et al.*, 2012; Y. Wang, Gilbreath, Kukutla, Yan, & Xu, 2011), they might acquire virulence factors from these pathogens via horizontal gene transfer to cope with the host's adaptive immune response. To identify the potential virulence mechanisms for colonization of *E. anophelis* NUHP1 in *Anopheles* malaria vector host as well as human, we did a blast search of the NUHP1 genome against the Virulence Factors of Pathogenic bacteria Database (VFDB) (Chen *et al.*, 2012) predicted 55 genes conferring to the virulence of this bacterium, which includes genes involved in capsule polysaccharide biosynthesis, iron siderophore synthesis, heavy metal resistance and oxidative stress response (Table 3.3). *E. anophelis* species carries a siderophore synthesis operon (ORF2289-ORF2298), which is predicted to encode a siderophore similar to the siderophores produced by *Yersinia* spp. (Figure 3.3). However, we also noticed that some of the predicted genes are actually involved in central metabolism rather than virulence such as *leuD*, *panD* and *panC*.

Figure 3.3 *E. anophelis* NUHP1 genome contains a siderophore biosynthesis operon (2421051 - 2432865 nt) similar to the siderophore biosynthesis operon of *Yersinia pestis* revealed by the antiSMASH server (<http://antismash.secondarymetabolites.org/>).

Reactive oxygen species is one of the major bactericidal mechanisms from the host immune system and bacterial pathogens have evolved oxidative stress response mechanism for survival during host colonization (Witko-Sarsat, Rieu, Descamps-Latscha, Lesavre, & Halbwachs-Mecarelli, 2000). The virulence factor prediction highlights that oxidative stress response may be essential for causing infections by *E. anophelis* NUHP1 as homologs of both KatA catalase and SodB-superoxide dismutase was found in the genome. Analysis using the RAST Server (Aziz *et al.*, 2008a) also showed that oxidative stress response is the largest group (37, 58%) among all the stress response mechanisms encoded by the *E. anophelis* NUHP1 genome (Figure S1, Table S2).

Table 3.3 Proteins involved in virulence encoded from *E. anophelis* NUHP1 identified by BLAST search against the Virulence Factors of Pathogenic bacteria Database (VFDB).

Database id	% Identity	Annotation	Source
VFG0032	51.79	bplG-probable sugar transferase	<i>Bordetella pertussis</i> Tohama I
VFG0036	44.69	bplC-lipopolysaccharide biosynthesis protein	<i>Bordetella pertussis</i> Tohama I
VFG0037	40.79	bplB-probable acetyltransferase	<i>Bordetella pertussis</i> Tohama I
VFG0077	52.08	clpP-ATP-dependent Clp protease proteolytic subunit	<i>Listeria monocytogenes</i> (serovar 1/2a) EGD-e
VFG0079	45.71	clpC-endopeptidase Clp ATP-binding chain C	<i>Listeria monocytogenes</i> (serovar 1/2a) EGD-e
VFG0080	49.27	clpE-ATP-dependent protease	<i>Listeria monocytogenes</i> (serovar 1/2a) EGD-e
VFG0082	60.16	aldA-aldehyde dehydrogenase	<i>Vibrio cholera</i> N16961
VFG0087	43.83	tagD-tagD protein	<i>Vibrio cholera</i> N16961
VFG0152	49.18	rhlR-transcriptional regulator RhlR	<i>Pseudomonas aeruginosa</i> PAO1
VFG0160	47.46	pvdE-pyoverdine biosynthesis protein PvdE	<i>Pseudomonas aeruginosa</i> PAO1
VFG0167	41.27	pchR-transcriptional regulator PchR	<i>Pseudomonas aeruginosa</i> PAO1
VFG0314	40.28	gluE-UDP-glucose 4-epimerase	<i>Helicobacter pylori</i> 26695
VFG0320	44.3	kdtB-lipopolysaccharide core biosynthesis protein (kdtB)	<i>Helicobacter pylori</i> 26695

VFG0431	53.38	tvb-Vi polysaccharide biosynthesis protein, UDP-glucose/GDP-mannose	<i>Salmonella enterica</i> (serovar typhi) CT18
VFG0574	53.42	mgtB-Mg ²⁺ transport protein	<i>Salmonella enterica</i> (serovar typhimurium) LT2
VFG0575	40.54	mgtC-Mg ²⁺ transport protein	<i>Salmonella enterica</i> (serovar typhimurium) LT2
VFG0576	42.57	ssb-ssDNA-binding protein controls activity of RecBCD nuclease	<i>Salmonella enterica</i> (serovar typhimurium) LT2
VFG0596	42.67	copR-Copper resistance; transcriptional regulatory protein	<i>Salmonella enterica</i> (serovar typhimurium) LT2
VFG0696	44.64	bexA-ATP-dependent polysaccharide export protein	<i>Haemophilus influenza</i> 1007 (type b)
VFG0869	43.41	aatC-AatC ATB binding protein of ABC transporter	<i>Escherichia coli</i> 042 (EAEC)
VFG1028	51.72	intI1-Tn21 integrase IntI1	<i>Shigella flexneri</i> (serotype 2a) YSH6000
VFG1066	42.59	orf50-unknown	<i>Shigella flexneri</i> (serotype 2a) YSH6000
VFG1248	49.35	fleQ-transcriptional regulator FleQ	<i>Pseudomonas aeruginosa</i> PAO1
VFG1300	43.05	cap8D-capsular polysaccharide synthesis enzyme Cap8D	<i>Staphylococcus aureus</i> MW2
VFG1306	45.45	cap8J-capsular polysaccharide synthesis enzyme Cap8J	<i>Staphylococcus aureus</i> MW2
VFG1342	48.89	cpsO-glycosyl transferase CpsO(V)	<i>Streptococcus agalactiae</i> 2603V/R
VFG1354	40.82	cbpE-choline binding protein E	<i>Streptococcus pneumonia</i> TIGR4
VFG1381	60.14	icl/aceA-aceA	<i>Mycobacterium tuberculosis</i> H37Rv
VFG1411	43.55	leuD- leuD	<i>Mycobacterium tuberculosis</i> H37Rv
VFG1416	57.01	panD-panD	<i>Mycobacterium tuberculosis</i> H37Rv
VFG1417	40.7	panC-panC	<i>Mycobacterium tuberculosis</i> H37Rv
VFG1511	61.18	ORF60-putative integrase	<i>Escherichia coli</i> 536 (UPEC)
VFG1586	44.12	orf52-hypothetical protein	<i>Escherichia coli</i> 536 (UPEC)
VFG1587	45.05	orf53- hypothetical protein	<i>Escherichia coli</i> 536 (UPEC)
VFG1668	45.96	orf45-putative lysil-tRNA synthetase LysU	<i>Escherichia coli</i> 536 (UPEC)

VFG1746	42.59	YPO0255-putative two-component response regulator	<i>Yersinia pestis</i> CO92
VFG1855	65.13	htpB-Hsp60, 60K heat shock protein HtpB	<i>Legionella pneumophila</i> Philadelphia 1
VFG1861	63.33	katA-catalase/(hydro)peroxidase	<i>Legionella pneumophila</i> Philadelphia 1
VFG1864	45.45	mip-macrophage infectivity potentiator (Mip)	<i>Legionella pneumophila</i> Philadelphia 1
VFG1866	42.13	rpoS-stationary phase specific sigma factor RpoS	<i>Legionella pneumophila</i> Philadelphia 1
VFG1867	44.9	sodB-superoxide dismutase	<i>Legionella pneumophila</i> Philadelphia 1
VFG1931	40.71	cadF-outer membrane fibronectin-binding protein	<i>Campylobacter jejuni</i> NCTC 11168
VFG1956	47.31	fcl-putative fucose synthetase	<i>Campylobacter jejuni</i> NCTC 11168
VFG1971	44.01	kpsF-KpsF protein	<i>Campylobacter jejuni</i> NCTC 11168
VFG1974	55.56	kpsT-putative capsule polysaccharide export ATP-binding protein	<i>Campylobacter jejuni</i> NCTC 11168
VFG2059	43.08	PA0073-probable ATP-binding component of ABC transporter	<i>Pseudomonas aeruginosa</i> PAO1
VFG2064	42.67	PA0078-hypothetical protein	<i>Pseudomonas aeruginosa</i> PAO1
VFG2085	45.22	vasH-sigma-54 dependent transcriptional regulator	<i>Vibrio cholera</i> N16961
VFG2110	47.27	vipD-VipB interferes with multivesicular body formation at the late endosome	<i>Legionella pneumophila</i> Philadelphia 1
VFG2225	67.14	gmd-GDP-mannose 4,6-dehydratase	<i>Brucella melitensis</i> 16M
VFG2228	44.64	wzt-O-antigen export system ATP-binding protein	<i>Brucella melitensis</i> 16M
VFG2276	45.65	colA-collagenase	<i>Clostridium perfringens</i> 13
VFG2361	45.65	galE-UDP-glucose 4-epimerase	<i>Yersinia enterocolitica</i> 8081
VFG2480	47.95	clpV-Clp-type ATPase chaperone protein	<i>Burkholderia pseudomallei</i> K96243
VFG2563	44.68	wzt2-ATP-binding ABC transporter capsular polysaccharide export protein	<i>Burkholderia pseudomallei</i> K96243

3.3.4 Comparative genomic analysis

Genome comparison between NUHP1 and *E. anophelis* assembly from mosquito

To have a better understanding of the role of mosquitoes in the pathogenesis of *E. anophelis* and the genomic features that enable *E. anophelis* adapts to mosquito gut environment, we compared the genome of *E. anophelis* isolate NUHP1 with the *E. anophelis* assembly from mosquito.

Comparative genomic analysis, shown in Figure 3.4, reveals that most of the unique sequences present in NUHP1 genome but not in the assembled contig are most likely genomic islands (GIs). These regions are found to have different GC contents compared to the neighboring region, as shown in the image (Figure 3.4). In accordance with this, the Mauve alignment also shows that all assembled contigs from the mosquito gut are represented by homologous sequences from NUHP1 full genome. However, there are some unique sequences in NUHP1 genome (Figure S2).

As most resistance genes of *E. anophelis* were reported to be located at the core genome of *Elizabethkingia* (González & Vila, 2012), this result suggested that multiple potential resistance genes are ancestral in the genus *Elizabethkingia* and that multiple resistance of *E. anophelis* might enable it to play an important role in its adaptation to mosquito.

Figure 3.4 Circular representation of sequence conservation between *E. anophelis* NUHP1 and *E. anophelis* assembly from mosquitoes for identifying genome regions with high flexibility. Circles are numbered from 1 (outermost circle) to 6 (innermost circle). Circle 1: Genomic islands. Circle 2: *E. anophelis* isolated from mosquitoes. Circle 3: *E. anophelis* NUHP1. Circle 4: GC skew (positive GC skew, green; negative GC skew, violet). Circle 5: GC content. Circle 6: Scale of NUHP1 genome.

Core genome-based phylogenetic structure of 16 *E. anophelis* isolates from NUH

The alignment of 2,622 core genes from all the genomes of 16 *E. anophelis* isolates from NUH were selected to deduce robust phylogenies. The *E. anophelis* isolates CSID_3015183678 (accession number CP014805) and FMS-007 (accession number CP006576) are used as reference, and *E. meningoseptica* ATCC13253 is an outgroup. Phylogenetic tree based on the alignments of the 2,622 core genes showed that *E. anophelis* isolates and *E. meningoseptica* were distinctly distributed into different groups (Fig. 3.5), which is in accordance with the previous studies (Breurec *et al.*, 2016; J. Teo *et al.*, 2014). Whereas CTICU7 and CTICU9 were separated from other NUH isolates, the rest NUH isolates were clustered into the same sublineage together with the clinical isolates

CSID_3015183678 and FMS-007 from other regions, suggesting CTICU7 and CTICU9 might belong to a different species. The close relation between NUH isolates and the other two *E. anophelis* nosocomial strains suggests that they might share a common evolutionary history. This result also showed that the genomes of some NUH isolates were very similar to each other, suggesting that they might be clonal. In contrast to the study in 2014 (J. Teo *et al.*, 2014) which suggested that NUHP1, NUHP2, NUHP3, NUH1, and NUH4 may be clonal because of the high similarity of their genomes, our results showed that they were in the distinct subgroups.

Figure 3.5 Phylogenetic tree based on multiple genome alignment of 2,622 core genes showing relationship of *Elizabethkingia spp.* . The open reading frames of all 16 genomes of the NUH isolates were predicted using Prokka v1.11 (Seemann, 2014), and the alignment of 2,622 core genes from all the genomes were performed using Roary v3.6.8 (Page *et al.*, 2015). The phylogenetic tree was constructed by RAxML-VI-HPC v8.2.9 (Stamatakis, 2006) based on the core gene alignment, with ATCC13253 as an outgroup. Bootstrap supporting values were calculated based on 1,000 replicates. The CSID_3015183678 and FMS-007 are reference strains.

Biofilm Formation of *E. anophelis* NUHP1 and Transcriptomic Analysis of Stress Response of NUHP1 to Hydrogen Peroxide

4.1 INTRODUCTION

Biofilms is ubiquitous in both natural and clinical environment. Bacteria grown in biofilm could utilize the substrates which could not be utilized by planktonic cells to enhance their growth (Rickard *et al.*, 2003). Furthermore, the biofilm matrix EPS act as barriers to protect cells inside the biofilms from environmental changes and hazardous substances such as antimicrobial agents (Jefferson, 2004). Biofilms are also perfect environment for horizontal gene transfer between different species due to the close contact between cells (Harrison, Turner, Marques, & Ceri, 2005).

Bacteria suffered the oxidative stress either from the exogenous environment such as the host immune system and competitors or from the byproduct of respiration during cellular activities. To survive in the environment with oxidative stress, bacteria have evolved mechanisms to protect themselves from the damage caused by ROS. They either keep the concentration of the ROS at tolerable levels by detoxifying enzymes and freeing radical-scavenging substrates or repairing oxidative damages with DNA and protein repair systems.

Among the *Elizabethkingia* spp., although *E. meningosepticum* has been reported to show strong biofilm formation capacity which may explain its potential pathogenicity (Balm *et al.*, 2013) and *Elizabethkingia* was observed to form biofilm in clinical setting, the factors related to biofilm formation by these non-motile bacteria is still not clear. In 2013, Teo *et al.* investigated an *E. anophelis* outbreak in two intensive care units (ICUs) at the National University Hospital (NUH), Singapore (J. Teo *et al.*, 2014). They found that three *E. anophelis* isolates from patients shared close genomic content with one ICU sink isolate, indicating that *E. anophelis* may also form biofilms to persist in the sinks as well as that *E. anophelis* may harbor resistance mechanisms towards disinfectants such as virex, acidified bleach and hydrogen peroxide, as the ICU sinks of the NUH are routinely cleaned by these disinfectants.

Hence, we investigated the biofilm formation ability of the patient isolates *E. anophelis* NUHP1 under various conditions. We also investigate the response of *E. anophelis* to oxidative resistance based on RNA-sequencing transcriptomic analysis.

4.2 MATERIALS AND METHODS

4.2.1 Bacterial strains

E. anophelis NUHP1, *Pseudomonas aeruginosa* PAO1, *E. coli* DH5α and *E. meningoseptica* ATCC 13253 (NITE)

4.2.2 Growth medium and conditions

The bacteria strains were streaked on Luria-Bertani (LB) agar (Lennox) and incubated for 16 h at 37°. Single colonies were picked from the LB agar plate and inoculated with Luria-Bertani (LB) medium for 16 h at 37°C and shaken at 200 rpm.

LB broth (Lennox): 1.0% tryptone, 0.5% yeast extract and 0.5% NaCl adjusted to 7.0.

ABTGC broth: 15.1mM (NH₄)₂SO₄, 33.7 mM Na₂HPO₄, 22.0 mM KH₂PO₄, 50 mM NaCl, 1 mM MgCl₂·6H₂O, 100 μM CaCl₂·2H₂O, 10 μM FeCl₃·6H₂O, 0.2% Casamino acid and 0.2% Glucose

Casitone-yeast extract (CYE broth): 5 g/L Casitone, 3 g/L Yeast Extract, 1 g/L MgSO₄·7H₂O

Tryptic Soy Broth (TSB): 17 g/L casein peptone (pancreatic), 2.5 g/L dipotassium hydrogen phosphate, 2.5 g/L glucose, 5 g/L sodium chloride and 3 g/L soya peptone (papain digest.)

Brain Heart Infusion Broth (BHI broth): 5 g/L beef heart, 12.5 g/L calf brains, 2.5 g/L disodium hydrogen phosphate, 2 g/L D(+)-glucose, 10 g/L peptone and 5 g/L sodium chloride

Nutrient Broth (NB): 1 g/L D(+)-glucose, 15 g/L peptone, 6 g/L sodium chloride, 3 g/L yeast extract

4.2.2 Biofilm formation assay

4.2.2.1 Static biofilm

Each bacteria of interest was inoculated in a 2ml LB culture and grew overnight at 37°C, 200 rpm. The overnight culture was diluted 1:100 in the desired media and 200 µl of each diluted culture was pipetted into each well in a fresh 96-well microtiter plate. After incubation for 48 hours, planktonic bacteria were pipetted out and the wells were successively washed with 0.9% NaCl three times. 200 µl of 0.1% crystal violet solution (pre-filtered through a 0.44 µm filter) to were add to each well and stain for 10-15 min at room temperature before discarding the solution. Wash each well with 0.9% NaCl for three times and air-dry the plates for 15 minutes. Finally, incubate each stained well with 200 µl of 95% ethanol for 10-15 minutes and measure the optical density (OD) of each of these samples at a wavelength of 600 nm. We compared static biofilm of *E. anophelis*, *P. aeruginosa* and *E. coli* in LB/ABTGC medium at 30/37°C and we also test static biofilm of *E. anophelis* in various medium. 5 µg/ml Dnase was added to TSB medium as indicated where appropriate.

To test the effect of hemoglobin on biofilm formation, *E. anophelis* NUHP1 and *E. meningoseptica* ATCC 13253 (NITE) biofilms were grown in µ-Slide 8 well microscopy chambers (ibidi, München, Germany) under static conditions at 37°C in ABTGC medium supplemented with 1µM FeCl₃ or 100ug/ml Hb respectively. Biofilms were stained with SYTO62 (Invitrogen, CA) and PI (Invitrogen, CA) for live/dead staining. Confocal images of 24 biofilms were captured (ZEISS LSM780 Confocal System) and analyzed using the Imaris software package (Bitplane, AG).

4.2.2.2 Flow cell biofilm

We used a flow cell system for confocal microscopy examination of biofilms. The flow cells were inoculated with a 1:100 dilution (in 10% TSB) of stationary-phase culture of *E. anophelis*, *P. aeruginosa* and *E. coli*, and flow was initiated after 1 h. The flow rate was 0.2 per min. After 3-day incubation at 37°C, biofilms were stained with LIVE/DEAD BacLight™ bacterial viability dye which makes use of two different stains: SYTO 9 and propidium iodide. Live cells were stained green by SYTO9, whereas dead cells that have a compromised/breached membrane were stained red by propidium iodide. Biofilms were visualized under LSM780 confocal system and analyzed using the Imaris software package (Bitplane, AG).

4.2.3 Transcriptomics and Quantitative RT-PCR analyses

A comparative transcriptome analysis between H₂O₂-treated *E. anophelis* NUHP1 cells and untreated controls were performed by using RNA-sequencing to understand the early transcriptional response of *E. anophelis* to oxidative stress. Overnight culture was inoculated into a well of 24 well plate with 1ml of LB medium to reach a starting OD of 0.01, and cultures were incubated at 37°C with shaking at 200 rpm. When the optical density at 600 nm reached 0.5, four of these cultures were immediately treated with H₂O₂ (19 mM), whereas the remaining four cultures were left untreated. After a further 10 min-incubation at 37°C with shaking at 200 rpm, cells from each culture were harvested by centrifugation and total RNA was extracted with RNeasy Protect Bacteria Mini Kit with on-column DNaseI digestion (Chua *et al.*, 2014). Gene expression analysis was conducted via Illumina RNA sequencing (RNA-Seq technology). RNA-Seq was conducted for three biological replicates of each sample. Libraries were produced using an Illumina TruSeq RNA Sample Prep Kit. The libraries were sequenced using the Illumina HiSeq 2500 platform with a paired-end protocol and read lengths of 100 nt. Analysis of the RNA-seq data was performed as previously described (Chua *et al.*, 2014). Briefly, the sequence reads were mapped onto the *E. anophelis* NUHP1 genome using the “RNA-Seq and expression analysis” application of CLC genomics Workbench 6.0 (CLC Bio, Aarhus, Denmark). The transcript count table was subjected to DESeq package (Anders & Huber, 2010) of R/Bioconductor (Gentleman *et al.*, 2004) for statistical analysis. The transcript counts were normalized to the effective library size. The differentially expressed genes were identified by performing a negative binomial test. Transcripts were stringently determined as differentially expressed when having a fold change larger than 4 and an adjusted p-value smaller than 0.01. Hierarchical clustering analysis was performed and a heatmap was drawn for the differentially expressed genes, using heatmap.2 package of R/Bioconductor (Gentleman *et al.*, 2004).

Quantitative reverse transcription PCR (qRT-PCR) was performed using a two-step method. First-strand cDNA was prepared from each RNA sample by using SuperScript® III First-Strand Synthesis System kit (Cat. No. 18080-400, Invitrogen) and SYBR Select Master Mix kit (Cat. No. 4472953, Applied Biosystems by Life Technologies) was used for RT-PCR in Applied Biosystems StepOne™ RT-PCR machine. qRT-PCR data were normalized to 16S rRNA and rpoD ($\Delta\Delta C_T$ method) prior to comparing treatment groups. PCR primers are listed in Table S3. All amplicons were 90-150 bp.

4.2.4 CAS Liquid Assay

The CAS liquid assay was performed as previously described, with modifications (Schwyn and Neilands 1987). All solutions and dilutions were made with 1mM PIPES (pH 5.6) unless otherwise stated. The water LC-MS CHROMASOLV (Fluka, Singapore) was used to prepare the PIPES solution. Six milliliters of 10mM HDTMA solution was placed in a 100-ml volumetric flask and diluted with 14ml 1mM PIPES. A mixture of 1.5ml ferric iron solution (1mM FeCl₃, 10mM HCl) and 7.5ml 2mM aqueous CAS (Tokyo Chemical Industry, Singapore) solution was slowly added under stirring. A 4.307 g quantity of anhydrous piperazine was dissolved in 20 ml PIPES solution to which 6.25ml of 12M HCl was carefully added. This buffer solution (pH =5.6) was washed into the 100-ml volumetric flask which was then filled with 1mM PIPES to make up 100ml of CAS assay solution. To demonstrate the efficiency of the CAS assay solution, a standard curve was prepared with purified siderophore deferroxamine mesylate salt (Sigma-Aldrich, Singapore). Hundred microliters of different concentrations of deferroxamine mesylate salt was mixed with equal volume of CAS assay solution in 96-well plate and the absorbance measured at 630 nm after reaching equilibrium. To determine the siderophore produced by *E. anophelis*, *E. anophelis* NUHP1 overnight culture was inoculated into LB medium with a starting optical density of OD₆₀₀=0.01 and incubated at 37 °C with 200 rpm shaking. When the cultures reached mid-log phase (OD₆₀₀=0.5), H₂O₂ was added so a final concentration of 19 mM was achieved for treatment. After incubating overnight (16–18 h), both H₂O₂-treated and untreated cultures were centrifuged and filtered through the 0.22-mm filters. A 0.5-ml aliquot of culture supernatants or the control LB medium (treated or untreated with H₂O₂) was mixed with 0.5 ml CAS assay solution. After reaching equilibrium the absorbance was measured at 630 nm. Experiments were performed in triplicate, and the results are shown as the mean ±standard deviation (SD). Student's t-test was used to determine the significance of the differences.

4.2 RESULTS AND DISCUSSION

4.2.3 Biofilm formation

Biofilm formation under static conditions

Although *E. anophelis* has been reported to colonize in the sink in the intensive care unit (J. Teo *et al.*, 2014), which suggests the relationship between biofilm formation and infections in patients, no studies on the factors involved in the biofilm formation of *E.*

anophelis have been carried out. In this study, biofilm-forming ability of *E. anophelis* was first compared with *P. aeruginosa* PAO1 and *E. coli* DH5 α under various growth conditions in a polystyrene surface. We tested biofilm-forming abilities of these three organisms in both rich media LB medium and minimal medium supplemented with glucose and Casamino Acids (ABTGC) combined with different temperature to mimic environment (30°C) or human host (37°C).

The effect of different temperature and medium on biofilm formation is showed in Figure 4.1. Under all tested conditions, *P. aeruginosa* exhibited highest biofilm formation ability, followed by *E. coli*, while *E. anophelis* showed the poorest biofilm-forming potential (Figure 4.1). Among the gram-negative bacteria, the biofilm formation of *Pseudomonas aeruginosa* and *Escherichia coli* have been intensively studied. This result was in agreement with previous study that a wide range of nutrients were found to allow *P. fluorescens* and *P. aeruginosa* to form biofilms (O'Toole & Kolter, 1998a, 1998b). On the other hand, some organisms could not form biofilms in low-nutrient medium such as *Escherichia coli* K-12 (Pratt & Kolter, 1998). However, in contrast, some other organisms such as *E. coli* O517:H7 only show biofilm formation ability in minimal medium (Dewanti & Wong, 1995). Since *E. anophelis* forms less biofilm compared with the other two bacterial species, it is possible that *E. anophelis* is less virulent than *P. aeruginosa*.

Furthermore, according to our study, *E. anophelis* biofilm grew better in minimal medium ABTGC than in rich medium LB (Figure 4.1) at 37 °C. This could be explained that biofilm formation was usually triggered by infertile conditions, so the minimal nutrient might be enough to support the biofilm formation of *E. anophelis*. However, different from *E. coli* O517:H7 which suffers nutrient stress in rich medium such as TSB, *E. anophelis* shows highest biofilm-forming potential in TSB medium (Figure 4.2). Similarly to *E. anophelis*, *E. meningoseptica* and *Hafnia alvei* displayed the same trend in minimal medium and nutrient-rich medium TSB (Anelet Jacobs & Hafizah Y Chenia, 2011; Vivas *et al.*, 2008). However, on the contrary to *E. meningoseptica* which also forms strong biofilm in the relatively nutrient-rich medium LB (P.-Y. Lin *et al.*, 2010), it is hard to explain why *E. anophelis* grew higher biofilm in ABTGC than in LB. It is possible that LB contains some inhibitors of *E. anophelis* biofilm. Hence, further investigation is necessary to identify the factors that influence biofilm formation of this bacterium.

The optimal growth temperature for *E. anophelis* are at 30-31 °C and 37 °C, however, in our study we found that the biofilm formation decreased at 30 °C compared to those grown

at 37°C. This result agree with the previous study that the *E. meningoseptica* isolated from *Oreochromis mossambicus* showed stronger adherence at 37°C than at room temperature (Anelet Jacobs & Hafizah Y Chenia, 2011). This may be because *E. anophelis* need to form biofilm to increase the chance of survival in the hosts, which might also explain that *E. anophelis* is hard to be eradicated during infection.

Figure 4.1 Static biofilm of *E. anophelis* NUHP1, *P. aeruginosa* PAO1 and *E. coli* DH5α in different medium at different temperature. *P < 0.05, Student's t-test

Biofilm formation in flow cell system

To better understand the nature of the *E. anophelis* biofilm, confocal microscopy was employed to observe and compare with *P. aeruginosa*, *E. coli* and *E. meningoseptica* whose biofilm formation abilities have been studied. Preliminary study was performed to determine the best media in which *E. anophelis* can form strong biofilm. Five medium were tested including LB, TSB, BHI, NB and CYE. TSB was chosen for flow cell biofilm assay as after 48h incubation at 37°C, *E. anophelis* showed highest biofilm formation potential in this media compared with the rest (Figure 4.2).

Figure 4.2 Static biofilm of *E. anophelis* in different medium.

Flow cell system together with confocal microscopy further confirmed the biofilm formation of *E. anophelis*. Figure 4.3A shows the biofilm structure of *E. anophelis* isolate NUHP1. *E. anophelis* displayed different pattern of biofilm development in flow cell compared with the other three strains. After 3 days of incubation, PAO1 formed flat biofilm, whereas *E. anophelis* NUHP1 and *E. meningoseptica* ATCC 13253 formed irregularly shaped three-dimensional biofilm with complex structures that did not show any typical morphology (Figure 4.3A) compared with the flat PAO1 biofilm (Figure 4.3D) and the macro colony in *E. coli* biofilm (Figure 4.3B). A similar result was also reported on the *E. meningoseptica* isolate CH2B, showing that CH2B formed microcolonies in flow cell condition with TSB medium after 24 hours, and the biofilm structure became thick and complicated after 48 hours of incubation (Anelet Jacobs & Hafizah Y Chenia, 2011). Therefore, since increased biofilm formation of *E. meningoseptica* was reported to be responsible for the poor outcome in patients, the similar trend of biofilm formation suggests biofilm may play a role in the pathogenesis caused by *E. anophelis*. Further study should be carried out to investigate the role of biofilm *in vivo*.

Figure 4.3 Live/Dead stain of flow cell biofilm. A: *E. anophelis* NUHP1 B: *E. coli* DH5α C: *E. meningoseptica* ATCC 13253 (NITE) D: *P. aeruginosa* PAO1. Scale bars, 20 μ m. Green are live cells that stained with Cyto 9 and dead cells that have a compromised/breached membrane will be stained in red by propidium iodide.

We found some red stained cells in the NUHP1 biofilm as shown in figure 4.3A. Since only dead cells that have a compromised/breached membrane will be stained in red by propidium iodide, it is proposed that eDNA play a role in the formation of NUHP1 biofilm. To validate that, 5 μ g/ml DNase was added in TSB media during NUHP1 static biofilm formation and the activities were compared with untreated biofilms. After incubated in 96 well plate for 48 hours, crystal violet assay showed that DNase largely inhibited the biofilm formation of NUHP1 (Figure 4.4). Although when eDNA was initially found in biofilm, it was simply considered as residues after cell lysis, later on, it was also demonstrated to be the constituent of the biofilm matrix as well as intercellular connector in many organisms such as *P. aeruginosa* and *S. aureus*. Our results also suggested that eDNA is an important component for biofilm formation of *E. anophelis*.

Figure 4. 4 Effect of DNase on *E. anophelis* biofilm biomass.

4.2.1 Transcriptomic analysis of stress response of *E. anophelis* to hydrogen peroxide

Another fact supporting the striking capacity of oxidative stress resistance is that hydrogen peroxide is routinely used to clean the sink of the hospital where *E. anophelis* NUHP1 was identified while could not eradicate the *E. anophelis* (J. Teo *et al.*, 2014). The minimal inhibitory concentration (MIC) of hydrogen peroxide is 38 mM for *E. anophelis* NUHP1, which is much higher than the MIC for *E. coli* DH5a (1 mM) and *P. aeruginosa* PAO1 (1 mM) strains. We exposed exponential growth phase NUHP1 cultures to sub-lethal concentration (19 mM) of hydrogen peroxide and then used RNA-seq based transcriptomic analysis to examine the most important genes for oxidative stress response. RNA-seq analysis indicated that out of 4,076 predicted *E. anophelis* genes, 142 displayed statistically significant mRNA level changes of ≥ 4 - fold with 104 of them displaying increased transcript levels (Table 4.1) and the rest displayed no change (Fig. 4.5). This response is comparable to that of *E. coli* exposed to identical treatment (1 mM H_2O_2 , 10 min), which resulted in 140 of 4,169 arrayed genes displaying mRNA levels increased >4 -fold (M. Zheng *et al.*, 2001).

Table 4.1 Effects of treatment with H_2O_2 on mRNA levels

Change in mRNA level	No. of genes (% of total) with indicated mRNA level change			
	≥ 4 -fold	≥ 4 - to 10-fold	≥ 10 - to 50-fold	≥ 50 -fold
Increase	104(2.6)	37(0.9)	40(1.0)	27(0.7)
Decrease	38(0.9)	38(0.9)	0(0.0)	0(0.0)
Total	142(3.5)	75(1.8)	40(1.0)	27(0.7)

Treatment of *E. anophelis* NUHP1 with H₂O₂ resulted in a dramatic decreased expression of genes involved in heavy metal efflux system such as Mg²⁺ transport ATPase (BD94_2835), Probable Co/Zn/Cd efflux system membrane fusion protein (BD94_2836), Cation efflux system protein CusA (BD94_2837), and Heavy metal RND efflux outer membrane protein (BD94_2838) (Table S6). In contrast, homologue proteins of two well-known antioxidative proteins, manganese superoxide dismutase (Aguirre & Culotta, 2012) (BD94_2310) and non-specific DNA-binding protein Dps (Martinez & Kolter, 1997) (BD94_3681), were induced 40 and 14.4 fold by H₂O₂ treatment, respectively (Table S4). The expression of a large set of iron uptake related genes such as the siderophore receptor (BD94_0071, BD94_2005), siderophore biosynthesis (BD94_2298, BD94_2299, BD94_2301), hemin utilization genes (BD94_2937, BD94_2938, BD94_2939) were highly induced by H₂O₂ treatment (Table S4). The above result suggests that iron uptake mechanisms might play an essential role in stress response to oxidative stress by *E. anopheles*.

Figure 4.5 Heat map of 142 genes whose mRNA level significantly changed. The differentially expressed genes (fold-change > 4, adjusted P-value < 0.01) between H₂O₂-treated and non-treated NUHP1 cells were identified by performing a negative binomial test using the DESeq package of R/Bioconductor.

4.2.2 RT-PCR analysis of stress response of *E. anophelis* to hydrogen peroxide

To further validate our RNA-seq methodology, real-time RT-PCR was used to determine relative mRNA levels of 18 genes selected from 20 genes whose mRNA levels displayed the highest fold changes in H₂O₂ treated *E. anophelis* compared with untreated controls (Table 4.2). RT-PCR and RNA-seq showed the same change tendency of most genes except for one gene. Transglycosylase associated protein decreased 5.78 fold with RNA-seq, while increased 2.21 fold in RT-PCR.

Overall, the values obtained by RT-PCR have a good correspondence with the results of the RNA-seq, taking into account the variation expected due to the different natures of the two methodologies.

Table 4.2 Top induced and reduced genes determined by RNA-seq and by qRT-PCR in H₂O₂ - treated cells.

Locus Tag	Fold Change (RNA-Seq)	Fold Change (RT-PCR)	Gene product description
BD94_0071	291.1	2976.5	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
BD94_1839	108.2	295.8	Methionine aminopeptidase (EC 3.4.11.18)
BD94_2005	126.8	5.9	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
BD94_2298	195.9	578.0	Siderophore biosynthesis L-2,4-diaminobutyrate decarboxylase
BD94_2299	156.3	64.4	Siderophore biosynthesis protein, monooxygenase
BD94_2301	200.3	46.6	Desferrioxamine E biosynthesis protein DesD @ Siderophore synthetase superfamily, group C @ Siderophore synthetase component, ligase
BD94_2937	88.8	21.8	ABC-type hemin transport system, ATPase component
BD94_2938	126.1	680.5	Hemin ABC transporter, permease protein
BD94_2939	100.3	19.8	Periplasmic hemin-binding protein

BD94_1092	-10.9	-26.3	Cytochrome c oxidase subunit CcoN (EC 1.9.3.1) / Cytochrome c oxidase subunit CcoO (EC 1.9.3.1)
BD94_1093	-11.8	-8.9	Cytochrome c oxidase subunit CcoP (EC 1.9.3.1)
BD94_1333	-4.5	-1.5	ATP phosphoribosyltransferase (EC 2.4.2.17)
BD94_2835	-11.1	-2.3	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)
BD94_2836	-13.8	-7.7	Probable Co/Zn/Cd efflux system membrane fusion protein
BD94_2837	-16.1	-74.3	Cobalt-zinc-cadmium resistance protein CzcA; Cation efflux system protein CusA
BD94_2838	-15.5	-50.7	Heavy metal RND efflux outer membrane protein, CzcC family
BD94_3514	-13.4	-6.9	Probable cytochrome-c peroxidase (EC 1.11.1.5)

4.2.3 Siderophore production is enhanced by oxidative stress in *E. anophelis*

We used the chrome azurol sulfonate (CAS) liquid assay, a universal siderophore detection method (Schwyn and Neilands 1987), to measure the siderophore produced by *E. anophelis*. The color of CAS assay solution changes from blue to yellow when siderophores in sample solutions chelate iron from CAS, which also leads to decrease in absorbance at 630 nm. The freshly prepared CAS solution was first mixed with a commercially available iron siderophore, deferoxamine, which gave a dose-dependent decrease of the absorbance at 630 nm (Fig. 4.6A). Supernatants of the *E. anophelis* cultivated in LB contained siderophore activity, which is able to decrease the absorbance at 630nm to a level close to 20 mM of deferoxamine (Fig. 4.6B). In accordance with the transcriptomic analysis, *E. anophelis* cultivated in the presence of sublethal H₂O₂ concentration (20mM) produced a significantly larger quantity of siderophore compared with *E. anophelis* cultivated in medium alone (Fig.4.6). We noted that the presence of H₂O₂ will lead to certain interference of the CAS assay of the LB medium control (Fig 4.6B). However, the concentration of H₂O₂ in the *E. anophelis* overnight cultures should be rather low due to the neutralization of secreted catalase.

Figure 4.6 Standard curve for the determination of siderophore (deferoxamine) concentration using a CAS solution. (A). Siderophore production by *E. anophelis* NUHP1 cultivated with and without the presence of H_2O_2 (B). Means and standard deviation (s.d.) from triplicate experiments are shown. * $P < 0.05$, Student's t-test.

The yersiniabactin-like siderophores are produced by *Yersinia* spp., certain strains of *Klebsiella pneumonia* (Bachman *et al.* 2011) and *E. coli* (Brumbaugh *et al.* 2015). Yersiniabactin-like siderophores could not be recognized by the host siderophore-binding protein lipocalin-2 and are thus essential for the virulence of *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, *K. pneumonia*, and *E. coli* (Bearden *et al.* 1997; Bachman *et al.* 2011; Brumbaugh *et al.* 2015). Yersiniabactin-like siderophores are able to reduce the respiratory oxidative stress response of innate immune cells (Paauw *et al.* 2009) and facilitate the survival of *K. pneumoniae* during pulmonary infection (Bachman *et al.*

2011). Yersiniabactin-like siderophores could also sequester heavy metals to prevent their toxicity (Chaturvedi *et al.* 2012; Bobrov *et al.* 2014). More interestingly, the copper–yersiniabactin complexes were shown to act as superoxide dismutase mimics, detoxifying the oxygen radicals (Chaturvedi *et al.* 2012). Further genetic analysis is required to confirm the production of yersiniabactin by *E. anophelis* under oxidative stress condition as the CAS assay is a general siderophore assay and not specific to the yersiniabactin.

Transcriptomic Analysis of *E. anophelis* in Response to Blood and Comparative Transcriptome Analysis of *E. anophelis* in Response to Blood and Hydrogen Peroxide

5.1 INTRODUCTION

Elizabethkingia anophelis was first identified from the midgut of mosquito *Anopheles gambiae* before it was reported to be an opportunistic pathogen, which causes infections in humans (Kampfer, Matthews *et al.* 2011).

It was reported that *Elizabethkingia spp.* is dominant in the gut of malaria vectors *Anopheles gambiae* and *Anopheles stephensi* with blood meal feeding (Ngwa *et al.*, 2013; Ying Wang *et al.*, 2011). For instance, in one of the study 16S rRNA analysis revealed that the microbial diversity is decreasing during mosquito development and *E. meningoseptica* accounted for over 80% of the 16S rRNA genes isolated from the midgut of both male and female mosquitoes. (Ngwa *et al.*, 2013). The colonization in the mosquito gut suggests that *Elizabethkingia spp.* can tolerate the oxidative stress that generated during the metabolism of blood meal.

On the other hand, bacteria also need to defense oxidative stress during the course of infection (KLEBANOFF, 1980; Thomas *et al.*, 1988). Transcriptomic analysis has been used to reveal the oxidative stress response of many bacteria to human blood. For example, in *E. faecalis*, genes associated with hydroperoxide resistance and DNA protection and genes encoding superoxide dismutase were upregulated upon blood exposure (Vebø *et al.*, 2009); iron storage and oxidative stress related genes and genes encoding catalase were highly upregulated in *Neisseria meningitidis* to protect it from oxidative stress during incubation with blood (Hedman *et al.*, 2012). In *Staphylococcus aureus*, *Yersinia pseudotuberculosis* and *Yersinia pestis*, similar results have also been obtained, whereby oxidative stress response was induced in bacteria upon the exposure to blood (Chauvaux *et al.*, 2007; Malachowa *et al.*, 2011; Rosso *et al.*, 2008).

In chapter 4 we have discussed the strong oxidative stress resistance of *E. anophelis* to H₂O₂ and the fact that blood meal feeding favors the growth of *Elizabethkingia spp.*. This suggested that the dominance of *E. anophelis* in the mosquito gut is due to their strong

capacity to resist oxidative stress. To better understand the response of *E. anophelis* to human blood during infection and localization in mosquitoes, we performed RNA-sequencing based transcriptome analysis of *E. anophelis* NUHP1 after incubation with blood. Meanwhile, we also compared the transcriptome of *E. anophelis* to blood and to H₂O₂.

5.2 MATERIALS AND METHODS

5.2.1 Sample harvest, RNA extraction and transcriptomic analysis of E. anophelis in response to mouse blood

Overnight culture was inoculated into a 50ml falcon tube with 15ml of LB medium to reach a starting OD of 0.01, and cultures were incubated at 37°C with shaking at 200 rpm. When the optical density at 600 nm reached 0.5, 5 ml of culture were taken out as control with a final concentration of 0.32% sodium citrate added. Spin down the rest culture at 6000 g for 8 minutes and resuspended the pellet in 1ml of freshly extracted mouse whole blood maintained at 37°C. All of the samples were incubated under the same condition mentioned above for another 30 minutes. Cells from control culture were then treated with 2 volume of RNA protect bacteria reagent (Qiagen) immediately after incubation. For the samples that are treated with mouse blood, the culture were first centrifuged at 300g for 5 min to separate the bacteria cells from blood cell before the supernatant were added with 2 volume of RNA protect bacteria reagent (Qiagen). Cells were centrifuged at 7000g for 12 minutes at 4 °C and the pellet were stored at -80 °C until RNA isolation.

For RNA extraction, the samples were first washed with 2 volumes of Erythrocyte Lysis (EL) buffer (Qiagen) and centrifuged at 4°C and 4500 rpm for 6 minutes (Echenique-Rivera *et al.*, 2011; Morag R. Graham, 2005). Before RNA was extracted the cell pellets were treated with 20 mg/ml lysozyme for 10 min with shaking on a vortexer at 1000 rpm. RNA was extracted with RNeasy Protect Bacteria Mini Kit (Qiagen) according to the manufacturer's protocol except that the samples were sonicated 2 seconds with 2 seconds pause for 15 times after the cells were resuspended in RLT buffer. DNA contamination was removed by on-column DNase digestion and post-treatment with TURBO DNA-free™ Kit (Thermo Fisher) The ribosome RNA was depleted with Ribo-Zero rRNA Removal Kit (Bacteria) (Illumina) before sequencing. The total RNA concentration and DNA contamination were examined by Qubit® 2.0 Fluorometer (Invitrogen) and NanoDrop 2000 Spectrophotometer (Thermo Scientific). The integrity of RNA was tested by Agilent 2100 Bioanalyzer (Agilent Technologies).

RNA-Seq was conducted for three biological replicates of each sample. Libraries were produced using an Illumina TruSeq RNA Sample Prep Kit. The libraries were sequenced using the Illumina HiSeq 2500 platform with a paired-end protocol and read lengths of 100 nt. Analysis of the RNA-seq data was performed as previously described (Chua *et al.*, 2014). Transcripts were stringently determined as differentially expressed when having a fold change larger than 4 and an adjusted p-value smaller than 0.01.

Quantitative reverse transcriptase PCR (qRT-PCR) was performed using a two-step method. First-strand cDNA was prepared from each RNA sample by using SuperScript® III First-Strand Synthesis System kit (Cat. No. 18080-400, Invitrogen) and SYBR Select Master Mix kit (Cat. No. 4472953, Applied Biosystems by Life Technologies) was used for RT-PCR in Applied Biosystems StepOne™ RT-PCR machine. qRT-PCR data were normalized to 16S rRNA and rpoD ($\Delta\Delta C_T$ method) prior to comparing treatment groups. PCR primers are listed in Table S3. All amplicons were 90-150 bp.

The overlap of genes with the expression at least 4- times fold change between the blood-treated and H₂O₂-treated NUHP1 was analysed by R with the VennDiagram package.

5.2.2 Time-Kill assay

Elizabethkingia anophelis strain NUHP1 was cultivated in iron-free ABTGC medium without addition of iron at 37 °C with 200 rpm shaking. Overnight cultures of NUHP1 were adjusted to 10⁹CFU/ml in fresh ABTGC medium with and without the supplementation of 10 µM hemoglobin (Hb). H₂O₂ was added to the Hb supplemented and control NUHP1 cultures at a final concentration of 20 mM, after which samples were harvested at time 5, 15, 30min and diluted as necessary and plated in LB agar plates for viable counts. Experiments were performed in triplicate, and the results are shown as the mean±SD.

5.2.3 Biofilm assay

To test the effect of hemoglobin on biofilm formation, *E. anophelis* NUHP1 and *E. meningoseptica* ATCC13253 (NITE) biofilms were grown in µ-Slide 8 well microscopy chambers (ibidi, München, Germany) under static conditions at 37°C in ABTGC medium supplemented with 10µM FeCl₃, 2.5µM Hb (with 10 µM Fe inside) or without any iron respectively. Biofilms were stained with SYTO9 (Invitrogen, CA) and PI (Invitrogen, CA) for live/dead staining.

5.3 RESULTS AND DISCUSSION

5.3.1 Transcriptomic analysis of *E. anophelis* in response to mouse blood

To obtain a comprehensive understanding of the response during *E. anophelis* incubation with blood, transcriptomic analysis was applied to determine the most important genes for NUHP1 in response to blood based on RNA-seq technology. As has been expected, a large amount of genes displayed at least 4-fold changes in mRNA level. This takes up to 12.6% (514 genes) of the total predicted *E. anophelis* genes (Table 5.1). Of the 514 genes, 319 genes (7.8% of the total predicted genes) displayed increased transcriptional levels and the rest 195 genes (4.8% of the total predicted genes) displayed decreased transcriptional levels (Table 5.1).

Table 5.1 Effects of treatment with mouse blood on mRNA levels

Change in mRNA level	No. of genes (% of total) with indicated mRNA level change			
	≥4-fold	≥4- to 10-fold	≥10- to 50-fold	≥50-fold
Increase	319(7.8)	192(4.7)	94(2.3)	33(0.8)
Decrease	195(4.8)	149(3.7)	39(1)	7(0.2)
Total	514(12.6)	341(8.4)	133(3.3)	40(1)

The changes of mRNA level of a large number of genes involved in iron uptake such as the siderophore receptor (BD94_0071 and BD94_2005), siderophore biosynthesis (BD94_2298 - BD94_2301), heme utilization genes (BD94_2937, BD94_2938 and BD94_2939) were also highly increased during incubation with blood compared with bacteria cultured in LB medium (Table 5.2 and Table S6). This is in consistent with transcriptomic analysis result that NUHP1 was treated with H₂O₂.

In previous studies, microarray-based transcriptomic analysis has revealed the upregulation of genes involved in iron and heme metabolism pathways in many bacteria species such as *Staphylococcus aureus* (Malachowa *et al.*, 2011), *Enterococcus faecalis* (Vebø *et al.*, 2009), *Yersinia pseudotuberculosis* (Rosso *et al.*, 2008) and *Yersinia pestis* (Chauvaux *et al.*, 2007) during incubation with human blood. The induced transcriptional levels of genes involved in iron and heme uptake could be easily explained by the fact that iron is the co-factor of many enzymes involved in numerous metabolic processes, which leads to its essential role in bacteria virulence (Corbin *et al.*, 2008; Torres *et al.*, 2007). The

concentration of iron required for the growth of most bacteria is between 0.4 μM and 4.0 μM . However, the level of free iron in blood is only 10^{-18} M, which is far below the minimal essential iron concentration for bacterial growth (Andrews, Robinson, & Rodríguez-Quñones, 2003; Skaar & Schneewind, 2004). Therefore, a functional iron uptake system is crucial for bacterial survival under such low iron conditions. The iron that bound to the host proteins will be deprived by the high-affinity iron chelators such as siderophores and then delivered to the bacterium. Hence, the induced expression of iron uptake-related genes such as siderophore receptors will help NUHP1 to adapt to the iron deficient condition in blood. This result suggests the potentially importance of iron uptake and homeostasis for the growth of *E. anophelis* in blood.

In addition, many genes associated with general metabolic processes are also induced to cope with the stress in blood, which is consistent with transcriptomic analysis of *Staphylococcus aureus* (Malachowa *et al.*, 2011), *Enterococcus faecalis* (Vebø *et al.*, 2009) and *Streptococcus agalactiae* (Mereghetti, Sitkiewicz, Green, & Musser, 2008) during culture in human blood. Consistent with the fact that the level of free amino acids (AAs) is relatively low in blood, our results revealed increased transcription of periplasmic oligopeptide-binding protein OppA as well as the upregulation of transcripts associated with arginine and serine catabolic after 30 minutes contact with blood, suggesting a strategy to ferment amino acids and acquire oligopeptides.

The expression of a catalase encoding gene (BD94_1895) was increased by 6-fold during incubation with blood (Table S6), indicating that NUHP1 has encountered oxidative stress. This result is opposite to the previous studies, which showed decreased transcription of catalase in both *Y. pestis* and *Y. pseudotuberculosis* during growth in plasma (Rosso *et al.*, 2008).

Manganese is also a co-factor that is important for the growth of bacteria *in vitro* (Abrantes, de Fátima Lopes, & Kok, 2011). Notably, upregulation of a gene that encodes manganese transport protein MntH (BD94_2742) was observed during the incubation of NUHP1 with blood (Table S6), suggesting the manganese homeostasis is also essential for growth of *E. anophelis*.

After being incubated in blood for 30 min, a drastic decline of expression of the genes responsible for various ions transport were observed. For example, *czcA* and *czcC* (BD94_2837 and BD94_2838) which are involved in cobalt-zinc-cadmium resistance were

downregulated for 573.4- and 370.4-fold, respectively. Similarly, the transcriptional level of genes that encode magnesium transport proteins (BD94_2834 and BD94_2835) were also greatly decreased (Table 5.2 and Table S7). These findings agree with the transcriptome remodeling by *Streptococcus agalactiae* in which the genes encode zinc and cobalt transport proteins were also downregulated during cultivation in blood (Mereghetti *et al.*, 2008). The increased intracellular Zn(II) may protect thiols from oxidation. It was reported that elevated zinc significantly increases resistance to high concentrations of H₂O₂ in *Bacillus subtilis* (Gaballa & Helmann, 2002). On the other hand, some antioxidative enzymes such as superoxide dismutase contains heavy metal based cofactors. For example, *E. coli* contains a periplasmic Cu/Zn-Sod (SodC) (Cornelis, Wei, Andrews, & Vinckx, 2011). The increased concentration of intracellular heavy metal may be essential for the function of antioxidative enzymes. Our results indicate that the downregulation of ions transport system may play an important role in oxidative stress resistance. Study should be carried out to further investigate the function of these ion transport systems.

To further validate our RNA-seq methodology, real-time RT-PCR was used to determine relative mRNA levels of 8 genes selected from 20 genes whose mRNA levels displayed the highest fold changes in H₂O₂ treated *E. anophelis* compared with untreated controls (Table 5.2). RT-PCR and RNA-seq showed the same change tendency of all genes we choose. Overall, the values obtained by RT-PCR were in accord with the results obtained from RNA-seq, taking into account the variation expected due to the different natures of the two methodologies.

Table 5.2 Top upregulated and downregulated genes determined by RNA-seq and by qRT-PCR in mouse blood-treated cells.

Locus ID	Fold change (RNA seq)	Fold change (RT-PCR)	Gene product description
BD94_2300	1746.7		Desferrioxamine E biosynthesis protein DesC @ Siderophore synthetase small component, acetyltransferase
BD94_2299	1657.2	1814.8	Siderophore biosynthesis protein, monooxygenase
BD94_2301	1403.9	1790.6	Desferrioxamine E biosynthesis protein DesD @ Siderophore synthetase superfamily, group C @ Siderophore synthetase component, ligase
BD94_2298	1027.5	1419.4	Siderophore biosynthesis L-2,4 diaminobutyrate decarboxylase
BD94_2939	319.2	240.2	Heme ABC transporter, cell surface heme and hemoprotein receptor HmuT
BD94_1897	282.5		putative iron-regulated membrane protein
BD94_1898	278.7		TonB-dependent siderophore receptor
BD94_1896	268		hypothetical protein
BD94_0934	267.7		putative outer membrane receptor
BD94_2938	237.9		Hemin ABC transporter, permease protein
BD94_2836	-989.8	-502.2	Probable Co/Zn/Cd efflux system membrane fusion protein
BD94_2837	-573.4	-471.8	Cobalt-zinc-cadmium resistance protein CzcA; Cation efflux system protein CusA
BD94_2834	-534.9		Mg(2+) transport ATPase protein C
BD94_2835	-405	-142.5	Mg(2+) transport ATPase, P-type (EC3.6.3.2)
BD94_2838	-370.4	-209.5	Heavy metal RND efflux outer membrane protein, CzcC family
BD94_2428	-120.7		hypothetical protein
BD94_2427	-59.8		hypothetical protein
BD94_1235	-46.9		TonB-dependent receptor
BD94_3334	-41.9		Oar protein
BD94_3516	-37.3		hypothetical protein

5.3.2 Comparison of *E. anophelis* transcriptome in response to mouse blood and to H₂O₂

To understand the differences of *E. anophelis*' responses to mouse blood and H₂O₂, we compared the transcriptome of blood-treated and H₂O₂-treated NUHP1. For this, we grouped the transcriptome data into four sets, namely A, B, C and D (Table S4-S7) and performed statistical analysis. Data set A contains 319 genes that were upregulated in blood-treated NUHP1 compared with untreated planktonic NUHP1. Data set B contains 104 genes that were upregulated in H₂O₂-treated NUHP1 compared with untreated planktonic NUHP1. Data set C contains 195 genes that were downregulated in blood-treated NUHP1 compared with untreated planktonic NUHP1. Data set D contains 38 genes that were downregulated in H₂O₂ treated NUHP1 compared with untreated planktonic NUHP1.

Data sets A and B have an overlap of 49 genes and data sets C and D have an overlap of 17 genes (Fig. 5.1, Table 5.3 and Table 5.4). Intriguingly, the iron uptake related genes were found to be greatly induced in both blood and H₂O₂ treated NUHP1 cells. In particular, BD94_2298 - BD94_2301 which encode siderophore biosynthesis proteins drew our attention as siderophore plays a crucial role in iron acquisition by bacteria from the environment (Visca, Leoni, Wilson, & Lamont, 2002). This observation is in agreement with the studies showing that genes associated with siderophore biosynthesis were upregulated in *S. aureus* after exposure to both H₂O₂ and human blood (Chang, Small, Toghrol, & Bentley, 2006; Malachowa *et al.*, 2011). On the other hand, when bacteria encounter oxidative stress, cellular components will be damaged by free hydroxyl radicals due to increased environmental iron content (Ming Zheng, Doan, Schneider, & Storz, 1999). Therefore, iron metabolism is one of the most important mechanisms for bacteria to survive under oxidative stress.

Figure 5.1 Venn diagram showing overlaps of data sets A–D that were derived from transcriptome analysis with RNA sequencing of the genes up regulated in blood treated NUHP1 (A), genes up regulated in H₂O₂ - treated NUHP1 (B), genes down regulated in blood treated NUHP1 (C), and genes down regulated in H₂O₂ treated NUHP1 (D). Genes of all data sets are listed in Tables S4-S7.

i. Data set A (dark ivory): Genes upregulated in NUHP1 cells treated with mouse blood compared with untreated NUHP1. Data set B (light ivory): Genes upregulated in NUHP1 cells treated with H₂O₂ compared with untreated NUHP1.

ii. Data set C (light grey): Genes downregulated in NUHP1 cells treated with mouse blood compared with untreated NUHP1. Data set D (dark grey): Genes downregulated in NUHP1 cells treated with H₂O₂ compared with untreated NUHP1.

iii. Data set A (dark ivory): Genes upregulated in NUHP1 cells treated with mouse blood compared with untreated NUHP1. Data set D (dark grey): Genes downregulated in NUHP1 cells treated with H₂O₂ compared with untreated NUHP1.

iv. Data set B (light ivory): Genes downregulated in NUHP1 cells treated with H₂O₂ compared with untreated NUHP1. Data set C (light grey): Genes upregulated in NUHP1 cells treated with mouse blood compared with untreated NUHP1. Overlapped genes between data sets A–D are listed in Table 5.3.

In accordance with iron metabolism, we also found that genes involved in heme synthesis and iron storage were upregulated in response to both blood- and H₂O₂-treatment (Figure 5.1A and Table 5.3). Heme is crucial in many cellular processes including electron transport and defense against ROS, since heme compounds with incorporated iron are important cofactors that function as the catalytic component of many enzymes involved in redox reactions (Elgrably-Weiss *et al.*, 2002). The heme uptake pathway is utilized by gram-negative bacteria to chelate heme from the hemophores of the hosts and transport heme into the bacteria cells (Wandersman & Delepelaire, 2004). Our finding agree with the previous studies on other organisms such as *Staphylococcus aureus* and *Salmonella enterica* that genes involved in heme uptake were induced by H₂O₂-driven oxidative stress (Chang *et al.*, 2006; Elgrably-Weiss *et al.*, 2002).

Consequently, the co-induction of these genes in both blood- and H₂O₂-treated NUHP1 cells indicates that H₂O₂-driven oxidative stress is the main stress in blood and NUHP1 defend oxidative stress by controlling intracellular iron levels.

Table 5.3 Overlaps of induced genes between mouse blood-treated and H₂O₂-treated NUHP1

Locus ID	Gene name and protein description	Fold change in data set A ^a	Fold change in data set B ^a
BD94_2300	Desferrioxamine E biosynthesis protein DesC @ Siderophore synthetase small component, acetyltransferase	1746.7	86.3
BD94_2299	Siderophore biosynthesis protein, monooxygenase	1657.2	156.3
BD94_2301	Desferrioxamine E biosynthesis protein DesD @ Siderophore synthetase superfamily, group C @ Siderophore synthetase component, ligase	1403.9	200.3
BD94_2298	Siderophore biosynthesis L-2,4- diaminobutyrate decarboxylase	1027.5	195.9
BD94_2939	Heme ABC transporter, cell surface heme and hemoprotein receptor HmuT	319.2	100.3
BD94_1898	TonB-dependent siderophore receptor	278.7	5.4
BD94_0934	putative outer membrane receptor	267.7	96.3
BD94_2938	Hemin ABC transporter, permease protein	237.9	126.1
BD94_2937	ABC-type hemin transport system, ATPase component	211.1	88.8
BD94_0072	hypothetical protein	167.7	248.3
BD94_2936	Methyltransferase (EC 2.1.1.-)	143.7	46.9
BD94_0935	hypothetical protein	133.0	50.8

BD94_0071	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins	116.5	291.1
BD94_1872	putative outer membrane receptor	113.1	99.1
BD94_0936	hypothetical protein	103.8	31.7
BD94_1901	iron-chelator utilization protein	98.7	5.6
BD94_2935	Hemin transport protein HmuS	85.8	60.5
BD94_0860	putative iron-regulated membrane protein	71.0	56.6
BD94_1900	Transcriptional regulator, AraC family	56.2	51.8
BD94_3020	putative iron-regulated membrane protein	52.0	51.7
BD94_1903	Ferrichrome-iron receptor	45.5	5.5
BD94_0937	Cytochrome c551 peroxidase (EC 1.11.1.5)	43.0	11.7
BD94_0867	Peptide methionine sulfoxide reductase MsrA (EC 1.8.4.11) / Peptide methionine sulfoxide reductase MsrB (EC 1.8.4.12)	40.0	6.1
BD94_0067	Ferrichrome-iron receptor	36.2	65.6
BD94_3023	TonB-dependent receptor	35.1	75.6
BD94_1873	FIG00405034: hypothetical protein	29.6	87.8
BD94_0068	putative iron-regulated membrane protein	27.0	30.7
BD94_0862	Ferrichrome-iron receptor	26.4	42.0
BD94_3022	hypothetical protein	25.3	76.2
BD94_2005	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins	25.0	126.8
BD94_0070	putative iron-regulated membrane protein	22.9	44.8
BD94_3021	TonB-dependent receptor, putative	22.9	76.9
BD94_0069	Aerobactin siderophore receptor IutA	21.5	25.1
BD94_1875	Cytochrome c551 peroxidase (EC 1.11.1.5)	18.7	87.6
BD94_0938	hypothetical protein	16.9	5.3
BD94_1904	PepSY-associated TM helix domain protein	16.2	4.1
BD94_1899	Transcriptional regulator, AraC family	16.0	39.4
BD94_4020	Aerobactin siderophore receptor IutA	9.8	64.0
BD94_2645	hypothetical protein	9.7	27.8
BD94_0768	Ferrichrome-iron receptor	7.6	50.2
BD94_0844	Bifunctional protein: zinc-containing alcohol dehydrogenase; quinone oxidoreductase (NADPH:quinone reductase) (EC 1.1.1.-); Similar to arginate lyase	6.2	7.9
BD94_0013	hypothetical protein	6.0	9.2
BD94_3664	Ankyrin 1	5.5	19.8
BD94_2346	putative Cytochrome bd2, subunit I	5.4	4.1
BD94_0845	hypothetical protein	4.6	5.6
BD94_1874	hypothetical protein	4.5	10.2
BD94_1871	Putative esterase	4.4	4.7
BD94_2347	Cytochrome d ubiquinol oxidase subunit II	4.3	6.0
BD94_0014	Ferrous iron transport protein B	4.3	11.9

^a Fold change of mRNA-levels was ≥ 4 ($P \leq 0.05$) in data sets A and B.

Among the 17 overlap genes that were downregulated in both blood- and H₂O₂-treated NUHP1 cells, the expression of genes associated with heavy metal efflux system dramatically decreased (Figure 5.1, Table 5.4, Table S6 and Table S8). Among those genes, the colocalization of BD94_2835, BD94_2836, BD94_2837 and BD94_2838 implies that they are from the same gene cluster.

Table 5.4 Overlaps of reduced genes between mouse blood-treated and H₂O₂-treated NUHP1

Locus ID	Gene name and protein description	Fold change in data set C ^a	Fold change in data set D ^a
BD94_2836	Probable Co/Zn/Cd efflux system membrane fusion protein	-989.8	-13.8
BD94_2837	Cobalt-zinc-cadmium resistance protein CzcA; Cation efflux system protein CusA	-573.4	-16.1
BD94_2835	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)	-405.0	-11.1
BD94_2838	Heavy metal RND efflux outer membrane protein, CzcC family	-370.4	-15.5
BD94_2428	hypothetical protein	-120.7	-4.7
BD94_1350	hypothetical protein	-17.6	-6.4
BD94_2430	hypothetical protein	-15.3	-5.9
BD94_3227	hypothetical protein	-15.3	-12.2
BD94_2280	hypothetical protein	-15.1	-5.6
BD94_2912	hypothetical protein	-13.6	-4.6
BD94_1351	hypothetical protein	-12.8	-7.7
BD94_0339	hypothetical protein	-7.9	-4.9
BD94_3513	hypothetical protein	-6.7	-8.8
BD94_3514	Probable cytochrome-c peroxidase (EC1.11.1.5)	-6.6	-13.4
BD94_3515	hypothetical protein	-6.4	-10.3
BD94_2374	protein of unknown function DUF306, Meta and HslJ	-5.3	-5.1
BD94_3904	hypothetical protein	-4.3	-14.3

^a Fold change of mRNA-levels was ≥ 4 ($P \leq 0.05$) in data sets C and D.

5.3.3 Hemoglobin utilization enhances growth, hydrogen peroxide tolerance and biofilm formation of *E. anophelis*

As the blood meal feeding to the *Anopheles gambiae* was found to drastically reduce the community diversity and favored growth of *Elizabethkingia spp.* in its gut (Y. Wang *et al.*, 2011) and the transcriptomic analysis revealed the importance of heme uptake related genes during response to both blood and H₂O₂ treatment, we hypothesized that the heme utilization based iron uptake might be essential for the growth and oxidative stress response of *Elizabethkingia spp.*. We thus compared the impact of ferric iron and the blood-associated iron source, hemoglobin, on the growth of *E. anophelis* NUHP1. In general, both *E. anophelis* NUHP1 grew very slowly in the minimal ABTGC medium. This might due to the fact some growth promoting factors was introduced from the rich LB medium to the minimal ABTGC medium. Hemoglobin was able to enhance the growth of both *E. anophelis* NUHP1 in a dose-dependent manner (Figure 5.2). Surprisingly, addition of ferric iron has no growth promotion effect on either *E. anophelis* NUHP1 (Figure 5.2).

Figure 5.2 Dose-dependent growth enhancement by FeCl₃ (A) and hemoglobin (Hb) (B) to *E. anophelis* NUHP1. The results were the average of duplicate measurements. Molar concentration is for the Fe element only.

Figure 5. 3 Time-kill curves of *E. anophelis* NUHP1 by 20 mM H₂O₂ in ABTGC medium with and without supplementation of 10 mM Hb.

Moreover, *E. anophelis* NUHP1 growing in the presence of hemoglobin had higher level of H₂O₂ tolerance compared to growing in the presence of ferric iron. The MIC value of H₂O₂ for *E. anophelis* NUHP1 growing in 40 μ M hemoglobin and 40 μ M FeCl₃ are 20 mM and 150 μ M, respectively. It has been proposed that biofilm formation of *Elizabethkingia* spp. might contribute to the infections (A. Jacobs & H. Y. Chenia, 2011). We also examine the impact of ferric iron and hemoglobin biofilm formation of *Elizabethkingia* spp. (Figure 5.4). *E. anophelis* NUHP1 is not a good biofilm former and could not form firmly attached biofilms on the substratum under the same growth condition as *E. meningoseptica* ATCC13253 (NITE) (Figure 5.4). *E. anophelis* NUHP1 formed biofilms with a biovolume of $1160.1 \pm 196.7 \mu\text{m}^3$ when supplemented with Ferric iron and $3708.7 \pm 621.4 \mu\text{m}^3$ when supplemented with hemoglobin. This represented an average of 3.2 fold increase in biovolume, with a range of 2.28 to 4.50 fold when hemoglobin was used as the iron source. Thus, supplementation of hemoglobin to the minimal medium dramatically enhanced the biofilm formation of *E. anophelis* NUHP1 (Figure 5.4). In contrast, the average biovolume of biofilm formed per $7228.4 \mu\text{m}^2$ square area in three technical replicates was calculated using the Imaris software package (Bitplane, AG). *E. meningoseptica* ATCC13253 (NITE) formed biofilms with an average biovolume and standard error of $4740.3 \pm 553.2 \mu\text{m}^3$ when supplemented with ferric iron and $6395.5 \pm 1724.3 \mu\text{m}^3$ when supplemented with hemoglobin. This represented an average of 1.35 fold increase in biovolume, with a range

of 0.88 to 1.94 fold when hemoglobin was used as the iron source. Thus hemoglobin did not have a significant effect on its biofilm formation.

Figure 5.4 Confocal images of 7,228.4 μm^2 substratum area of 24 h *E. anophelis* NUHP1 and *E. meningoseptica* ATCC13253 biofilms grown in iron free ABTGC medium and ABTGC medium supplemented with different iron sources. Representative confocal images from triplicate experiments are shown for each condition. Scale bars: 20 μm .

Conclusion and Future Plan

6.1. Conclusion

In conclusion, we report the complete genome sequence of *E. anophelis* isolate NUHP1 from a patient, which is the first complete genome of the *Elizabethkingia* genus. The genome of *E. anophelis* strain NUHP1 consists of a circular chromosome with a size of 4,369,828 base pairs (bp). A large number of antibiotic resistant genes and virulence genes are predicted from the NUHP1 genome. Our results proved that this strain can resist many antibiotics commonly used to treat infections caused by Gram-negative bacteria. Large-sized genomic islands carrying transposon elements and stress response regions were found in the genome of NUHP1.

Furthermore, we investigated the biofilm formation ability of *E. anophelis* NUHP1 under various conditions and the similar trend of biofilm formation between *E. anophelis* and *E. meningoseptica* suggests that biofilm formation also plays an important role in the pathogenesis of *E. anophelis*. We also found that extracellular DNA is an important component of biofilms formed by *E. anophelis*.

RNA-sequencing based transcriptomic analysis of *E. anophelis* NUHP1 during hydrogen peroxide treatment revealed that NUHP1 has acquired resistance or stress response mechanisms towards disinfectants. This might explain the prevalence of *E. anophelis* infections in ICUs since the ICU sinks of the NUH are routinely cleaned by using virex, acidified bleach and hydrogen peroxide. Expressions of genes involved in synthesis of a yersiniabactin-like iron siderophore and heme utilization are highly induced as a protective mechanism toward oxidative stress caused by hydrogen peroxide treatment. Chromeazurol sulfonate assay verified that siderophore production of *E. anophelis* is increased in the presence of oxidative stress.

To better understand the capacity of *Elizabethkingia anophelis* to respond to human blood during infection and localization in mosquitoes, we performed a RNA-sequencing based transcriptome analysis of *Elizabethkingia anophelis* NUHP1 after incubation with mouse blood. Meanwhile, we also compared the transcriptome of response of *E. anophelis* to blood and to H₂O₂. The overlap of upregulated genes in response to both hydrogen peroxide

and blood treatment further implies that the dominance of *E. anophelis* in the mosquitoes is due to their strong resistance to oxidative stress.

In addition, upregulated genes involved in biosynthesis of a yersiniabactin-like iron siderophore and heme utilization during hydrogen peroxide treatment were also observed to be upregulated during blood treatment. This further strengthens that siderophore and heme utilization play important roles in response to oxidative stress, which could be induced by treatment of both hydrogen peroxide and blood. Time kill assay and biofilm assay proved that hemoglobin utilization rather than ferric iron enhances growth, hydrogen peroxide tolerance and biofilm formation in *E. anophelis*.

Taken together, our study emphasizes that *E. anophelis* is an important pathogen with a strong resistance to various classes of antimicrobials and the capability to form biofilm, which greatly contributes to its high mortality and prevalence in clinical outbreaks. In addition, we also showed that *E. anophelis* displayed strong resistance to oxidative stress induced by blood, which probably enables *E. anophelis* to survive in the gut of mosquitoes and utilize mosquitoes as one of its transmission medium. Siderophore production and hemoglobin utilization are important for oxidative stress tolerance in *E. anophelis*. Our results provide novel clues on the transmission and pathogenicity of *E. anophelis* and shed lights on better prevention and treatment strategies for *E. anophelis* infections in clinical settings.

6.2. Future Plan

Since hemoglobin rather than ferric iron greatly facilitates the growth, hydrogen peroxide tolerance and biofilm formation of *E. anophelis* NUHP1 we emphasized that heme utilization pathways play an essential role in stress response and virulence of the emerging pathogen *E. anophelis*.

Iron acquisition via catecholate siderophores has been proved to play a fundamental role in bacterial colonization of the murine intestinal tract (Pi *et al.*, 2012). Iron availability increases the pathogenic potential of pathogens at the intestinal epithelial interface also have been reported where increased iron concentration enhanced the growth as well as the adhesion and invasion abilities of enteric pathogens (Kortman, Boleij, Swinkels, & Tjalsma, 2012). Iron level is directly connected to virulence of many pathogens (Beasley, Marolda, Cheung, Buac, & Heinrichs, 2011; Kortman *et al.*, 2012; Wyckoff, Mey, & Payne, 2007). The redundancy in iron transport systems has made it more difficult to determine the role

of individual systems *in vivo* and *in vitro*. To verify the role of hemoglobin in infection caused by *E. anophelis*, in the future we will study colonization and virulence gene expression of *E. anophelis* NUHP1 *in vivo* using gut and skin colonization models of mice. *E. anophelis* NUHP1 will be cultured under iron-limiting conditions and in the presence of increasing concentrations of either ferric chloride or hemoglobin and then inoculated into mice gut or skin wound. Then ability of *E. anophelis* NUHP1 to colonize in gut or skin wound in different iron conditions will be assessed by serial dilution and plating to determine CFUs and biofilm imaging analysis, followed by gene expression analysis to determine the colonization factors *in vivo*.

Genomic manipulation on *E. anophelis* is a challenge we have encountered during the process to study the molecular mechanism of this bacterium in response to hydrogen peroxide. The failure of plasmid transformations and various transposon systems to modify this microorganism indicates the difficulty in modification of its genome. Therefore, in order to better understand the relationship between *E. anophelis* and mosquitos in response to oxidative stress, in the future we will infect mosquitos with *E. anophelis* NUHP1 and discover the transcriptional response of *E. anophelis* *in vivo*.

- Abrantes, M. C., de Fátima Lopes, M., & Kok, J. (2011). Impact of manganese, copper and zinc ions on the transcriptome of the nosocomial pathogen *Enterococcus faecalis* V583. *PLoS One*, 6(10), e26519.
- Adachi, A., Mori, T., Shimizu, T., Yokoyama, A., Takayama, N., Ikeda, Y., & Okamoto, S. (2004). *Chryseobacterium meningosepticum* septicemia in a recipient of allogeneic cord blood transplantation. *Scand J Infect Dis*, 36(6-7), 539-540.
- Aguirre, J. D., & Culotta, V. C. (2012). Battles with iron: manganese in oxidative stress protection. *J Biol Chem*, 287(17), 13541-13548. doi:10.1074/jbc.R111.312181
- Ahimou, F., Semmens, M. J., Haugstad, G., & Novak, P. J. (2007). Effect of protein, polysaccharide, and oxygen concentration profiles on biofilm cohesiveness. *Applied and environmental microbiology*, 73(9), 2905-2910.
- Alikhan, N. F., Petty, N. K., Ben Zakour, N. L., & Beatson, S. A. (2011). BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics*, 12, 402. doi:10.1186/1471-2164-12-402
- Alipour, M., Suntres, Z. E., & Omri, A. (2009). Importance of DNase and alginate lyase for enhancing free and liposome encapsulated aminoglycoside activity against *Pseudomonas aeruginosa*. *Journal of Antimicrobial Chemotherapy*, dkp165.
- Anderl, J. N., Franklin, M. J., & Stewart, P. S. (2000). Role of antibiotic penetration limitation in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. *Antimicrobial Agents and Chemotherapy*, 44(7), 1818-1824.
- Anders, S., & Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biol*, 11(10), R106. doi:10.1186/gb-2010-11-10-r106
gb-2010-11-10-r106 [pii]
- Andrews, S. C., Robinson, A. K., & Rodríguez-Quinones, F. (2003). Bacterial iron homeostasis. *FEMS microbiology reviews*, 27(2-3), 215-237.
- Anelet Jacobs, H. Y. C. (2011). Biofilm formation and adherence characteristics of an *Elizabethkingia meningoseptica* isolate from *Oreochromis mossambicus*. *Ann Clin Microbiol Antimicrob*, 10(16).
- Anjem, A., & Imlay, J. A. (2012). Mononuclear iron enzymes are primary targets of hydrogen peroxide stress. *Journal of Biological Chemistry*, 287(19), 15544-15556.
- Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., . . . Zagnitko, O. (2008a). The RAST server: Rapid annotations using subsystems technology. *BMC Genomics*, 9. doi:Artn 75
Doi 10.1186/1471-2164-9-75
- Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., . . . Zagnitko, O. (2008b). The RAST Server: rapid annotations using subsystems technology. *BMC Genomics*, 9, 75. doi:10.1186/1471-2164-9-75
- Balm, M. N., Salmon, S., Jureen, R., Teo, C., Mahdi, R., Seetoh, T., . . . Fisher, D. A. (2013). Bad design, bad practices, bad bugs: frustrations in controlling an outbreak of *Elizabethkingia meningoseptica* in intensive care units. *J Hosp Infect*, 85(2), 134-140. doi:10.1016/j.jhin.2013.05.012
- Beasley, F. C., Marolda, C. L., Cheung, J., Buac, S., & Heinrichs, D. E. (2011). *Staphylococcus aureus* transporters Hts, Sir, and Sst capture iron liberated from human transferrin by Staphyloferrin A, Staphyloferrin B, and catecholamine stress hormones, respectively, and contribute to virulence. *Infect Immun*, 79(6), 2345-2355. doi:10.1128/IAI.00117-11
- Bernardet, J.-F., P. Segers, M. Vancanneyt, F. Berthe, K. Kersters, P. Vandamme. (1996). Cutting a Gordian knot: Emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int. J. Syst. Bacteriol*, 46, 128-148.
- Bielski, B., Arudi, R. L., & Sutherland, M. W. (1983). A study of the reactivity of HO₂/O₂-with unsaturated fatty acids. *Journal of Biological Chemistry*, 258(8), 4759-4761.

- Billal, D. S., Feng, J., Leprohon, P., Légaré, D., & Ouellette, M. (2011). Whole genome analysis of linezolid resistance in *Streptococcus pneumoniae* reveals resistance and compensatory mutations. *BMC Genomics*, 12(1), 1.
- Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 13(1), 42-51.
- Bloch, K. C., Nadarajah, R., & Jacobs, R. (1997). *Chryseobacterium meningosepticum*: An emerging pathogen among immunocompromised adults - Report of 6 cases and literature review. *Medicine*, 76(1), 30-41. doi:10.1097/00005792-199701000-00003
- Bobossi-Serengbe G, G. J., Beyam NE, Bercion R. (2006). First documented case of *Chryseobacterium meningosepticum* meningitis in Central African Republic. *Med. Trop.*, 66, 182-184.
- Boissiere, A., Tchioffo, M. T., Bachar, D., Abate, L., Marie, A., Nsango, S. E., . . . Morlais, I. (2012). Midgut microbiota of the malaria mosquito vector *Anopheles gambiae* and interactions with *Plasmodium falciparum* infection. *PLoS Pathog*, 8(5), e1002742. doi:10.1371/journal.ppat.1002742
- Breurec, S., Criscuolo, A., Diancourt, L., Rendueles-Garcia, O., Vandenbergert, M., Passet, V., . . . Brisse, S. (2016). Genomic epidemiology and global diversity of the emerging bacterial pathogen *Elizabethkingia anophelis*. doi:10.1101/044792
- Cabiscol, E., Tamarit, J., & Ros, J. (2000). Oxidative stress in bacteria and protein damage by reactive oxygen species. *International microbiology: the official journal of the Spanish Society for Microbiology*, 3(1), 3-8.
- Cabiscol, E., Tamarit, J., & Ros, J. (2010). Oxidative stress in bacteria and protein damage by reactive oxygen species. *International Microbiology*, 3(1), 3-8.
- Candeias, L. P., & Steenken, S. (1993). Electron transfer in di (deoxy) nucleoside phosphates in aqueous solution: rapid migration of oxidative damage (via adenine) to guanine. *Journal of the American Chemical Society*, 115(6), 2437-2440.
- Cartwright, E. J., Prabhu, R. M., Zinderman, C. E., Schobert, W. E., Jensen, B., Noble-Wang, J., . . . Drug Administration Tissue Safety Team, I. (2010). Transmission of *Elizabethkingia meningoseptica* (formerly *Chryseobacterium meningosepticum*) to tissue-allograft recipients: a report of two cases. *J Bone Joint Surg Am*, 92(6), 1501-1506. doi:10.2106/JBJS.I.00502
- Chang Chien, H. Y., Chiu, N. C., Li, W. C., & Huang, F. Y. (2000). Characteristics of neonatal bacterial meningitis in a teaching hospital in Taiwan from 1984-1997. *J Microbiol Immunol Infect*, 33(2), 100-104.
- Chang, W., Small, D. A., Toghrol, F., & Bentley, W. E. (2006). Global transcriptome analysis of *Staphylococcus aureus* response to hydrogen peroxide. *J Bacteriol*, 188(4), 1648-1659.
- Chauvaux, S., Rosso, M.-L., Frangeul, L., Lacroix, C., Labarre, L., Schiavo, A., . . . Coppée, J.-Y. (2007). Transcriptome analysis of *Yersinia pestis* in human plasma: an approach for discovering bacterial genes involved in septicemic plague. *Microbiology*, 153(9), 3112-3124.
- Chen, L., Xiong, Z., Sun, L., Yang, J., & Jin, Q. (2012). VFDB 2012 update: toward the genetic diversity and molecular evolution of bacterial virulence factors. *Nucleic Acids Res*, 40(Database issue), D641-645. doi:10.1093/nar/gkr989
- Choi, H.-J., Kim, S.-J., Mukhopadhyay, P., Cho, S., Woo, J.-R., Storz, G., & Ryu, S.-E. (2001). Structural basis of the redox switch in the OxyR transcription factor. *Cell*, 105(1), 103-113.
- Chua, S. L., Liu, Y., Yam, J. K., Chen, Y., Vejborg, R. M., Tan, B. G., . . . Yang, L. (2014). Dispersed cells represent a distinct stage in the transition from bacterial biofilm to planktonic lifestyles. *Nat Commun*, 5, 4462. doi:10.1038/ncomms5462
- Corbin, B. D., Seeley, E. H., Raab, A., Feldmann, J., Miller, M. R., Torres, V. J., . . . Gerads, R. (2008). Metal chelation and inhibition of bacterial growth in tissue abscesses. *Science*, 319(5865), 962-965.
- Cornelis, P., Wei, Q., Andrews, S. C., & Vinckx, T. (2011). Iron homeostasis and management of oxidative stress response in bacteria. *Metallomics*, 3(6), 540-549.
- Costerton, J. W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., Lappin-Scott, H.M. (1995). Microbial biofilms. *Annual Reviews in Microbiology*, 49, 711-745.

- Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science*, 284(5418), 1318-1322.
- Crosa, J. H., & Walsh, C. T. (2002). Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. *Microbiology and Molecular Biology Reviews*, 66(2), 223-249.
- da Silva, P. S., & Pereira, G. H. (2013). Elizabethkingia meningoseptica: Emergent bacteria causing pneumonia in a critically ill child. *Pediatr Int*, 55(2), 231-234. doi:10.1111/j.1442-200X.2012.03650.x
- Dakin, H. (1906). The oxidation of amido-acids with the production of substances of biological importance. *Journal of Biological Chemistry*, 1(2), 171-176.
- Demple, B., Johnson, A., & Fung, D. (1986). Exonuclease III and endonuclease IV remove 3' blocks from DNA synthesis primers in H₂O₂-damaged Escherichia coli. *Proceedings of the National Academy of Sciences*, 83(20), 7731-7735.
- Dewanti, R., & Wong, A. C. (1995). Influence of culture conditions on biofilm formation by Escherichia coli O157: H7. *International journal of food microbiology*, 26(2), 147-164.
- Díaz, A., Loewen, P. C., Fita, I., & Carpena, X. (2012). Thirty years of heme catalases structural biology. *Archives of biochemistry and biophysics*, 525(2), 102-110.
- Dizdaroglu, M., Rao, G., Halliwell, B., & Gajewski, E. (1991). Damage to the DNA bases in mammalian chromatin by hydrogen peroxide in the presence of ferric and cupric ions. *Archives of biochemistry and biophysics*, 285(2), 317-324.
- Dooley, J. R., Nims, L. J., Lipp, V. H., Beard, A., & Delaney, L. T. (1980). Meningitis of infants caused by Flavobacterium meningosepticum: report of a patient and analysis of 63 infections. *J Trop Pediatr*, 26(1), 24-30.
- Driffield, K., Miller, K., Bostock, J., O'Neill, A., & Chopra, I. (2008). Increased mutability of Pseudomonas aeruginosa in biofilms. *Journal of Antimicrobial Chemotherapy*, 61(5), 1053-1056.
- du Moulin, G. C. (1979). Airway colonization by Flavobacterium in an intensive care unit. *J Clin Microbiol*, 10(2), 155-160.
- Echenique-Rivera, H., Muzzi, A., Del Tordello, E., Seib, K. L., Francois, P., Rappuoli, R., . . . Serruto, D. (2011). Transcriptome analysis of Neisseria meningitidis in human whole blood and mutagenesis studies identify virulence factors involved in blood survival. *PLoS Pathog*, 7(5), e1002027. doi:10.1371/journal.ppat.1002027
- Edward Hadas, M. D., Jadwiga Winiecka-Krusnell, Anna Sutek. (2004). Acanthamoeba spp. as vehicles of pathogenic bacteria. *Acta Parasitologica*, 49(4).
- Elbadawi, L. I. (2016). A Large and Primarily Community Associated Outbreak of Elizabethkingia anophelis Infections, Wisconsin, 2015-2016. Paper presented at the IDWeek 2016.
- Elgrably-Weiss, M., Park, S., Schlosser-Silverman, E., Rosenshine, I., Imlay, J., & Altuvia, S. (2002). A Salmonella enterica serovar typhimurium hemA mutant is highly susceptible to oxidative DNA damage. *J Bacteriol*, 184(14), 3774-3784.
- Ferguson, A. D., Hofmann, E., Coulton, J. W., Diederichs, K., & Welte, W. (1998). Siderophore-mediated iron transport: crystal structure of FhuA with bound lipopolysaccharide. *Science*, 282(5397), 2215-2220.
- Flemming, H.-C., Neu, T. R., & Wozniak, D. J. (2007). The EPS matrix: the "house of biofilm cells". *J Bacteriol*, 189(22), 7945-7947.
- Flemming, H.-C., & Wingender, J. (2001). Relevance of microbial extracellular polymeric substances (EPSs)-Part I: Structural and ecological aspects. *Water science and technology*, 43(6), 1-8.
- Flint, D., Smyk-Randall, E., Tuminello, J., Draczynska-Lusiak, B., & Brown, O. (1993). The inactivation of dihydroxy-acid dehydratase in Escherichia coli treated with hyperbaric oxygen occurs because of the destruction of its Fe-S cluster, but the enzyme remains in the cell in a form that can be reactivated. *Journal of Biological Chemistry*, 268(34), 25547-25552.
- Flint, D. H., Tuminello, J., & Emptage, M. (1993). The inactivation of Fe-S cluster containing hydro-lyases by superoxide. *Journal of Biological Chemistry*, 268(30), 22369-22376.
- Floyd, J. L., Smith, K. P., Kumar, S. H., Floyd, J. T., & Varela, M. F. (2010). LmrS is a multidrug efflux pump of the major facilitator superfamily from Staphylococcus aureus. *Antimicrobial Agents and Chemotherapy*, 54(12), 5406-5412.

- Fournier, P.-E., Drancourt, M., & Raoult, D. (2007). Bacterial genome sequencing and its use in infectious diseases. *The Lancet infectious diseases*, 7(11), 711-723.
- Frank, T., Gody, J. C., Nguyen, L. B., Berthet, N., Le Fleche-Mateos, A., Bata, P., . . . Breurec, S. (2013). First case of *Elizabethkingia anophelis* meningitis in the Central African Republic. *Lancet*, 381(9880), 1876. doi:10.1016/S0140-6736(13)60318-9
- Frank, T., Gody, J. C., Nguyen, L. B. L., Berthet, N., Le Fleche-Mateos, A., Bata, P., . . . Breurec, S. (2013). First case of *Elizabethkingia anophelis* meningitis in the Central African Republic. *The Lancet*, 381(9880), 1876.
- Fraser, S. L., & Jorgensen, J. H. (1997). Reappraisal of the antimicrobial susceptibilities of *Chryseobacterium* and *Flavobacterium* species and methods for reliable susceptibility testing. *Antimicrob Agents Chemother*, 41(12), 2738-2741.
- Frølund, B., Palmgren, R., Keiding, K., & Nielsen, P. H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water research*, 30(8), 1749-1758.
- Gaballa, A., & Helmann, J. D. (2002). A peroxide - induced zinc uptake system plays an important role in protection against oxidative stress in *Bacillus subtilis*. *Molecular Microbiology*, 45(4), 997-1005.
- Gaupp, R., Ledala, N., & Somerville, G. A. (2012). Staphylococcal response to oxidative stress. *Front Cell Infect Microbiol*, 2, 33. doi:10.3389/fcimb.2012.00033
- Gentleman, R. C., Carey, V. J., Bates, D. M., Bolstad, B., Dettling, M., Dudoit, S., . . . Zhang, J. (2004). Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*, 5(10), R80. doi:gb-2004-5-10-r80 [pii] 10.1186/gb-2004-5-10-r80
- González, L. J., & Vila, A. J. (2012). Carbapenem resistance in *Elizabethkingia meningoseptica* is mediated by metallo- β -lactamase BlaB. *Antimicrobial Agents and Chemotherapy*, 56(4), 1686-1692.
- Green, O., Murray, P., & Gea-Banacloche, J. C. (2008). Sepsis caused by *Elizabethkingia miricola* successfully treated with tigecycline and levofloxacin. *Diagn Microbiol Infect Dis*, 62(4), 430-432. doi:10.1016/j.diagmicrobio.2008.07.015
- Greenberg, J. T., Monach, P., Chou, J. H., Josephy, P. D., & Demple, B. (1990). Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 87(16), 6181-6185.
- Gu, M., & Imlay, J. A. (2013). Superoxide poisons mononuclear iron enzymes by causing mismetallation. *Molecular Microbiology*, 89(1), 123-134.
- Güngör, S., Özen, M., Akinci, A., & Durmaz, R. (2004). A *Chryseobacterium Meningosepticum* Outbreak in a Neonatal Ward. *Infection Control and Hospital Epidemiology*, 24(8), 613-617.
- Hakenbeck, R. (1995). Target-mediated resistance to β -lactam antibiotics. *Biochemical pharmacology*, 50, 1121-1127.
- Harrison, J., Turner, R., Marques, L., & Ceri, H. (2005). A new understanding of these microbial communities is driving a revolution that may transform the science of microbiology. *Am Sci*, 93(6), 508-515.
- Hassan, H. M., & Fridovich, I. (1979). Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. *Archives of biochemistry and biophysics*, 196(2), 385-395.
- Hassett, D., Charniga, L., Bean, K., Ohman, D., & Cohen, M. S. (1992). Response of *Pseudomonas aeruginosa* to pyocyanin: mechanisms of resistance, antioxidant defenses, and demonstration of a manganese-cofactored superoxide dismutase. *Infection and immunity*, 60(2), 328-336.
- Hedman, A. K., Li, M.-S., Langford, P. R., & Kroll, J. S. (2012). Transcriptional profiling of serogroup B *Neisseria meningitidis* growing in human blood: an approach to vaccine antigen discovery. *PLoS One*, 7(6), e39718.
- Herbig, A. F., & Helmann, J. D. (2001). Roles of metal ions and hydrogen peroxide in modulating the interaction of the *Bacillus subtilis* PerR peroxide regulon repressor with operator DNA. *Molecular Microbiology*, 41(4), 849-859.

- Hidalgo, L., Hopkins, K. L., Gutierrez, B., Ovejero, C. M., Shukla, S., Douthwaite, S., . . . Gonzalez-Zorn, B. (2013). Association of the novel aminoglycoside resistance determinant RmtF with NDM carbapenemase in Enterobacteriaceae isolated in India and the UK. *Journal of Antimicrobial Chemotherapy*, dkt078.
- Hogg, M., Wallace, S. S., & Doublié, S. (2005). Bumps in the road: how replicative DNA polymerases see DNA damage. *Current opinion in structural biology*, 15(1), 86-93.
- Hoque, S., Graham, J., Kaufmann, M., & Tabaqchali, S. (2001). Chryseobacterium (Flavobacterium) meningosepticum outbreak associated with colonization of water taps in a neonatal intensive care unit. *Journal of Hospital Infection*, 47(3), 188-192.
- Hoque, S. N., Graham, J., Kaufmann, M. E., & Tabaqchali, S. (2001). Chryseobacterium (Flavobacterium) meningosepticum outbreak associated with colonization of water taps in a neonatal intensive care unit. *J Hosp Infect*, 47(3), 188-192. doi:10.1053/jhin.2000.0908
- Hsu, M.-S., Liao, C.-H., Huang, Y.-T., Liu, C.-Y., Yang, C.-J., Kao, K.-L., & Hsueh, P.-R. (2011). Clinical features, antimicrobial susceptibilities, and outcomes of Elizabethkingia meningoseptica (Chryseobacterium meningosepticum) bacteremia at a medical center in Taiwan, 1999–2006. *European journal of clinical microbiology & infectious diseases*, 30(10), 1271-1278.
- Hsu, M. S., Liao, C. H., Huang, Y. T., Liu, C. Y., Yang, C. J., Kao, K. L., & Hsueh, P. R. (2011). Clinical features, antimicrobial susceptibilities, and outcomes of Elizabethkingia meningoseptica (Chryseobacterium meningosepticum) bacteremia at a medical center in Taiwan, 1999-2006. *Eur J Clin Microbiol Infect Dis*, 30(10), 1271-1278. doi:10.1007/s10096-011-1223-0
- Hutchinson, F. (1985). Chemical changes induced in DNA by ionizing radiation. *Progress in nucleic acid research and molecular biology*, 32, 115-154.
- Imlay, J., & Fridovich, I. (1992). Exogenous quinones directly inhibit the respiratory NADH dehydrogenase in Escherichia coli. *Archives of biochemistry and biophysics*, 296(1), 337-346.
- Imlay, J. A. (2013). The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nature Reviews Microbiology*, 11(7), 443-454.
- Jacobs, A., & Chenia, H. Y. (2011). Biofilm formation and adherence characteristics of an Elizabethkingia meningoseptica isolate from Oreochromis mossambicus. *Ann Clin Microbiol Antimicrob*, 10, 16. doi:10.1186/1476-0711-10-16
- Jacobs, A., & Chenia, H. Y. (2011). Biofilm formation and adherence characteristics of an Elizabethkingia meningoseptica isolate from Oreochromis mossambicus. *Ann Clin Microbiol Antimicrob*, 10(1), 1.
- Jefferson, K. K. (2004). What drives bacteria to produce a biofilm? *FEMS microbiology letters*, 236(2), 163-173.
- Johnson, C. (2016). *Elizabethkingia anophelis: Experience of an Academic Health System in Southeastern Wisconsin*. Paper presented at the IDWeek 2016.
- Kämpfer, P., Glaeser, S. P., Busse, H.-J., & McInroy, J. A. (2015). Elizabethkingia endophytica sp. nov., isolated from Zea mays and emended description of Elizabethkingia anophelis Kämpfer et al. 2011. *International Journal of Systematic and Evolutionary Microbiology*, 65(7), 2187-2193. doi:10.1099/ijs.0.000236
- Kämpfer, P., Matthews, H., Glaeser, S. P., Martin, K., Lodders, N., & Faye, I. (2011). Elizabethkingia anophelis sp. nov., isolated from the midgut of the mosquito Anopheles gambiae. *Int J Syst Evol Microbiol*, 61(Pt 11), 2670-2675. doi:10.1099/ijs.0.026393-0
- Kämpfer, P., Matthews, H., Glaeser, S. P., Martin, K., Lodders, N., & Faye, I. (2011). Elizabethkingia anophelis sp. nov., isolated from the midgut of the mosquito Anopheles gambiae. *International Journal of Systematic and Evolutionary Microbiology*, 61(11), 2670-2675.
- Kim, K. K., Kim, M. K., Lim, J. H., Park, H. Y., & Lee, S. T. (2005). Transfer of Chryseobacterium meningosepticum and Chryseobacterium miricola to Elizabethkingia gen. nov. as Elizabethkingia meningoseptica comb. nov. and Elizabethkingia miricola comb. nov. *Int J Syst Evol Microbiol*, 55(Pt 3), 1287-1293. doi:10.1099/ijs.0.63541-0
- King, E. O. (1959). Studies on a group of previously unclassified bacteria associated with meningitis in infants. *Am. J. Clin. Pathol.*, 31, 241-247.

- Kirby, J. T., Sader, H. S., Walsh, T. R., & Jones, R. N. (2004). Antimicrobial susceptibility and epidemiology of a worldwide collection of *Chryseobacterium* spp: report from the SENTRY Antimicrobial Surveillance Program (1997-2001). *J Clin Microbiol*, 42(1), 445-448.
- KLEBANOFF, S. J. (1980). Oxygen metabolism and the toxic properties of phagocytes. *Annals of Internal Medicine*, 93(3), 480-489.
- Kodama, Y., Nishimura, M., Nakashima, K., Ito, N., Fukano, R., Okamura, J., & Inagaki, J. (2013). [Central intravenous catheter-related bacteremia due to *Chryseobacterium indologenes* after cord blood transplantation]. [*Rinsho ketsueki*] *The Japanese journal of clinical hematology*, 54(3), 305-310.
- Kojima, S., & Nikaido, H. (2013). Permeation rates of penicillins indicate that *Escherichia coli* porins function principally as nonspecific channels. *Proceedings of the National Academy of Sciences*, 110(28), E2629-E2634.
- Kortman, G. A., Boleij, A., Swinkels, D. W., & Tjalsma, H. (2012). Iron availability increases the pathogenic potential of *Salmonella typhimurium* and other enteric pathogens at the intestinal epithelial interface. *PLoS One*, 7(1), e29968. doi:10.1371/journal.pone.0029968
- Kukutla, P., Lindberg, B. G., Pei, D., Rayl, M., Yu, W., Steritz, M., . . . Xu, J. (2013). Draft Genome Sequences of *Elizabethkingia anophelis* Strains R26T and Ag1 from the Midgut of the Malaria Mosquito *Anopheles gambiae*. *Genome Announc*, 1(6). doi:10.1128/genomeA.01030-13
- Kukutla, P., Lindberg, B. G., Pei, D., Rayl, M., Yu, W., Steritz, M., . . . Xu, J. (2014). Insights from the genome annotation of *Elizabethkingia anophelis* from the malaria vector *Anopheles gambiae*. *PLoS One*, 9(5), e97715. doi:10.1371/journal.pone.0097715
- Kuo, C., Mashino, T., & Fridovich, I. (1987). alpha, beta-Dihydroxyisovalerate dehydratase. A superoxide-sensitive enzyme. *Journal of Biological Chemistry*, 262(10), 4724-4727.
- Lamont, I. L., Beare, P. A., Ochsner, U., Vasil, A. I., & Vasil, M. L. (2002). Siderophore-mediated signaling regulates virulence factor production in *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences*, 99(10), 7072-7077.
- Langille, M. G., & Brinkman, F. S. (2009). IslandViewer: an integrated interface for computational identification and visualization of genomic islands. *Bioinformatics*, 25(5), 664-665. doi:10.1093/bioinformatics/btp030
- Lau, S. K., Chow, W. N., Foo, C. H., Curreem, S. O., Lo, G. C., Teng, J. L., . . . Woo, P. C. (2016). *Elizabethkingia anophelis* bacteremia is associated with clinically significant infections and high mortality. *Sci Rep*, 6, 26045. doi:10.1038/srep26045
- Lau, S. K., Wu, A. K., Teng, J. L., Tse, H., Curreem, S. O., Tsui, S. K., . . . Woo, P. C. (2015). Evidence for *Elizabethkingia anophelis* transmission from mother to infant, Hong Kong. *Emerg Infect Dis*, 21(2), 232-241. doi:10.3201/eid2102.140623
- Lauro, F. M., Elie-Fadrosh, E. A., Richter, T. K., Vitulo, N., Ferreira, S., Johnson, J. H., & Bartlett, D. H. (2014). Ecotype diversity and conversion in *Photobacterium profundum* strains. *PLoS One*, 9(5), e96953. doi:10.1371/journal.pone.0096953
- Lee, J.-W., & Helmann, J. D. (2006). The PerR transcription factor senses H₂O₂ by metal-catalysed histidine oxidation. *Nature*, 440(7082), 363-367.
- Li, Y., Kawamura, Y., Fujiwara, N., Naka, T., Liu, H., Huang, X., . . . Ezaki, T. (2003). *Chryseobacterium miricola* sp. nov., a novel species isolated from condensation water of space station Mir. *Syst Appl Microbiol*, 26(4), 523-528. doi:10.1078/072320203770865828
- Ligeza, A., Tikhonov, A. N., Hyde, J. S., & Subczynski, W. K. (1998). Oxygen permeability of thylakoid membranes: electron paramagnetic resonance spin labeling study. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1365(3), 453-463.
- Lin, P.-Y., Chen, H.-L., Huang, C.-T., Su, L.-H., & Chiu, C.-H. (2010). Biofilm production, use of intravascular indwelling catheters and inappropriate antimicrobial therapy as predictors of fatality in *Chryseobacterium meningosepticum* bacteraemia. *International journal of antimicrobial agents*, 36(5), 436-440.
- Lin, P. Y., Chen, H. L., Huang, C. T., Su, L. H., & Chiu, C. H. (2010a). Biofilm production, use of intravascular indwelling catheters and inappropriate antimicrobial therapy as predictors of fatality in *Chryseobacterium meningosepticum* bacteraemia. *Int J Antimicrob Agents*, 36(5), 436-440. doi:DOI 10.1016/j.ijantimicag.2010.06.033

- Lin, P. Y., Chen, H. L., Huang, C. T., Su, L. H., & Chiu, C. H. (2010b). Biofilm production, use of intravascular indwelling catheters and inappropriate antimicrobial therapy as predictors of fatality in *Chryseobacterium meningosepticum* bacteraemia. *Int J Antimicrob Agents*, 36(5), 436-440. doi:10.1016/j.ijantimicag.2010.06.033
- Lin, Y. T., Chiu, C. H., Chan, Y. J., Lin, M. L., Yu, K. W., Wang, F. D., & Liu, C. Y. (2009). Clinical and microbiological analysis of *Elizabethkingia meningoseptica* bacteremia in adult patients in Taiwan. *Scand J Infect Dis*, 41(9), 628-634. doi:10.1080/00365540903089476
- Lindh, J. M., Borg-Karlson, A. K., & Faye, I. (2008). Transstadial and horizontal transfer of bacteria within a colony of *Anopheles gambiae* (Diptera: Culicidae) and oviposition response to bacteria-containing water. *Acta Trop*, 107(3), 242-250. doi:10.1016/j.actatropica.2008.06.008
- Loman, N. J., Constantinidou, C., Chan, J. Z., Halachev, M., Sergeant, M., Penn, C. W., . . . Pallen, M. J. (2012). High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity. *Nature Reviews Microbiology*, 10(9), 599-606.
- Lucet, J. C., Decre, D., Fichelle, A., Joly-Guillou, M. L., Pernet, M., Deblangy, C., . . . Regnier, B. (1999). Control of a prolonged outbreak of extended-spectrum beta-lactamase-producing enterobacteriaceae in a university hospital. *Clin Infect Dis*, 29(6), 1411-1418. doi:10.1086/313511
- Malachowa, N., Whitney, A. R., Kobayashi, S. D., Sturdevant, D. E., Kennedy, A. D., Braughton, K. R., . . . Otto, M. (2011). Global changes in *Staphylococcus aureus* gene expression in human blood. *PLoS One*, 6(4), e18617.
- Malaka Z. Amer, M. B., Abdalla Bukhari, Dalal Nemenqani. (2011). Neonatal meningitis caused by *Elizabethkingia meningoseptica* in Saudi Arabia. *J Infect Dev Ctries*, 5(10), 745-747.
- Martinez, A., & Kolter, R. (1997). Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps. *J Bacteriol*, 179(16), 5188-5194.
- Massé, E., & Gottesman, S. (2002). A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 99(7), 4620-4625.
- McArthur, A. G., Waglechner, N., Nizam, F., Yan, A., Azad, M. A., Baylay, A. J., . . . Wright, G. D. (2013). The comprehensive antibiotic resistance database. *Antimicrob Agents Chemother*, 57(7), 3348-3357. doi:10.1128/AAC.00419-13
- Mereghetti, L., Sitkiewicz, I., Green, N. M., & Musser, J. M. (2008). Extensive adaptive changes occur in the transcriptome of *Streptococcus agalactiae* (group B streptococcus) in response to incubation with human blood. *PLoS One*, 3(9), e3143.
- Mireji, P. O., Keating, J., Hassanali, A., Mbogo, C. M., Muturi, M. N., Githure, J. I., & Beier, J. C. (2010). Biological cost of tolerance to heavy metals in the mosquito *Anopheles gambiae*. *Med Vet Entomol*, 24(2), 101-107. doi:10.1111/j.1365-2915.2010.00863.x
- Mireji, P. O., Keating, J., Hassanali, A., Mbogo, C. M., Nyambaka, H., Kahindi, S., & Beier, J. C. (2008). Heavy metals in mosquito larval habitats in urban Kisumu and Malindi, Kenya, and their impact. *Ecotoxicology and Environmental Safety*, 70(1), 147-153. doi:DOI 10.1016/j.ecoenv.2007.03.012
- Mohammad I. Issack, Y. N. (2011). An outbreak of *Elizabethkingia meningoseptica* neonatal meningitis in Mauritius. *J Infect Dev Ctries*, 5(12), 834-839.
- Molin, S., & Tolker-Nielsen, T. (2003). Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. *Current opinion in biotechnology*, 14(3), 255-261.
- Morag R. Graham, K. V., Stephen F. Porcella, William T. Barry, Brian B. Gowen, Claire R. Johnson, Fred A. Wright, and James M. Musser. (2005). Group A *Streptococcus* Transcriptome Dynamics during growth in human blood reveals bacterial adaptive and survival strategies. *American Journal of Pathology*, 166(2).
- Ngwa, C., Glöckner, V., Abdelmohsen, U. R., Scheuermayer, M., Fischer, R., Hentschel, U., & Pradel, G. (2013). 16S rRNA gene-based identification of *Elizabethkingia meningoseptica* (Flavobacteriales: Flavobacteriaceae) as a dominant midgut bacterium of the Asian malaria vector *Anopheles stephensi* (Diptera: Culicidae) with antimicrobial activities. *Journal of medical entomology*, 50(2), 404-414.

- Nichols, D. S., & McMeekin, T. A. (2002). Biomarker techniques to screen for bacteria that produce polyunsaturated fatty acids. *Journal of microbiological methods*, 48(2), 161-170.
- Nicholson, A. C., Whitney, A. M., Emery, B. D., Bell, M. E., Gartin, J. T., Humrighouse, B. W., . . . McQuiston, J. R. (2016). Complete Genome Sequences of Four Strains from the 2015-2016 Elizabethkingia anophelis Outbreak. *Genome Announc*, 4(3). doi:10.1128/genomeA.00563-16
- Norris, A. L., & Serpersu, E. H. (2013). Ligand promiscuity through the eyes of the aminoglycoside N3 acetyltransferase IIa. *Protein Science*, 22(7), 916-928.
- Nulens, E., Bussels, B., Bols, A., Gordts, B., & Van Landuyt, H. W. (2001). Recurrent bacteremia by Chryseobacterium indologenes in an oncology patient with a totally implanted intravascular device. *Clin Microbiol Infect*, 7(7), 391-393.
- O'Rourke, E. J., Chevalier, C., Pinto, A. V., Thiberge, J. M., Ielpi, L., Labigne, A., & Radicella, J. P. (2003). Pathogen DNA as target for host-generated oxidative stress: role for repair of bacterial DNA damage in Helicobacter pylori colonization. *Proceedings of the National Academy of Sciences*, 100(5), 2789-2794.
- O'Toole, G. A., & Kolter, R. (1998a). Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. *Molecular Microbiology*, 30(2), 295-304.
- O'Toole, G. A., & Kolter, R. (1998b). Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Molecular Microbiology*, 28(3), 449-461.
- Ochsner, U. A., Johnson, Z., & Vasil, M. L. (2000). Genetics and regulation of two distinct haem-uptake systems, phu and has, in Pseudomonas aeruginosa. *Microbiology*, 146(1), 185-198.
- Page, A. J., Cummins, C. A., Hunt, M., Wong, V. K., Reuter, S., Holden, M. T., . . . Parkhill, J. (2015). Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics*, 31(22), 3691-3693.
- Pereira, G. H., Garcia Dde, O., Abboud, C. S., Barbosa, V. L., & Silva, P. S. (2013). Nosocomial infections caused by Elizabethkingia meningoseptica: an emergent pathogen. *Braz J Infect Dis*, 17(5), 606-609. doi:10.1016/j.bjid.2013.02.011
- Pi, H., Jones, S. A., Mercer, L. E., Meador, J. P., Caughron, J. E., Jordan, L., . . . Klebba, P. E. (2012). Role of catecholate siderophores in gram-negative bacterial colonization of the mouse gut. *PLoS One*, 7(11), e50020. doi:10.1371/journal.pone.0050020
- Pishchany, G., & Skaar, E. P. (2012). Taste for Blood: Hemoglobin as a Nutrient Source for Pathogens. *PLoS Pathog*, 8(3). doi:ARTN e1002535
DOI 10.1371/journal.ppat
- Pomposiello, P. J., Bennik, M. H., & Demple, B. (2001). Genome-Wide Transcriptional Profiling of the Escherichia coli Responses to Superoxide Stress and Sodium Salicylate. *J Bacteriol*, 183(13), 3890-3902.
- Pratt, L. A., & Kolter, R. (1998). Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type I pili. *Molecular Microbiology*, 30(2), 285-293.
- Putnam, C. D., Arvai, A. S., Bourne, Y., & Tainer, J. A. (2000). Active and inhibited human catalase structures: ligand and NADPH binding and catalytic mechanism. *Journal of molecular biology*, 296(1), 295-309.
- Rajendran, P., Muthukrishnan, J., & Gunasekaran, P. (2003). Microbes in heavy metal remediation. *Indian J Exp Biol*, 41(9), 935-944.
- Randall, C. P., Mariner, K. R., Chopra, I., & O'Neill, A. J. (2013). The target of daptomycin is absent from Escherichia coli and other gram-negative pathogens. *Antimicrobial Agents and Chemotherapy*, 57(1), 637-639.
- Rani, A., Sharma, A., Rajagopal, R., Adak, T., & Bhatnagar, R. K. (2009). Bacterial diversity analysis of larvae and adult midgut microflora using culture-dependent and culture-independent methods in lab-reared and field-collected Anopheles stephensi-an Asian malarial vector. *BMC Microbiol*, 9, 96. doi:10.1186/1471-2180-9-96
- Rasmussen, T. B., Bjarnsholt, T., Skindersoe, M. E., Hentzer, M., Kristoffersen, P., Kote, M., . . . Givskov, M. (2005). Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *J Bacteriol*, 187(5), 1799-1814. doi:10.1128/JB.187.5.1799-1814.2005

- Rice, A. J., Park, A., & Pinkett, H. W. (2014). Diversity in ABC transporters: Type I, II and III importers. *Critical reviews in biochemistry and molecular biology*, 49(5), 426-437.
- Rickard, A. H., Gilbert, P., High, N. J., Kolenbrander, P. E., & Handley, P. S. (2003). Bacterial coaggregation: an integral process in the development of multi-species biofilms. *Trends in microbiology*, 11(2), 94-100.
- Rosso, M.-L., Chauvaux, S., Dessein, R., Laurans, C., Frangeul, L., Lacroix, C., . . . Coppée, J.-Y. (2008). Growth of *Yersinia pseudotuberculosis* in human plasma: impacts on virulence and metabolic gene expression. *BMC microbiology*, 8(1), 1.
- Salysers, A. A., & AmabileCuevas, C. F. (1997). Why are antibiotic resistance genes so resistant to elimination? *Antimicrobial Agents and Chemotherapy*, 41(11), 2321-2325.
- Schreckenberger PC, D. M., Weyant RS, Hollis DG. . (2007). *Acinetobacter*, *Achromobacter*, *Chryseobacterium*, *Moraxella*, and other nonfermentative gram-negative rods. *Manual of clinical microbiology*, 770-802.
- Schryvers, A. B., & Stojiljkovic, I. (1999). Iron acquisition systems in the pathogenic *Neisseria*. *Molecular Microbiology*, 32(6), 1117-1123.
- Seaver, L. C., & Imlay, J. A. (2001). Alkyl hydroperoxide reductase is the primary scavenger of endogenous hydrogen peroxide in *Escherichia coli*. *J Bacteriol*, 183(24), 7173-7181.
- Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics*, btu153.
- Shah, M. A., Mutreja, A., Thomson, N., Baker, S., Parkhill, J., Dougan, G., . . . Wren, B. W. (2014). Genomic epidemiology of *Vibrio cholerae* O1 associated with floods, Pakistan, 2010. *Emerg Infect Dis*, 20(1), 13-20.
- Sies, H. (1993). Damage to plasmid DNA by singlet oxygen and its protection. *Mutation Research/Genetic Toxicology*, 299(3-4), 183-191.
- Sies, H., & Menck, C. F. (1992). Singlet oxygen induced DNA damage. *Mutation Research/DNAging*, 275(3-6), 367-375.
- Simpson, W., Olczak, T., & Genco, C. A. (2000). Characterization and expression of HmuR, a TonB-dependent hemoglobin receptor of *Porphyromonas gingivalis*. *J Bacteriol*, 182(20), 5737-5748.
- Skaar, E. P., & Schneewind, O. (2004). Iron-regulated surface determinants (Isd) of *Staphylococcus aureus*: stealing iron from heme. *Microbes and Infection*, 6(4), 390-397.
- Smith, A. D., & Wilks, A. (2012). Extracellular heme uptake and the challenges of bacterial cell membranes. *Current topics in membranes*, 69, 359.
- Snyder, L., Loman, N. J., Faraj, L., Levi, K., Weinstock, G., Boswell, T., . . . Ala'Aldeen, D. (2013). Epidemiological investigation of *Pseudomonas aeruginosa* isolates from a six-year-long hospital outbreak using high-throughput whole genome sequencing. *Euro Surveill*, 18(42), 20611.
- Sobota, J. M., & Imlay, J. A. (2011). Iron enzyme ribulose-5-phosphate 3-epimerase in *Escherichia coli* is rapidly damaged by hydrogen peroxide but can be protected by manganese. *Proceedings of the National Academy of Sciences*, 108(13), 5402-5407.
- Spangler, S. K., Visalli, M. A., Jacobs, M. R., & Appelbaum, P. C. (1996). Susceptibilities of non-*Pseudomonas aeruginosa* gram-negative nonfermentative rods to ciprofloxacin, ofloxacin, levofloxacin, D-ofloxacin, sparfloxacin, ceftazidime, piperacillin, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, and imipenem. *Antimicrob Agents Chemother*, 40(3), 772-775.
- Spratt, B. G. (1994). Resistance to antibiotics mediated by target alterations. *Science*, 264(5157), 388-393.
- Stamatakis, A. (2006). RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22(21), 2688-2690.
- Stewart, P. S., & Costerton, J. W. (2001). Antibiotic resistance of bacteria in biofilms. *The Lancet*, 358(9276), 135-138.
- Sutherland, I. W. (2001). Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology*, 147(1), 3-9.
- Tardat, B., & Touati, D. (1991). Two global regulators repress the anaerobic expression of MnSOD in *Escherichia coli*: Fur (ferric uptake regulation) and Arc (aerobic respiration control). *Molecular Microbiology*, 5(2), 455-465.

- Teo, J., Tan, S. Y.-Y., Liu, Y., Tay, M., Ding, Y., Li, Y., . . . Yang, L. (2014). Comparative genomic analysis of malaria mosquito vector-associated novel pathogen *Elizabethkingia anophelis*. *Genome Biol Evol*, 6(5), 1158-1165.
- Teo, J., Tan, S. Y.-Y., Tay, M., Ding, Y., Kjelleberg, S., Givskov, M., . . . Yang, L. (2013). First case of *E. anophelis* outbreak in an intensive-care unit. *The Lancet*, 382(9895), 855-856. doi:10.1016/s0140-6736(13)61858-9
- Teo, J., Tan, S. Y., Liu, Y., Tay, M., Ding, Y., Li, Y., . . . Yang, L. (2014). Comparative genomic analysis of malaria mosquito vector-associated novel pathogen *Elizabethkingia anophelis*. *Genome Biol Evol*, 6(5), 1158-1165. doi:10.1093/gbe/evu094
- Thomas, E. L., Lehrer, R. I., & Rest, R. F. (1988). Human neutrophil antimicrobial activity. *Review of Infectious Diseases*, 10(Supplement 2), S450-S456.
- Torres, V. J., Stauff, D. L., Pishchany, G., Bezbradica, J. S., Gordy, L. E., Iturregui, J., . . . Skaar, E. P. (2007). A *Staphylococcus aureus* regulatory system that responds to host heme and modulates virulence. *Cell Host & Microbe*, 1(2), 109-119.
- Tsuchido, T., & Takano, M. (1988). Sensitization by heat treatment of *Escherichia coli* K-12 cells to hydrophobic antibacterial compounds. *Antimicrobial Agents and Chemotherapy*, 32(11), 1680-1683.
- Tzouveleakis, L., Markogiannakis, A., Psychogiou, M., Tassios, P., & Daikos, G. (2012). Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clinical Microbiology Reviews*, 25(4), 682-707.
- Vandamme, P., Bernardet, J. F., Segers, P., Kersters, K., & Holmes, B. (1994). New Perspectives in the Classification of the Flavobacteria - Description of *Chryseobacterium* Gen-Nov, *Bergeyella* Gen-Nov, and *Empedobacter* Nom Rev. *International Journal of Systematic Bacteriology*, 44(4), 827-831.
- Vandamme, P., J.-F. Bernardet, P. Segers, K. Kersters, B. Holmes. (1994). New perspectives in the classification of the flavobacteria: description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. *Int. J. Syst. Bacteriol*, 44, 827-831.
- Vargiu, A. V., & Nikaido, H. (2012). Multidrug binding properties of the AcrB efflux pump characterized by molecular dynamics simulations. *Proceedings of the National Academy of Sciences*, 109(50), 20637-20642.
- Vasil, M. L., & Ochsner, U. A. (1999). The response of *Pseudomonas aeruginosa* to iron: genetics, biochemistry and virulence. *Molecular Microbiology*, 34(3), 399-413.
- Vebø, H. C., Snipen, L., Nes, I. F., & Brede, D. A. (2009). The transcriptome of the nosocomial pathogen *Enterococcus faecalis* V583 reveals adaptive responses to growth in blood. *PLoS One*, 4(11), e7660.
- Vilain, S., Pretorius, J. M., Theron, J., & Brözel, V. S. (2009). DNA as an adhesin: *Bacillus cereus* requires extracellular DNA to form biofilms. *Applied and environmental microbiology*, 75(9), 2861-2868.
- Visca, P., Leoni, L., Wilson, M. J., & Lamont, I. L. (2002). Iron transport and regulation, cell signalling and genomics: lessons from *Escherichia coli* and *Pseudomonas*. *Molecular Microbiology*, 45(5), 1177-1190.
- Vivas, J., Padilla, D., Real, F., Bravo, J., Grasso, V., & Acosta, F. (2008). Influence of environmental conditions on biofilm formation by *Hafnia alvei* strains. *Veterinary Microbiology*, 129(1), 150-155.
- Wahlberg, D. Biological Health Hazard—*Elizabethkingia anophelis* (fatal): Wisconsin.
- Walker, T. M., Ip, C. L., Harrell, R. H., Evans, J. T., Kapatai, G., Dedicoat, M. J., . . . Crook, D. W. (2013). Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study. *The Lancet infectious diseases*, 13(2), 137-146.
- Wandersman, C., & Delepelaire, P. (2004). Bacterial iron sources: from siderophores to hemophores. *Annu. Rev. Microbiol.*, 58, 611-647.
- Wang, Y., Gilbreath III, T. M., Kukutla, P., Yan, G., & Xu, J. (2011). Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. *PLoS One*, 6(9), e24767.
- Wang, Y., Gilbreath, T. M., 3rd, Kukutla, P., Yan, G., & Xu, J. (2011). Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. *PLoS One*, 6(9), e24767. doi:10.1371/journal.pone.0024767

- Wiegand, I., Hilpert, K., & Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature protocols*, 3(2), 163-175.
- Wiener, J., Quinn, J. P., Bradford, P. A., Goering, R. V., Nathan, C., Bush, K., & Weinstein, R. A. (1999). Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. *JAMA*, 281(6), 517-523.
- Wilks, A., & O'Neill, M. J. (2014). Extracellular heme uptake and metabolism in bacterial pathogenesis. *Handbook of Porphyrin Science with Applications to Chemistry, Physics, Materials Science, Engineering, Biology and Medicine*, 26, 267-315.
- Wingender, J., Strathmann, M., Rode, A., Leis, A., & Flemming, H.-C. (2001). [25] Isolation and biochemical characterization of extracellular polymeric substances from *Pseudomonas aeruginosa*. *Methods in enzymology*, 336, 302-314.
- Witko-Sarsat, V., Rieu, P., Descamps-Latscha, B., Lesavre, P., & Halbwachs-Mecarelli, L. (2000). Neutrophils: molecules, functions and pathophysiological aspects. *Lab Invest*, 80(5), 617-653.
- Wood, L. F., Leech, A. J., & Ohman, D. E. (2006). Cell wall - inhibitory antibiotics activate the alginate biosynthesis operon in *Pseudomonas aeruginosa*: roles of σ^{22} (AlgT) and the AlgW and Prc proteases. *Molecular Microbiology*, 62(2), 412-426.
- Wyckoff, E. E., Mey, A. R., & Payne, S. M. (2007). Iron acquisition in *Vibrio cholerae*. *Biometals*, 20(3-4), 405-416. doi:10.1007/s10534-006-9073-4
- Xie, Z.-Y., Zhou, Y.-C., Wang, S.-F., Mei, B., Xu, X.-D., Wen, W.-Y., & Feng, Y.-Q. (2009). First isolation and identification of *Elizabethkingia meningoseptica* from cultured tiger frog, *Rana tigrina rugulosa*. *Veterinary Microbiology*, 138(1-2), 140-144. doi:10.1016/j.vetmic.2009.02.011
- Yang, L., Barken, K. B., Skindersoe, M. E., Christensen, A. B., Givskov, M., & Tolker-Nielsen, T. (2007). Effects of iron on DNA release and biofilm development by *Pseudomonas aeruginosa*. *Microbiology*, 153(5), 1318-1328.
- Zhang, X., & Bishop, P. L. (2003). Biodegradability of biofilm extracellular polymeric substances. *Chemosphere*, 50(1), 63-69.
- Zheng, M., Doan, B., Schneider, T. D., & Storz, G. (1999). OxyR and SoxRS regulation of fur. *J Bacteriol*, 181(15), 4639-4643.
- Zheng, M., Wang, X., Templeton, L. J., Smulski, D. R., LaRossa, R. A., & Storz, G. (2001). DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J Bacteriol*, 183(15), 4562-4570. doi:10.1128/JB.183.15.4562-4570.2001
- Zhu, L., Lin, J., Ma, J., Cronan, J. E., & Wang, H. (2010). Triclosan resistance of *Pseudomonas aeruginosa* PAO1 is due to FabV, a triclosan-resistant enoyl-acyl carrier protein reductase. *Antimicrobial Agents and Chemotherapy*, 54(2), 689-698.

Figure S1 Distribution of stress response genes revealed by the RAST Server

Figure S2 Mauve alignment of genomes of *E. anophelis* NUHP1 and *E. anophelis* assembly from mosquitoes

Table S1 List of GIs as predicted by IslandViewer program

Start	End	Size	GI Prediction Program	Locus Tag (BD94_)	Product
381265	395424	14159	IslandPath-DIMOB	356	SSU ribosomal protein S21p
			IslandPath-DIMOB	357	Integrase, site-specific recombinase
			IslandPath-DIMOB	358	Ribosome hibernation protein YhbH
			IslandPath-DIMOB	359	Translation elongation factor Tu
			IslandPath-DIMOB	360	hypothetical protein
			IslandPath-DIMOB	361	Transcription antitermination protein NusG
			IslandPath-DIMOB	362	hypothetical protein
			IslandPath-DIMOB	363	Outer membrane protein W precursor
			IslandPath-DIMOB	364	Transcriptional regulator, HxlR family
			IslandPath-DIMOB	365	Transmembrane efflux protein
			IslandPath-DIMOB	366	LSU ribosomal protein L11p (L12e)
			IslandPath-DIMOB	367	LSU ribosomal protein L1p (L10Ae)
			IslandPath-DIMOB	368	LSU ribosomal protein L10p (P0)
			IslandPath-DIMOB	369	LSU ribosomal protein L7/L12 (P1/P2)

			IslandPath-DIMOB	370	Tetraacyldisaccharide 4'-kinase
			IslandPath-DIMOB	371	Protein YicC
			IslandPath-DIMOB	372	Trans-aconitate 2-methyltransferase
			IslandPath-DIMOB	373	Guanylate kinase
466849	530231	63382	IslandPath-DIMOB	450	putative dolichol-P-glucose synthetase
			IslandPath-DIMOB	451	FKBP-type peptidyl-prolyl cis-trans isomerase SlyD
			IslandPath-DIMOB	452	hypothetical protein
			IslandPath-DIMOB	453	Integrase
			IslandPath-DIMOB	454	Single-stranded DNA-binding protein
			IslandPath-DIMOB	455	Mycobacteriophage Barnyard protein gp56
			IslandPath-DIMOB	456	hypothetical protein
			multiple methods	457	hypothetical protein
			multiple methods	458	hypothetical protein
			multiple methods	459	hypothetical protein
			multiple methods	460	hypothetical protein
			multiple methods	461	hypothetical protein

multiple methods	462	ThiF family protein, ubiquitin-activating enzyme
multiple methods	463	hypothetical protein
multiple methods	464	hypothetical protein
multiple methods	465	hypothetical protein
multiple methods	466	Lactoylglutathione lyase
multiple methods	467	hypothetical protein
multiple methods	468	Conjugative transposon protein TraQ
multiple methods	469	Conjugative transposon protein TraO
multiple methods	470	Conjugative transposon protein TraN
multiple methods	471	Conjugative transposon protein TraM
multiple methods	472	hypothetical protein
multiple methods	473	hypothetical protein
multiple methods	474	Conjugative transposon protein TraK
multiple methods	475	Conjugative transposon protein TraJ
multiple methods	476	Conjugative transposon protein TraI
multiple methods	477	Conjugative transposon protein TraG

multiple methods	478	Conjugative transposon protein TraE
multiple methods	479	helix-turn-helix domain protein
IslandPath-DIMOB	480	hypothetical protein
IslandPath-DIMOB	481	Nucleotidyltransferase
IslandPath-DIMOB	482	Conjugative transposon protein TraD
IslandPath-DIMOB	483	Conjugative transposon protein TraB
IslandPath-DIMOB	484	Conjugative transposon protein TraB
IslandPath-DIMOB	485	Conjugative transposon protein TraA
IslandPath-DIMOB	486	hypothetical protein clusted with conjugative transposons, BF0131
IslandPath-DIMOB	487	Putative conjugative transposon mobilization protein
IslandPath-DIMOB	488	Putative mobilization protein BF0133
IslandPath-DIMOB	489	hypothetical protein
IslandPath-DIMOB	490	hypothetical protein
IslandPath-DIMOB	491	hypothetical protein
IslandPath-DIMOB	492	Cobalt-zinc-cadmium resistance protein CzcA; Cation efflux system protein CusA

IslandPath-DIMOB	493	Probable Co/Zn/Cd efflux system membrane fusion protein
IslandPath-DIMOB	494	Lead, cadmium, zinc and mercury transporting ATPase; Copper-translocating P-type ATPase
IslandPath-DIMOB	495	hypothetical protein
IslandPath-DIMOB	496	TonB-dependent receptor
IslandPath-DIMOB	497	hypothetical protein
IslandPath-DIMOB	498	hypothetical protein
IslandPath-DIMOB	499	nickel resistance protein
IslandPath-DIMOB	500	Tetracycline resistance element mobilization regulatory protein rteC
multiple methods	501	putative DNA methylase
multiple methods	502	hypothetical protein
multiple methods	503	hypothetical protein
IslandPath-DIMOB	504	hypothetical protein
IslandPath-DIMOB	505	hypothetical protein
multiple methods	506	hypothetical protein
multiple methods	507	DNA topoisomerase III

			multiple methods	508	hypothetical protein
			multiple methods	509	hypothetical protein
			multiple methods	510	hypothetical protein
672477	681819	9342	SIGI-HMM	660	hypothetical protein
			SIGI-HMM	661	hypothetical protein
			SIGI-HMM	662	hypothetical protein
			SIGI-HMM	663	hypothetical protein
			SIGI-HMM	664	hypothetical protein
			SIGI-HMM	665	ABC transporter ATP-binding protein
			SIGI-HMM	666	putative two-component system sensor protein, no kinase domain
871560	877078	5518	SIGI-HMM	846	putative transcriptional regulator
			SIGI-HMM	847	hypothetical protein
			SIGI-HMM	848	hypothetical protein
			SIGI-HMM	849	hypothetical protein
			SIGI-HMM	850	hypothetical protein
			SIGI-HMM	851	hypothetical protein

896916	901242	4326	IslandPath-DIMOB	863	hypothetical protein
			IslandPath-DIMOB	864	Integron integrase
			IslandPath-DIMOB	865	Mobile element protein
			IslandPath-DIMOB	866	hypothetical protein
			IslandPath-DIMOB	867	Peptide methionine sulfoxide reductase MsrA / Peptide methionine sulfoxide reductase MsrB
			IslandPath-DIMOB	868	membrane protein
1153368	1158323	4955	SIGI-HMM	1100	hypothetical protein
			SIGI-HMM	1101	hypothetical protein
			SIGI-HMM	1102	hypothetical protein
			SIGI-HMM	1103	hypothetical protein
			SIGI-HMM	1104	hypothetical protein
1601758	1623141	21383	IslandPath-DIMOB	1517	Probable Co/Zn/Cd efflux system membrane fusion protein
			IslandPath-DIMOB	1518	hypothetical protein
			IslandPath-DIMOB	1519	hypothetical protein
			IslandPath-DIMOB	1520	hypothetical protein
			IslandPath-DIMOB	1521	hypothetical protein

IslandPath-DIMOB	1522	Probable Co/Zn/Cd efflux system membrane fusion protein
IslandPath-DIMOB	1523	MlpB
IslandPath-DIMOB	1524	Probable Co/Zn/Cd efflux system membrane fusion protein
IslandPath-DIMOB	1525	hypothetical protein
IslandPath-DIMOB	1526	hypothetical protein
IslandPath-DIMOB	1527	Membrane protein involved in the export of O-antigen and teichoic acid
IslandPath-DIMOB	1528	Lead, cadmium, zinc and mercury transporting ATPase; Copper-translocating P-type ATPase
IslandPath-DIMOB	1529	Multicopper oxidase
IslandPath-DIMOB	1530	hypothetical protein
multiple methods	1531	hypothetical protein
multiple methods	1532	Zinc uptake regulation protein ZUR
multiple methods	1533	hypothetical protein
multiple methods	1534	FIG01093033: hypothetical protein
multiple methods	1535	probable copper-transporting ATPase
multiple methods	1536	Arsenical resistance operon repressor

			multiple methods	1537	hypothetical protein
			multiple methods	1538	putative DNA methylase
			IslandPath-DIMOB	1539	hypothetical protein
			IslandPath-DIMOB	1540	Putative cytoplasmic protein
			IslandPath-DIMOB	1541	hypothetical protein
			IslandPath-DIMOB	1542	hypothetical protein
			IslandPath-DIMOB	1543	hypothetical protein
			IslandPath-DIMOB	1544	hypothetical protein
			IslandPath-DIMOB	1545	hypothetical protein
			IslandPath-DIMOB	1546	Conjugative transposon protein TraA
1668515	1672766	4251	SIGI-HMM	1586	hypothetical protein
			SIGI-HMM	1587	hypothetical protein
			SIGI-HMM	1588	hypothetical protein
2437079	2452848	15769	IslandPath-DIMOB	2311	hypothetical protein
			IslandPath-DIMOB	2312	hypothetical protein
			IslandPath-DIMOB	2313	hypothetical protein

			IslandPath-DIMOB	2314	putative transposase
			IslandPath-DIMOB	2315	tyrosine type site-specific recombinase
			IslandPath-DIMOB	2316	Mobilizable transposon, tnpC protein
			IslandPath-DIMOB	2317	excisionase
			IslandPath-DIMOB	2318	hypothetical protein
			IslandPath-DIMOB	2319	DNA primase
			IslandPath-DIMOB	2320	hypothetical protein
			IslandPath-DIMOB	2321	Mobilization protein BmpH
			IslandPath-DIMOB	2322	hypothetical protein
			IslandPath-DIMOB	2323	RNA polymerase ECF-type sigma factor
			IslandPath-DIMOB	2324	hypothetical protein
			IslandPath-DIMOB	2325	hypothetical protein
3230189	3238189	8000	SIGI-HMM	3032	hypothetical protein
			SIGI-HMM	3033	hypothetical protein
			SIGI-HMM	3034	hypothetical protein
			SIGI-HMM	3035	hypothetical protein

			SIGI-HMM	3036	hypothetical protein
			SIGI-HMM	3037	hypothetical protein
			SIGI-HMM	3038	hypothetical protein
			SIGI-HMM	3039	hypothetical protein
			SIGI-HMM	3040	hypothetical protein
			SIGI-HMM	3041	hypothetical protein
			SIGI-HMM	3042	hypothetical protein
			SIGI-HMM	3043	hypothetical protein
3491319	3502927	11608	SIGI-HMM	3283	hypothetical protein
			SIGI-HMM	3284	hypothetical protein
			SIGI-HMM	3285	Glycosyltransferase
			SIGI-HMM	3286	hypothetical protein
			SIGI-HMM	3287	hypothetical protein
			SIGI-HMM	3288	Proposed peptidoglycan lipid II flippase MurJ
			SIGI-HMM	3289	hypothetical protein
			SIGI-HMM	3290	hypothetical protein

			SIGI-HMM	3291	GDP-L-fucose synthetase
			SIGI-HMM	3292	GDP-mannose 4,6 dehydratase
			SIGI-HMM	3293	Mannose-1-phosphate guanylyltransferase (GDP)
3705960	3712970	7010	SIGI-HMM	3483	hypothetical protein
			SIGI-HMM	3484	hypothetical protein
			SIGI-HMM	3485	hypothetical protein
			SIGI-HMM	3486	hypothetical protein
			SIGI-HMM	3487	hypothetical protein
			SIGI-HMM	3488	hypothetical protein
			SIGI-HMM	3489	hypothetical protein
			SIGI-HMM	3490	hypothetical protein
			SIGI-HMM	3491	hypothetical protein
			SIGI-HMM	3492	hypothetical protein
			SIGI-HMM	3493	hypothetical protein
			SIGI-HMM	3494	hypothetical protein
			SIGI-HMM	3495	hypothetical protein

3944192	4002655	58463	IslandPath-DIMOB	3697	hypothetical protein
			IslandPath-DIMOB	3698	N-acetylmuramoyl-L-alanine amidase
			IslandPath-DIMOB	3699	hypothetical protein
			multiple methods	3700	hypothetical protein
			multiple methods	3701	hypothetical protein
			multiple methods	3702	hypothetical protein
			multiple methods	3703	Peptidase, M23/M37 family
			multiple methods	3704	Putative mobilization protein
			multiple methods	3705	hypothetical protein
			multiple methods	3706	Conjugative transposon protein TraN
			multiple methods	3707	Conjugative transposon protein TraM
			multiple methods	3708	hypothetical protein
			multiple methods	3709	Conjugative transposon protein TraK
			multiple methods	3710	Conjugative transposon protein TraJ
			multiple methods	3711	hypothetical protein
			multiple methods	3712	hypothetical protein

multiple methods	3713	hypothetical protein
multiple methods	3714	hypothetical protein
multiple methods	3715	Conjugative transposon protein TraG
multiple methods	3716	hypothetical protein
multiple methods	3717	Conjugative transposon protein TraE
multiple methods	3718	hypothetical protein
multiple methods	3719	Conjugative transposon protein TraA
multiple methods	3720	Putative conjugative transposon mobilization protein
multiple methods	3721	hypothetical protein
multiple methods	3722	hypothetical protein
multiple methods	3723	putative hemolysin secretion protein
multiple methods	3724	putative hemolysin secretion protein
multiple methods	3725	Transcriptional regulator, MarR family
multiple methods	3726	Integron integrase
multiple methods	3727	Ethidium bromide-methyl viologen resistance protein EmrE
IslandPath-DIMOB	3728	Mobile element protein

IslandPath-DIMOB	3729	Integron integrase
IslandPath-DIMOB	3730	Aminoglycoside 6'-N-acetyltransferase
IslandPath-DIMOB	3731	hypothetical protein
IslandPath-DIMOB	3732	Mobile element protein
IslandPath-DIMOB	3733	Integron integrase
IslandPath-DIMOB	3734	aminoglycoside 6-adenylyltransferase
IslandPath-DIMOB	3735	Mobile element protein
IslandPath-DIMOB	3736	Mobile element protein
IslandPath-DIMOB	3737	Mobile element protein
IslandPath-DIMOB	3738	Mobile element protein
IslandPath-DIMOB	3739	hypothetical protein
IslandPath-DIMOB	3740	Mobile element protein
IslandPath-DIMOB	3741	Mobile element protein
IslandPath-DIMOB	3742	DNA primase
IslandPath-DIMOB	3743	hypothetical protein
IslandPath-DIMOB	3744	hypothetical protein

			IslandPath-DIMOB	3745	hypothetical protein
			IslandPath-DIMOB	3746	hypothetical protein
			IslandPath-DIMOB	3747	hypothetical protein
			IslandPath-DIMOB	3748	hypothetical protein
			IslandPath-DIMOB	3749	hypothetical protein
			IslandPath-DIMOB	3750	Integrase
4323123	4327929	4806	SIGI-HMM	4031	hypothetical protein
			SIGI-HMM	4032	hypothetical protein
			SIGI-HMM	4033	Restriction modification enzyme

Table S2 Stress response genes

category	subcategory	subsystem	role	<i>E. anophelis</i> gene number
Stress Response	Osmotic stress	Osmoregulation	Outer membrane protein A precursor	409,410
Stress Response	Osmotic stress	Osmoregulation	Aquaporin Z	720
Stress Response	Osmotic stress	Osmoregulation	Glycerol uptake facilitator protein	2505
Stress Response	Oxidative stress	Protection from Reactive Oxygen Species	Peroxidase	1895
Stress Response	Oxidative stress	Protection from Reactive Oxygen Species	Manganese superoxide dismutase	1758,2310,3828
Stress Response	Oxidative stress	Protection from Reactive Oxygen Species	Cytochrome c551 peroxidase	937,1875
Stress Response	Oxidative stress	Protection from Reactive Oxygen Species	Catalase	1895,3831
Stress Response	Oxidative stress	Oxidative stress	Iron-binding ferritin-like antioxidant protein	3681
Stress Response	Oxidative stress	Oxidative stress	Organic hydroperoxide resistance transcriptional regulator	1922
Stress Response	Oxidative stress	Oxidative stress	Alkyl hydroperoxide reductase subunit C-like protein	2275
Stress Response	Oxidative stress	Oxidative stress	Manganese superoxide dismutase	1758,2310,3828
Stress Response	Oxidative stress	Oxidative stress	Zinc uptake regulation protein ZUR	1151,1532,1765,2733
Stress Response	Oxidative stress	Oxidative stress	Non-specific DNA-binding protein Dps	3681
Stress Response	Oxidative stress	Oxidative stress	Ferroxidase	3681
Stress Response	Oxidative stress	Oxidative stress	Ferric uptake regulation protein FUR	440
Stress Response	Oxidative stress	Oxidative stress	Hydrogen peroxide-inducible genes activator	3830
Stress Response	Oxidative stress	Oxidative stress	Peroxidase	1895

Stress Response	Oxidative stress	Oxidative stress	transcriptional regulator, Crp/Fnr family	946,1914,2801,3871
Stress Response	Oxidative stress	Oxidative stress	Catalase	1895,3831
Stress Response	Oxidative stress	Oxidative stress	Organic hydroperoxide resistance protein	1921
Stress Response	Oxidative stress	Glutathione: Non-redox reactions	Lactoylglutathione lyase	466
Stress Response	Oxidative stress	Glutathione: Non-redox reactions	Similar to Hydroxyacylglutathione hydrolase, but in an organism lacking glutathione biosynthesis	390
Stress Response	Oxidative stress	Redox-dependent regulation of nucleus processes	Nicotinate phosphoribosyltransferase	3682
Stress Response	Oxidative stress	Redox-dependent regulation of nucleus processes	Nicotinamidase	2031
Stress Response	Oxidative stress	Redox-dependent regulation of nucleus processes	NAD-dependent protein deacetylase of SIR2 family	2444
Stress Response	Oxidative stress	Redox-dependent regulation of nucleus processes	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	725
Stress Response	Oxidative stress	Glutathione: Redox cycle	Glutathione peroxidase	2621
Stress Response	Cold shock	Cold shock, CspA family of proteins	Cold shock protein CspG	1831
Stress Response	Cold shock	Cold shock, CspA family of proteins	Cold shock protein CspA	2887
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Hypothetical radical SAM family enzyme, NOT coproporphyrinogen III oxidase, oxygen-independent	3384
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Chaperone protein DnaK	771
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Chaperone protein DnaJ	2744

Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Ribosomal RNA small subunit methyltransferase E	3154
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	tmRNA-binding protein SmpB	3619
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Heat shock protein GrpE	2743
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Translation elongation factor LepA	4054
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Ribosome-associated heat shock protein implicated in the recycling of the 50S subunit (S4 paralog)	757
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Nucleoside 5-triphosphatase RdgB (dHAPTP, dITP, XTP-specific)	2207
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Ribosomal protein L11 methyltransferase	1182
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	Signal peptidase-like protein	3951
Stress Response	Heat shock	Heat shock dnaK gene cluster extended	rRNA small subunit methyltransferase I	2
Stress Response	Detoxification	D-tyrosyl-tRNA(Tyr) deacylase	D-tyrosyl-tRNA(Tyr) deacylase	3427
Stress Response	Detoxification	Uptake of selenate and selenite	Sulfate and thiosulfate import ATP-binding protein CysA	3458
Stress Response	Detoxification	Uptake of selenate and selenite	DedA protein	1165
Stress Response	Stress Response - no subcategory	Dimethylarginine metabolism	Ornithine aminotransferase	267
Stress Response	Stress Response - no subcategory	Dimethylarginine metabolism	NG,NG-dimethylarginine dimethylaminohydrolase 1	3629

Stress Response	Stress Response - no subcategory	Hfl operon	GTP-binding protein HflX	117
Stress Response	Periplasmic Stress	Periplasmic Stress Response	Survival protein SurA precursor (Peptidyl-prolyl cis-trans isomerase SurA)	1286
Stress Response	Periplasmic Stress	Periplasmic Stress Response	Outer membrane protein H precursor	199
Stress Response	Periplasmic Stress	Periplasmic Stress Response	HtrA protease/chaperone protein	3692

Table S3 Primers for RT-PCR

gene name	sequence	product
rpoD_1_F	TCAGGCATTGGCAGAACAGT	127bp of rpoD gene
rpoD_1_R	TTCTTCTGGTGAAGGCGGTC	127bp of rpoD gene
TonB_F	TGGATTGGGGCAGATTGCTT	114bp of TonB gene
TonB_R	TCTGTTGCCAGCTTTGGACT	114bp of TonB gene
rhb_F	GAAACTCTGAACAGCCCCGA	138bp of rhb gene
rhb_R	TCCGGCTACCATCGCTTTAC	138bp of rhb gene
CzcACusA_F	GCTCGATCCGCTAACCTCTC	128bp of CzcACusA gene
CzcACusA_R	CGGAAACCTGTTTGATCCGC	128bp of CzcACusA gene
CzcC_F	ACTGTCCAAATCCGACAGCA	123bp of CzcC gene
CzcC_R	TGTTCAGCACTCTCTGCCTG	123bp of CzcC gene
16srRNA_F	CGTGTGCAACCTGCCTTTAT	149bp of 16srDNA
16srRNA_R	GAGCCGTTACCTCACCAACT	149bp of 16srDNA
DesD_F	TGGCTATTCTCTGGCTGCTG	145bp of DesD gene
DesD_R	GTTCTCTCCGTGTGGCATGT	145bp of DesD gene
Htp_F	GCATGCTGGCAGTATCTGGA	90bp of Htp gene
Htp_R	TAGCTCCGGACGTTAGTCCT	90bp of Htp gene
Sbm_F	GCACCGGAACAACCTCCGTAT	148bp of Sbm gene
Sbm_R	ATTTCGGCAGCACTCTGACC	148bp of Sbm gene
MetAP_F	CAGGTGAGTGAAGCTGTTGC	138bp of MetAP gene
MetAP_R	AGTAAGGAGTGGTGCCGATT	138bp of MetAP gene
TBDP_F	GCTGGATGCCACCTGTAGAA	91bp of TBDP gene
TBDP_R	ACTCCACCCATTGCATCAGAG	91bp of TBDP gene
HutB_F	AAGAGCGGGTTAATGGGAGC	117bp of HutB gene
HutB_R	TGATGCCAAACCACCATCCA	117bp of HutB gene
Hts_F	CTTTCCCGGGTAATGGCACA	145bp of Hts gene
Hts_R	TAGCGGAATTGGCTCGTTTG	145bp of Hts gene
MFP_F	CATACGTTTCGTCAGCACCG	118bp of MFP gene
MFP_R	ACTCCGGGTTACCTCATCCA	118bp of MFP gene
CCP_F	CGGTATCGACGACAGACTGG	90bp of CCP gene
CCP_R	TGGCTTACACCATCCCATGT	90bp of CCP gene
CcoP_F	ATTATGCCCATCAGGACGCA	146bp of CcoP gene
CcoP_R	GAACAACCTGCTCCCCTTCGG	146bp of CcoP gene

CcoO_F	TCATGGGTGCACTTCTGGTC	107bp of CcoO gene
CcoO_R	ATAGGCCACTCATGTTCCGC	107bp of CcoO gene
Mta_F	CACCGGAGCAGATATTGCCA	103bp of Mta gene
Mta_R	TCCAAACGTCCTTCTGCCAT	103bp of Mta gene
Tap_F	TCGGACTCATTGCAGGAGC	146bp of Tap gene
Tap_R	GCCGGTAACATCTACGCCA	146bp of Tap gene
HisG_F	AGCACCTACCGTACTCCCAT	108bp of HisG gene
HisG_R	GCGCCAAGGTCTTTCAACTG	108bp of HisG gene

Table S4 Genes up regulated at least 2-fold in H₂O₂ treated NUHP1 cells.^a

Locus Tag (BD_94)	Fold Change	adjusted <i>p</i>-value	baseMeans _CK^b	baseMeans _H₂O₂	Gene product description
0003	2.0	1.2E-07	211.0	428.3	Alpha-L-fucosidase (EC 3.2.1.51)
0013	9.2	1.6E-26	12.6	115.7	hypothetical protein
0014	11.9	9.6E-78	97.4	1159.5	Ferrous iron transport protein B
0015	6.5	1.8E-05	3.3	21.4	hypothetical protein
0030	2.9	0.0012	9.3	26.8	RNA polymerase ECF-type sigma factor
0031	2.1	0.0041	33.3	70.5	hypothetical protein
0066	14.8	2.4E-72	38.5	569.9	hypothetical protein
0067	65.6	1.6E-58	11.1	729.3	Ferrichrome-iron receptor
0068	30.7	9.2E-56	7.0	215.2	putative iron-regulated membrane protein
0069	25.1	6.1E-81	17.8	445.7	Ferric aerobactin receptor
0070	44.8	1.5E-67	5.2	232.6	putative iron-regulated membrane protein
0071	291.1	3.2E-77	8.2	2375.0	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
0072	248.3	2.7E-144	9.6	2389.0	hypothetical protein
0106	2.1	6.8E-07	114.2	236.8	Phage shock protein E precursor
0135	2.3	9.3E-07	57.4	132.7	PaaD-like protein (DUF59) involved in Fe-S cluster assembly
0179	3.9	6.8E-07	489.6	1899.6	FIG00648854: hypothetical protein
0263	2.6	0.0001	19.6	50.3	probable glycosyl transferase
0264	3.1	1.2E-05	15.2	46.4	Glycosyl transferase, group 1
0265	2.2	0.0008	25.9	56.0	Eps11J
0279	2.1	0.0175	15.2	31.3	FIG01092102: hypothetical protein
0297	2.5	1.2E-11	142.8	359.2	LMBE-RELATED PROTEIN
0332	3.8	8.3E-09	16.3	61.6	hypothetical protein
0344	2.4	5.0E-10	126.2	302.1	hypothetical protein
0345	3.2	5.2E-15	77.9	247.6	hypothetical protein
0346	11.6	4.9E-39	93.1	1076.4	hypothetical protein
0352	2.1	3.0E-07	135.2	281.2	Excinuclease ABC subunit C
0355	2.2	2.1E-09	170.2	382.2	ATP-dependent DNA helicase UvrD/PcrA
0392	2.2	2.1E-09	197.5	438.5	Ferrochelataase, protoheme ferrolyase (EC 4.99.1.1)
0393	2.1	3.2E-08	228.6	475.2	NifU-like domain protein

0395	2.2	3.1E-09	174.2	388.0	DNA repair and recombination protein, putative helicase
0396	2.1	1.2E-06	103.4	215.2	Luciferase-like monooxygenase (EC 1.14.-.-)
0397	2.2	8.4E-09	138.6	310.8	Apolipoprotein N-acyltransferase (EC 2.3.1.-)
0450	2.4	6.4E-08	73.0	172.1	putative dolichol-P-glucose synthetase
0671	2.0	6.0E-05	61.5	124.0	Modification methylase HphIB (EC 2.1.1.72)
0687	14.0	1.7E-15	18.5	259.4	Ferrichrome-iron receptor
0702	2.3	1.8E-08	102.3	235.2	UDP-N-acetylenolpyruvoylglucosamine reductase (EC 1.1.1.158)
0733	2.3	1.8E-07	76.1	173.3	hypothetical protein
0734	2.4	6.8E-05	27.0	64.4	Outer membrane hemin receptor
0763	2.1	8.8E-09	458.4	940.0	aminopeptidase
0766	2.7	1.5E-15	238.2	654.5	Septum site-determining protein MinD
0768	50.2	1.3E-166	146.7	7359.6	Ferrichrome-iron receptor
0785	2.2	9.1E-07	69.8	154.7	Phosphohistidine phosphatase SixA
0826	3.6	4.7E-13	62.9	227.5	Hemolysins and related proteins containing CBS domains
0827	3.9	8.5E-09	15.2	59.2	hypothetical protein
0837	3.8	1.6E-17	55.6	208.7	Arylsulfatase (EC 3.1.6.1)
0844	7.9	3.1E-49	67.5	531.9	Bifunctional protein: zinc-containing alcohol dehydrogenase; quinone oxidoreductase (NADPH:quinone reductase) (EC 1.1.1.-); Similar to arginate lyase
0845	5.6	4.3E-37	95.7	531.3	hypothetical protein
0846	39.8	9.2E-133	33.7	1343.3	putative transcriptional regulator
0851	3.0	1.2E-07	27.0	79.8	hypothetical protein
0852	6.3	9.5E-36	237.4	1498.9	hypothetical protein
0853	39.1	2.0E-112	69.7	2724.0	hypothetical protein
0854	25.9	2.1E-40	4.8	124.2	hypothetical protein
0855	29.0	5.2E-62	8.2	236.2	hypothetical protein
0856	19.9	1.1E-27	15.9	318.0	hypothetical protein
0857	13.7	1.8E-19	10.0	137.4	patatin family protein
0858	20.4	3.7E-44	13.3	271.9	L-alanoyl-D-glutamate peptidase
0859	4.2	6.8E-08	10.8	45.5	hypothetical protein
0860	56.6	7.7E-38	2.6	146.6	hypothetical protein

0862	42.0	3.9E-67	11.9	497.5	Ferrichrome-iron receptor
0867	6.1	0.0002	2.6	15.9	Peptide methionine sulfoxide reductase MsrA (EC 1.8.4.11) / Peptide methionine sulfoxide reductase MsrB (EC 1.8.4.12)
0876	2.8	4.4E-05	16.7	46.8	3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157); 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35);Ontology_term=KEGG_ENZYME:1.1.1.157,KEGG_ENZYME:1.1.1.35
0881	4.1	2.0E-07	91.7	378.7	2-oxoglutarate/malate translocator
0882	5.6	3.7E-06	28.9	160.5	Succinate dehydrogenase cytochrome b subunit
0883	5.7	7.6E-09	164.2	936.3	Succinate dehydrogenase flavoprotein subunit (EC 1.3.99.1)
0884	4.6	1.5E-07	91.4	423.4	Succinate dehydrogenase iron-sulfur protein (EC 1.3.99.1)
0927	2.3	0.0029	17.0	38.4	putative ribonucleoprotein-related protein
0928	2.5	0.0300	7.1	17.5	hypothetical protein
0929	2.4	0.0002	22.2	53.3	Protein RtcB
0934	96.3	4.0E-64	3.0	285.0	TonB-dependent receptor
0935	50.8	5.3E-91	7.8	394.6	hypothetical protein
0936	31.7	1.5E-49	7.4	235.4	hypothetical protein
0937	11.7	1.1E-38	15.2	177.1	Cytochrome c551 peroxidase (EC 1.11.1.5)
0938	5.3	6.4E-23	32.6	172.8	hypothetical protein
0965	2.3	0.0162	16.3	38.3	cAMP-binding proteins - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinases
0994	2.6	0.0017	13.7	35.6	RND efflux system, inner membrane transporter CmeB
0995	3.0	1.5E-05	15.6	46.7	Probable RND efflux membrane fusion protein
1041	16.6	0.0021	80.4	1333.1	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (EC 2.1.1.14)
1069	4.3	4.6E-34	349.9	1512.1	Thioredoxin reductase (EC 1.8.1.9)
1216	2.8	1.1E-05	23.0	65.3	Malate permease
1224	3.1	0.0008	9.6	30.1	Vng1746c
1292	4.2	3.2E-14	34.4	144.9	hypothetical protein

1293	2.3	0.0004	737.7	1720.5	hypothetical protein
1299	14.7	6.9E-31	14.8	218.1	hypothetical protein
1309	2.4	8.3E-08	59.0	143.7	Protein secretion chaperonin CsaA
1317	2.7	2.1E-10	70.4	189.1	2-hydroxychromene-2-carboxylate isomerase/DsbA-like thioredoxin domain
1370	2.0	2.3E-06	104.9	213.5	Sterol desaturase
1405	2.4	0.0019	37.8	91.7	hypothetical protein
1511	2.5	0.0352	6.3	16.0	hypothetical protein
1571	2.2	3.6E-06	62.3	135.7	hypothetical protein
1629	3.5	0.0069	6.3	22.1	hypothetical protein
1741	2.9	0.0281	4.8	13.8	Glycosyltransferase (EC 2.4.1.-)
1744	10.1	3.4E-30	14.8	150.1	bacterial regulatory protein, DeoR family
1759	4.8	2.5E-07	7.4	35.6	beta-lactamase domain protein
1760	9.8	7.7E-07	5.9	57.9	Permeases of the major facilitator superfamily
1797	2.4	1.5E-09	96.4	235.2	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)
1837	5.0	3.4E-14	26.0	130.4	cAMP-binding proteins - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinases
1838	3.5	9.8E-17	68.2	236.6	Putative esterase
1839	108.2	2.4E-77	2.2	241.2	Methionine aminopeptidase (EC 3.4.11.18)
1840	63.6	5.9E-55	3.7	236.5	Peptide deformylase (EC 3.5.1.88)
1871	4.7	3.4E-28	78.3	368.2	Putative esterase
1872	99.1	4.5E-33	3.3	330.8	TonB-dependent receptor
1873	87.8	7.7E-130	9.2	812.0	hypothetical protein
1874	10.2	6.0E-72	125.7	1280.8	hypothetical protein
1875	87.6	5.7E-81	5.2	455.2	Cytochrome c551 peroxidase (EC 1.11.1.5)
1876	3.2	0.0431	3.3	10.6	hypothetical protein
1895	3.8	1.1E-28	404.9	1523.3	Catalase (EC 1.11.1.6) / Peroxidase (EC 1.11.1.7)
1898	5.4	8.5E-07	5.6	29.9	Ferrichrome-iron receptor
1899	39.4	6.6E-22	3.3	131.5	Transcriptional regulator, AraC family
1900	51.8	1.7E-19	1.9	95.8	Transcriptional regulator, AraC family
1901	5.6	0.0054	1.9	10.4	Iron utilization protein

1902	3.0	0.0381	4.1	12.1	Multi antimicrobial extrusion protein (Na ⁺)/drug antiporter), MATE family of MDR efflux pumps
1903	5.5	0.0008	4.1	22.2	Ferrichrome-iron receptor
1904	4.1	0.0005	4.8	19.8	hypothetical protein
1918	2.3	0.0253	10.0	22.7	Conserved hyperthetical protein
1920	3.1	7.6E-09	28.2	88.0	Mil3930 protein
1921	2.4	3.0E-06	40.8	98.0	Organic hydroperoxide resistance protein
2001	2.2	3.8E-06	64.9	140.4	hypothetical protein
2005	126.8	9.6E-130	21.4	2715.9	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
2027	2.1	0.0005	31.1	65.9	Similar to (S)-2,3-di-O-geranylgeranylglyceryl phosphate synthase
2060	3.0	1.3E-19	330.6	994.7	hypothetical protein
2078	2.7	0.0110	7.0	19.4	Putative membrane protein YeiH
2102	2.1	1.6E-06	158.9	338.2	hypothetical protein
2143	6.3	6.7E-05	3.0	18.8	hypothetical protein
2209	2.4	1.2E-07	58.6	141.3	hypothetical protein
2237	2.2	0.0154	31.9	70.3	SanA protein
2273	5.1	7.8E-28	57.1	288.7	hypothetical protein
2274	5.4	1.4E-11	62.6	337.6	Thioredoxin
2275	3.4	2.8E-26	1092.5	3706.5	Alkyl hydroperoxide reductase subunit C-like protein
2287	2.1	3.1E-07	149.3	306.6	hypothetical protein
2288	2.3	0.0022	15.9	37.1	transporter, LysE family
2298	195.9	4.1E-135	3.3	655.7	Siderophore biosynthesis L-2,4-diaminobutyrate decarboxylase
2299	156.3	5.1E-148	5.5	867.2	Siderophore biosynthesis protein, monooxygenase
2300	86.3	8.5E-84	3.3	288.2	Desferrioxamine E biosynthesis protein DesC @ Siderophore synthetase small component, acetyltransferase
2301	200.3	6.1E-182	7.4	1484.9	Desferrioxamine E biosynthesis protein DesD @ Siderophore synthetase superfamily, group C @ Siderophore synthetase component, ligase
2304	3.0	0.0191	4.8	14.6	TonB-dependent siderophore receptor

2307	7.6	2.0E-28	20.4	155.7	Possible glyoxylase family protein (Lactoylglutathione lyase) (EC 4.4.1.5)
2308	24.3	1.5E-89	24.5	593.8	NADPH-dependent FMN reductase
2309	18.9	4.0E-98	61.1	1155.6	hypothetical
2310	40.0	5.4E-48	20.4	814.3	Manganese superoxide dismutase (EC 1.15.1.1)
2311	3.2	4.9E-05	25.6	82.9	hypothetical protein
2312	3.4	2.1E-07	26.7	91.5	hypothetical protein
2318	3.4	0.0009	13.3	44.9	hypothetical protein
2319	3.1	0.0015	7.8	24.2	DNA primase (EC 2.7.7.-)
2330	2.9	7.2E-05	14.8	42.3	VgrG protein
2346	4.1	2.2E-12	90.4	370.1	putative Cytochrome bd2, subunit I
2347	6.0	1.4E-27	34.5	205.3	Cytochrome d ubiquinol oxidase subunit II (EC 1.10.3.-)
2370	2.6	1.2E-06	34.5	88.5	Probable multidrug resistance protein
2392	2.1	0.0002	163.1	335.3	Predicted signal-transduction protein containing cAMP-binding and CBS domains
2449	2.6	0.0489	5.2	13.5	hypothetical protein
2487	2.2	3.7E-09	190.2	418.5	Na ⁺ /H ⁺ antiporter NhaA type
2505	4.5	1.7E-06	36.7	165.8	Glycerol uptake facilitator protein
2588	2.8	2.9E-07	30.0	83.5	Cell division inhibitor
2645	27.8	1.1E-27	2.6	72.2	hypothetical protein
2683	3.6	8.4E-05	38.9	139.2	TonB-dependent receptor
2684	2.9	0.0092	20.4	59.0	hypothetical protein
2721	2.3	0.0414	7.4	17.4	Death on curing protein, Doc toxin
2748	2.8	6.2E-06	21.5	60.1	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
2775	2.2	0.0012	26.7	58.2	High-affinity choline uptake protein BetT
2889	2.7	3.5E-05	18.2	49.9	putative transport protein
2934	2.3	6.9E-05	33.8	76.2	protein of unknown function
2935	60.5	4.5E-105	16.7	1010.7	Hemin transport protein HmuS
2936	46.9	2.3E-84	7.4	347.2	Methyltransferase (EC 2.1.1.-)
2937	88.8	6.8E-101	5.9	525.9	ABC-type hemin transport system, ATPase component
2938	126.1	2.0E-64	5.2	652.8	Hemin ABC transporter, permease protein

2939	100.3	1.2E-97	10.4	1039.0	Periplasmic hemin-binding protein
2991	2.4	0.0001	23.4	56.5	FIG000605: protein co-occurring with transport systems (COG1739)
3020	51.7	1.7E-34	4.1	210.0	putative iron-regulated membrane protein
3021	76.9	2.0E-84	10.3	794.1	TonB-dependent receptor, putative
3022	76.2	6.9E-51	4.4	338.6	hypothetical protein
3023	75.6	5.7E-32	9.6	728.8	TonB-dependent receptor
3024	2.6	0.0008	843.2	2193.2	Peptidase, S41 family
3025	2.5	5.3E-05	180.4	454.9	hypothetical protein
3027	33.0	1.0E-68	8.2	269.8	hypothetical protein
3028	21.0	1.7E-39	9.6	202.2	hypothetical protein
3029	15.1	3.9E-14	2.6	39.0	hypothetical protein
3030	6.9	6.0E-22	16.3	112.7	hypothetical protein
3031	3.5	5.1E-11	128.4	450.3	hypothetical protein
3065	2.1	2.0E-05	154.6	327.9	hypothetical protein
3217	24.1	3.3E-46	13.7	330.4	DNA polymerase III alpha subunit (EC 2.7.7.7)
3218	18.6	3.0E-34	14.5	269.1	DNA polymerase IV (EC 2.7.7.7)
3219	5.1	1.3E-14	35.9	182.2	hypothetical protein
3244	2.6	0.0003	15.6	40.8	Sulfite reductase [NADPH] flavoprotein alpha-component (EC 1.8.1.2)
3245	3.7	0.0002	7.0	25.8	Siroheme synthase / Precorrin-2 oxidase (EC 1.3.1.76) / Sirohydrochlorin ferrochelatase (EC 4.99.1.4) / Uroporphyrinogen-III methyltransferase (EC 2.1.1.107)
3246	2.7	1.1E-05	21.9	59.6	Cysteine synthase (EC 2.5.1.47)
3247	3.6	5.9E-07	13.0	47.3	Serine acetyltransferase (EC 2.3.1.30)
3248	2.4	0.0010	28.9	69.4	Sulfate adenylyltransferase subunit 1 (EC 2.7.7.4)
3249	2.5	6.9E-05	24.1	59.5	Sulfate adenylyltransferase subunit 2 (EC 2.7.7.4)
3252	2.0	1.4E-06	120.7	246.2	B. burgdorferi predicted coding region BB0756
3308	2.0	0.0002	47.5	96.0	putative glucose transferase
3356	2.8	0.0002	13.7	38.6	Putative cytoplasmic protein
3384	2.7	1.9E-06	27.8	74.8	Hypothetical radical SAM family enzyme, NOT

					coproporphyrinogen III oxidase, oxygen-independent
3401	2.0	0.0062	30.4	61.2	TonB-dependent receptor
					Phenylacetate-CoA
3454	2.3	0.0008	46.7	109.5	oxygenase/reductase, PaaK subunit
3455	3.2	1.2E-05	26.4	85.2	Phenylacetate-coenzyme A ligase (EC 6.2.1.30)
3465	2.1	3.9E-07	125.0	259.8	hypothetical protein
3505	4.4	3.4E-10	56.7	249.1	hypothetical protein
3506	16.9	4.8E-35	36.7	621.2	hypothetical protein
3507	8.8	1.8E-29	414.5	3651.6	hypothetical protein
3508	12.1	1.9E-38	219.1	2650.2	hypothetical protein
3509	12.5	4.6E-13	5.6	69.3	hypothetical protein
3510	16.8	6.2E-10	3.7	62.4	hypothetical protein
3561	2.5	5.3E-05	38.5	98.0	DNA polymerase IV (EC 2.7.7.7)
3573	2.2	0.0002	31.5	69.0	Sensory box histidine kinase
3574	2.3	0.0071	12.2	28.5	hypothetical protein
3597	2.1	3.8E-05	59.3	121.6	5,10-methylenetetrahydrofolate reductase (EC 1.5.1.20)
					5-methyltetrahydrofolate--
3598	2.7	1.2E-13	168.9	450.2	homocysteine methyltransferase (EC 2.1.1.13)
3599	2.1	2.2E-07	133.8	280.0	Linoleoyl-CoA desaturase (EC 1.14.19.3)
					Aspartokinase (EC 2.7.2.4) /
3603	2.4	3.1E-06	36.0	88.2	Homoserine dehydrogenase (EC 1.1.1.3)
3664	19.8	2.2E-110	99.5	1966.3	hypothetical protein
3665	15.4	1.6E-74	42.2	648.8	hypothetical protein
					Putative NADPH-dependent
3666	13.6	1.8E-80	70.3	958.1	reductase flavoprotein component, possibly involved in thiamine biosynthesis
					Thiamin biosynthesis lipoprotein
3667	9.8	6.4E-33	25.9	253.0	ApbE
					Non-specific DNA-binding
3681	14.4	3.3E-61	44.9	647.5	protein Dps / Iron-binding ferritin-like antioxidant protein / Ferroxidase (EC 1.16.3.1)
3686	2.0	0.0187	16.6	33.4	hypothetical protein
3694	2.4	3.1E-05	48.9	118.8	TonB-dependent receptor
3715	2.1	0.0017	40.0	84.4	Conjugative transposon protein TraG

3720	2.6	9.8E-05	29.7	75.9	Putative conjugative transposon mobilization protein BF0132
3721	2.8	0.0002	13.7	38.4	hypothetical protein
3743	2.0	0.0360	15.2	30.5	hypothetical protein
3764	7.1	1.2E-29	28.8	205.6	hypothetical protein
3765	4.7	6.9E-15	103.2	484.1	TonB-dependent receptor
3799	2.0	5.9E-05	59.3	119.6	Peptidyl-tRNA hydrolase (EC 3.1.1.29)
3802	2.5	1.6E-09	73.8	187.9	putative auxin-regulated protein
3803	2.6	0.0406	28.9	73.9	putative transporter
3827	2.6	0.0024	11.5	29.7	Hypothetical Nudix-like regulator
3872	2.1	5.4E-07	233.7	492.9	Type cbb3 cytochrome oxidase biogenesis protein CcoI; Copper-translocating P-type ATPase (EC 3.6.3.4); FUPA29 P-type ATPase
3912	4.0	9.7E-31	409.0	1621.3	Fumarate hydratase class II (EC 4.2.1.2)
3913	5.5	9.9E-25	126.8	698.7	aminoglycoside 6-adenylyltransferase
3941	2.0	0.0045	23.7	47.8	hypothetical protein
3973	2.6	3.6E-12	256.6	656.8	Cysteine desulfurase (EC 2.8.1.7), SufS subfamily
3974	2.5	1.1E-10	105.2	266.7	hypothetical protein
3975	2.2	2.8E-08	133.4	293.6	PlcB, ORFX, ORFP, ORFB, ORFA, Idh gene
3976	2.9	6.8E-17	231.5	664.2	Iron-sulfur cluster assembly protein SufD
3977	2.9	9.0E-11	50.0	147.4	S23 ribosomal protein
3978	2.5	1.4E-07	240.7	605.3	Iron-sulfur cluster assembly ATPase protein SufC
3979	2.6	9.0E-16	438.1	1160.9	Iron-sulfur cluster assembly protein SufB
3980	2.8	2.4E-13	98.9	281.3	probable iron binding protein from the HesB_IscA_SufA family
4020	64.0	1.1E-153	23.7	1514.3	Aerobactin siderophore receptor IutA

^a:Only genes that were differentially expressed (Fold Change > 2; adjusted p value < 0.05), are displayed. Expression values have been calculated using the R package DESeq (Anders & Huber, 2010).

^b: 'baseMeans' are the absolute expression values averaged for both the 3 replicates of a condition as calculated by the DESeq package.

Table S5 Genes down regulated at least 2-fold in H₂O₂ treated NUHP1 cells. ^a

Locus Tag (BD94_)	Fold Change	adjusted p-value	baseMeans _CK b	baseMeans _H2O2	Gene product description
0005	-2.0	1.6E-08	696.4	340.8	hypothetical protein
0033	-2.5	0.0008	803.8	321.8	hypothetical protein
0055	-2.2	0.0003	1947.0	880.0	LSU ribosomal protein L21p
0108	-4.3	1.6E-08	252.2	58.1	TonB-dependent receptor
0166	-2.2	5.1E-08	273.8	126.4	Two-component system sensor histidine kinase
0200	-2.4	2.8E-14	2800.5	1162.1	Outer membrane protein assembly factor YaeT precursor
0339	-4.9	1.2E-06	935.5	189.5	hypothetical protein
0341	-6.2	0.0035	698.3	111.9	hypothetical protein
0363	-5.1	1.2E-32	401.7	78.9	Outer membrane protein W precursor
0366	-2.3	5.4E-06	3233.8	1420.2	LSU ribosomal protein L11p (L12e)
0367	-2.4	0.0001	4094.9	1708.1	LSU ribosomal protein L1p (L10Ae)
0368	-2.4	4.8E-06	3783.7	1591.4	LSU ribosomal protein L10p (P0)
0369	-2.2	2.5E-06	1575.3	720.2	LSU ribosomal protein L7/L12 (P1/P2)
0378	-7.1	1.7E-11	814.2	115.3	hypothetical protein
0524	-2.4	6.0E-07	118.3	50.0	3,4-dihydroxy-2-butanone 4-phosphate synthase (EC 4.1.99.12)
0583	-2.5	0.0313	34.2	13.5	Bifunctional protein: zinc-containing alcohol dehydrogenase; quinone oxidoreductase (NADPH:quinone reductase) (EC 1.1.1.-); Similar to arginate lyase
0586	-3.6	1.3E-11	94.2	26.2	hypothetical protein
0654	-2.3	7.4E-05	70.1	30.9	hypothetical protein
0724	-2.4	3.1E-14	11847.1	4997.4	TonB-dependent receptor
0891	-2.8	6.5E-16	685.4	248.2	hypothetical protein
0955	-2.1	0.0040	109.3	52.5	putative cell surface protein
1019	-2.2	6.9E-06	122.2	56.4	hypothetical protein
1026	-2.1	0.0002	79.0	37.6	hypothetical protein
1028	-2.4	2.9E-05	294.5	123.9	hypothetical protein
1092	-10.9	1.2E-71	3279.9	301.6	Cytochrome c oxidase subunit CcoN (EC 1.9.3.1) / Cytochrome c oxidase subunit CcoO (EC 1.9.3.1)
1093	-11.8	5.5E-21	901.1	76.7	Cytochrome c oxidase subunit CcoP (EC 1.9.3.1)
1132	-2.8	7.6E-07	640.1	226.8	Lipoprotein
1152	-4.4	2.6E-05	164.2	37.1	hypothetical protein

1154	-6.4	5.1E-07	583.7	91.1	hypothetical protein
1170	-3.1	1.9E-08	139.9	45.8	nodulin 21-related protein
1184	-3.5	1.1E-10	243.4	70.0	Peptidase, family M23 (EC 3.4.24.-)
1199	-2.5	4.9E-13	1184.8	476.4	Glutamine synthetase type III, GlnN (EC 6.3.1.2)
1259	-4.5	4.9E-06	1920.4	430.3	hypothetical protein
1316	-2.6	4.6E-07	93.1	36.1	membrane protein, putative
1329	-2.9	2.2E-05	44.9	15.6	Imidazole glycerol phosphate synthase amidotransferase subunit (EC 2.4.2.-)
1330	-3.6	1.1E-11	95.3	26.2	Histidinol-phosphatase (EC 3.1.3.15) / Imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19)
1331	-4.0	1.9E-11	79.0	20.0	Histidinol-phosphate aminotransferase (EC 2.6.1.9)
1332	-3.2	8.3E-09	76.8	24.0	Histidinol dehydrogenase (EC 1.1.1.23)
1333	-4.5	3.8E-06	66.4	14.9	ATP phosphoribosyltransferase (EC 2.4.2.17)
1349	-2.6	3.5E-08	121.2	46.9	hypothetical protein
1350	-6.4	5.7E-11	76.1	11.9	hypothetical protein
1351	-7.7	4.0E-43	335.7	43.9	hypothetical protein
1369	-2.3	1.9E-09	902.6	389.0	Proteinase inhibitor I11, ecotin precursor
1432	-3.0	5.6E-05	527.5	175.5	Probable low-affinity inorganic phosphate transporter
1433	-3.8	1.1E-07	546.6	143.5	Phosphate transport regulator (distant homolog of PhoU)
1445	-3.0	2.1E-17	1881.6	619.9	hypothetical protein
1446	-2.6	3.9E-17	3699.2	1401.4	OmpA/MotB domain protein
1459	-2.2	1.8E-07	187.7	84.3	Prolyl oligopeptidase(EC:3.4.21.26)
1504	-2.7	0.0007	32.6	12.2	FIG01092293: hypothetical protein
1505	-3.0	1.7E-06	54.1	18.0	monooxygenase, putative
1506	-2.6	0.0001	45.2	17.6	hypothetical protein
1507	-2.4	0.0004	46.7	19.5	Arsenate reductase (EC 1.20.4.1)
1509	-2.2	0.0017	43.7	19.8	Arsenical-resistance protein ACR3
1648	-2.1	0.0177	28.2	13.4	FIG01092293: hypothetical protein
1649	-2.6	0.0002	41.2	15.9	monooxygenase, putative
1650	-2.1	0.0226	31.6	15.1	hypothetical protein
1651	-2.6	0.0006	35.6	13.8	Arsenate reductase (EC 1.20.4.1)
1652	-2.7	0.0062	21.5	8.0	Phosphinothricin N-acetyltransferase (EC 2.3.1.-)
1653	-2.1	0.0057	41.9	19.9	Arsenical-resistance protein ACR3

1673	-2.5	0.0003	208.7	81.9	hypothetical protein
1729	-2.5	0.0266	17.1	6.8	hypothetical protein
1733	-2.0	0.0018	57.9	28.3	hypothetical protein
1748	-2.2	0.0331	22.3	10.3	hypothetical protein
1757	-2.1	1.5E-07	283.5	134.7	Cytochrome oxidase biogenesis protein Sco1/SenC/PrrC, putative copper metallochaperone
1764	-2.3	0.0162	23.7	10.3	TonB-dependent receptor
1779	-2.1	0.0004	72.4	35.2	hypothetical protein
1790	-2.4	0.0062	30.8	12.7	hypothetical protein
1892	-2.3	0.0383	38.7	16.9	hypothetical protein
1945	-2.2	1.7E-11	1192.4	532.0	putative outer membrane protein
1948	-2.6	2.9E-09	326.0	124.9	3-oxoacyl-[acyl-carrier-protein] synthase, KASIII (EC 2.3.1.41)
1966	-2.6	2.4E-09	168.1	65.6	ApaG protein
1973	-3.9	8.1E-14	750.3	194.0	hypothetical protein
2125	-2.1	1.8E-07	1755.7	844.3	TPR-domain containing protein
2152	-2.1	0.0120	30.4	14.3	Transcriptional regulator, AraC family
2161	-2.0	1.5E-06	252.8	126.1	Putative periplasmic protein
2162	-2.2	0.0052	34.2	15.4	amino acid permease-associated region
2180	-2.3	8.1E-11	582.1	254.0	hypothetical protein
2224	-2.3	3.4E-06	107.9	47.3	Transcriptional regulator, MarR family
2227	-3.8	4.8E-08	50.1	13.3	hypothetical protein
2231	-2.4	9.6E-08	2855.1	1206.8	Enoyl-CoA hydratase [isoleucine degradation] (EC 4.2.1.17) / 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35)
2232	-3.3	5.3E-10	263.2	79.7	Transcriptional regulator, MarR family
2257	-2.6	2.0E-12	294.5	111.2	Aspartokinase (EC 2.7.2.4) / Homoserine dehydrogenase (EC 1.1.1.3)
2258	-2.4	1.1E-05	83.9	35.1	Homoserine kinase (EC 2.7.1.39)
2280	-5.6	7.8E-12	460.1	82.8	hypothetical protein
2281	-2.2	0.0008	240.0	109.6	hypothetical protein
2295	-2.0	0.0145	36.4	18.2	Transcriptional regulator, AraC family
2336	-2.0	0.0011	59.3	29.3	Acriflavin resistance plasma membrane protein
2338	-2.6	1.7E-12	1094.8	429.0	hypothetical protein

2339	-2.6	2.0E-07	956.7	367.7	efflux transporter, RND family, MFP subunit
2340	-2.5	4.2E-09	1990.7	802.6	RND efflux system, inner membrane transporter CmeB
2341	-2.1	1.1E-08	950.7	450.7	RND efflux system, outer membrane lipoprotein, NodT family
2359	-2.2	0.0005	53.4	23.8	hypothetical protein
2360	-3.5	1.1E-17	242.0	68.7	Cytochrome c551/c552
2365	-5.7	0.0005	13.7	2.4	putative TonB-dependent receptor
2374	-5.1	3.0E-15	136.3	26.9	protein of unknown function DUF306, Meta and HslJ
2394	-2.8	0.0028	6559.0	2354.7	hypothetical protein
2423	-2.3	4.1E-06	1124.8	493.6	D-alanyl-D-alanine carboxypeptidase (EC 3.4.16.4)
2427	-2.2	3.8E-08	6561.4	2969.4	hypothetical protein
2428	-4.7	3.6E-42	8643.4	1822.1	hypothetical protein
2430	-5.9	1.6E-18	1755.0	298.7	hypothetical protein
2473	-2.5	4.1E-10	1832.8	724.1	SSU ribosomal protein S6p
2474	-2.9	2.1E-08	1289.5	442.6	SSU ribosomal protein S18p @ SSU ribosomal protein S18p, zinc-independent
2475	-2.4	1.9E-07	2276.9	932.9	LSU ribosomal protein L9p
2495	-2.4	2.0E-07	189.7	78.1	hypothetical protein
2533	-2.8	1.0E-11	1691.9	612.5	TonB-dependent receptor
2534	-2.2	4.3E-07	892.2	414.5	Trehalase (EC 3.2.1.28)
2539	-2.4	3.1E-07	366.4	154.4	PhnB protein; putative DNA binding 3-demethylubiquinone-9 3-methyltransferase domain protein
2544	-3.2	3.3E-08	69.0	21.4	hypothetical protein
2552	-2.7	3.4E-06	69.0	26.0	Ammonium transporter
2556	-2.6	4.3E-05	7100.9	2732.0	SSU ribosomal protein S1p
2568	-2.0	0.0001	105.7	52.7	hypothetical protein
2733	-3.1	0.0221	13.3	4.4	Zinc uptake regulation protein ZUR
2741	-2.0	0.0002	319.9	159.4	hypothetical protein
2758	-2.2	0.0204	23.7	10.8	hypothetical protein
2759	-5.4	3.3E-05	319.2	59.4	hypothetical protein
2796	-2.1	0.0045	40.1	19.0	Arsenate reductase (EC 1.20.4.1)
2797	-2.4	0.0008	46.7	19.8	FIG01092293: hypothetical protein
2834	-3.5	5.5E-09	1045.8	297.1	Mg(2+) transport ATPase protein C
2835	-11.1	3.5E-80	6816.5	615.2	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)
2836	-13.8	6.4E-105	6691.3	483.5	Probable Co/Zn/Cd efflux system membrane fusion protein

2837	-16.1	4.9E-107	14038.2	873.7	Cobalt-zinc-cadmium resistance protein CzcA; Cation efflux system protein CusA
2838	-15.5	5.3E-56	5431.4	349.7	Heavy metal RND efflux outer membrane protein, CzcC family
2850	-2.3	1.4E-11	945.2	414.0	hypothetical protein
2858	-3.9	1.5E-13	484.5	125.4	hypothetical protein
2859	-3.9	7.2E-09	2470.5	637.7	Response regulator
2864	-2.7	1.8E-06	322.5	120.5	Prephenate and/or arogonate dehydrogenase (unknown specificity) (EC 1.3.1.12)(EC 1.3.1.43)
2865	-2.1	8.0E-05	98.7	48.0	hypothetical protein
2873	-2.0	0.0004	76.1	37.2	PROBABLE SIGNAL PEPTIDE PROTEIN
2874	-2.4	0.0036	59.1	25.1	protein of unknown function DUF1501
2890	-6.6	1.1E-09	514.5	78.1	Transglycosylase associated protein
2891	-2.7	4.5E-14	420.9	156.2	Aspartokinase (EC 2.7.2.4)
2911	-3.6	5.6E-21	1558.3	438.8	Rhodanese-like domain protein
2912	-4.6	5.9E-34	883.9	191.4	hypothetical protein
2970	-2.4	0.0068	27.8	11.8	Ribonuclease (EC 3.1.27.-)
2972	-2.1	0.0148	47.9	22.4	MlpB
3016	-2.2	0.0025	43.4	20.1	Acetyltransferase, GNAT family
3064	-3.7	1.3E-24	593.8	160.6	Aminopeptidase
3072	-8.6	2.3E-05	1840.4	213.4	Probable zinc protease pqqL (EC 3.4.99.-)
3074	-2.4	5.4E-11	378.5	156.9	putative lipoprotein
3113	-2.2	4.8E-07	182.0	84.1	hypothetical protein
3125	-2.1	3.4E-08	645.4	312.3	Dihydrodipicolinate reductase (EC 1.3.1.26)
3150	-2.0	5.5E-09	1274.9	630.7	Transcriptional regulator, AsnC family
3176	-2.3	0.0001	63.1	27.5	hypothetical protein
3227	-12.2	2.0E-07	2497.9	204.0	hypothetical protein
3258	-6.7	9.3E-21	673.4	101.0	hypothetical protein
3334	-7.4	0.0428	128.3	17.2	TonB-dependent receptor
3366	-2.3	7.0E-05	2093.2	899.4	SSU ribosomal protein S16p
3400	-3.9	5.5E-09	657.5	168.6	hypothetical protein
3407	-2.9	4.6E-05	57.2	20.0	Threonine dehydratase biosynthetic (EC 4.3.1.19)
3408	-3.6	6.6E-08	153.0	42.0	Ketol-acid reductoisomerase (EC 1.1.1.86)
3409	-4.7	2.8E-09	46.4	9.8	Acetolactate synthase small subunit (EC 2.2.1.6)

3410	-3.4	7.4E-09	110.3	32.4	Acetolactate synthase large subunit (EC 2.2.1.6)
3411	-3.6	3.1E-13	124.7	35.1	Dihydroxy-acid dehydratase (EC 4.2.1.9)
3418	-2.5	4.3E-06	77.9	31.4	hypothetical protein
3451	-2.3	0.0005	51.2	22.6	hypothetical protein
3452	-2.8	2.8E-05	259.2	91.2	Phenylacetate-CoA oxygenase, PaaG subunit
3453	-3.9	1.8E-13	110.5	28.2	hypothetical protein
3513	-8.8	1.1E-13	44.5	5.1	hypothetical protein
3514	-13.4	4.4E-21	60.1	4.5	Probable cytochrome-c peroxidase (EC 1.11.1.5)
3515	-10.3	3.4E-17	53.0	5.1	hypothetical protein
3516	-3.0	6.1E-06	173.6	58.2	hypothetical protein
3517	-3.8	1.6E-08	191.2	49.7	hypothetical protein
3518	-4.3	4.8E-07	151.5	34.9	hypothetical protein
3519	-4.0	0.0033	111.4	27.9	hypothetical protein
3520	-3.2	0.0004	134.4	42.4	hypothetical protein
3535	-11.5	1.4E-06	991.1	86.3	hypothetical protein
3580	-2.6	0.0456	13.7	5.3	FIG01080692: hypothetical protein
3621	-2.5	6.2E-12	400.5	161.2	putative lipoprotein
3625	-2.9	0.0003	31.2	10.6	TonB-dependent receptor
3644	-2.0	3.1E-07	3668.0	1803.3	LSU ribosomal protein L18p (L5e)
3645	-2.1	5.1E-05	4103.3	1999.4	LSU ribosomal protein L6p (L9e)
3646	-2.0	2.8E-05	2741.3	1355.7	SSU ribosomal protein S8p (S15Ae)
3652	-2.1	2.5E-06	782.5	367.9	LSU ribosomal protein L29p (L35e)
3653	-2.0	0.0005	2853.8	1407.9	LSU ribosomal protein L16p (L10e)
3677	-2.0	8.5E-05	13220.3	6547.8	Translation elongation factor G
3678	-2.1	0.0002	2056.8	980.2	SSU ribosomal protein S7p (S5e)
3692	-4.0	4.8E-11	10919.4	2734.7	HtrA protease/chaperone protein
3757	-2.3	0.0068	55.9	24.0	Alpha-mannosidase (EC 3.2.1.24)
3760	-2.2	0.0222	22.6	10.2	mannose-6-phosphate isomerase, class I
3785	-5.2	0.0021	158.0	30.5	hypothetical protein
3801	-3.5	8.8E-05	4161.2	1189.0	hypothetical protein
3859	-4.1	3.3E-20	210.4	51.6	hypothetical protein
3864	-3.0	0.0004	78.1	26.1	Ornithine carbamoyltransferase (EC 2.1.3.3)
3865	-3.3	0.0002	101.9	31.1	Acetylornithine aminotransferase (EC 2.6.1.11)
3866	-2.3	3.6E-05	90.0	39.2	N-acetyl-gamma-glutamyl-phosphate reductase (EC 1.2.1.38)

3868	-2.6	0.0202	17.1	6.6	hypothetical protein
3897	-3.1	1.8E-11	1101.8	357.8	peptidase M48, Ste24p
3898	-2.3	3.1E-08	192.6	82.4	hypothetical protein
3904	-14.3	5.4E-20	298.1	20.9	hypothetical protein
3911	-3.3	1.1E-24	1472.5	439.7	Fumarate hydratase class I, aerobic (EC 4.2.1.2)
3955	-2.2	2.9E-05	3815.5	1759.5	LSU ribosomal protein L25p
3983	-5.3	5.5E-16	647.4	123.2	hypothetical protein
4019	-2.6	5.1E-10	1354.2	515.3	Invasion associated protein p60

^a: Only genes that were differentially expressed (Fold Change < -2; adjusted p value < 0.05), are displayed. Expression values have been calculated using the R package DESeq.

^b: 'baseMeans' are the absolute expression values averaged for both the 3 replicates of a condition as calculated by the DESeq package.

Table S6 Genes up regulated at least 4-fold in mouse blood treated NUHP1 cells. ^a

Locus Tag (BD94_)	Fold change	P-value	Normalized means_CK	Normalized means_blood	Feature ID
0013	6	2E-126	187.4	950.8	hypothetical protein
0014	4.3	2.8E-49	1615.2	9259.7	Ferrous iron transport protein B
0057	4.3	4.5E-34	502.8	2098.7	hypothetical protein
0058	5.6	2.4E-24	17.1	72.3	hypothetical protein
0060	9.7	3E-160	159.8	1347.2	hypothetical protein
0067	36.2	2E-224	268.2	10760.1	Ferrichrome-iron receptor
0068	27	1E-198	100.6	2354.7	putative iron-regulated membrane protein
0069	21.5	5E-160	219.1	4524.4	Aerobactin siderophore receptor IutA
0070	22.9	3E-149	132.8	2626.9	putative iron-regulated membrane protein
0071	116.5	0	154.8	18698.6	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
0072	167.7	0	100.7	17988.8	hypothetical protein
0188	5.9	2.9E-25	72.2	346.9	hypothetical protein
0210	20.7	8.5E-98	20.6	378.8	4-Hydroxy-2-oxoglutarate aldolase (EC 4.1.3.16) @ 2-dehydro-3-deoxyphosphogluconate aldolase (EC 4.1.2.14)
0211	19.8	2E-121	34.6	573.3	2-dehydro-3-deoxygluconate kinase (EC 2.7.1.45)
0212	73.2	1E-127	15.2	978.4	D-mannonate oxidoreductase (EC 1.1.1.57)
0213	45.2	2E-176	28.4	1138.1	Mannonate dehydratase (EC 4.2.1.8)
0214	22.1	9E-198	81.3	1609.6	Uronate isomerase (EC 5.3.1.12)
0234	5.3	3.5E-53	74.1	317	hypothetical protein
0243	7.5	1.3E-35	17.6	97.5	hypothetical protein
0255	7.7	4.5E-74	106.3	666	FIG00649718: hypothetical protein
0341	4.1	2.3E-30	446.3	1738.1	hypothetical protein
0353	6.3	1.5E-54	72.3	359.3	hypothetical protein
0435	4.1	2E-129	817.2	3691.1	hypothetical protein
0562	6.1	8.5E-23	147.7	749.3	hypothetical protein
0566	6	4E-122	2897.5	22266.4	putative outer membrane protein, probably involved in nutrient binding

0567	7.8	7E-162	1417.9	14912.6	hypothetical protein
0568	5.5	2E-139	1727.6	13440.2	putative outer membrane protein, probably involved in nutrient binding
0569	6.7	4E-164	706.6	5154.4	hypothetical protein
0654	9	5E-41	118.7	882.8	hypothetical protein
0656	24.1	2E-133	54.5	1183.4	hypothetical protein
0657	17.2	9E-145	111.3	1673.9	hypothetical protein
0658	23.3	5E-122	39.1	763.4	hypothetical protein
0659	34.7	1E-212	44.8	1384.9	hypothetical protein
0660	17.8	1E-126	200.8	3451.1	hypothetical protein
0661	5.4	2.1E-51	200.8	909.4	Lanthionine biosynthesis cyclase LanC
0677	17.1	3E-100	26.2	394.6	hypothetical protein
0678	10.8	9.7E-83	43.1	384.3	hypothetical protein
0712	4.6	1.7E-16	14.6	54.3	hypothetical protein
0719	5.8	1.7E-21	17.4	75.4	Isocitrate lyase (EC 4.1.3.1)
0720	5.3	2E-147	438.2	2174.9	Aquaporin Z
0722	4.1	7.4E-41	454.6	1760.7	UDP-2,3-diacylglucosamine diphosphatase (EC 3.6.1.54)
0724	13	4E-165	1992.6	31197.2	Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA (TC 3.A.1.5.1)
0727	19.2	7.1E-80	11.2	159.5	TonB family protein / TonB-dependent receptor
0728	6.3	3.4E-26	15.2	71.7	Putative outer membrane protein, probably involved in nutrient binding
0768	7.6	4.1E-66	2693.3	26577.6	Ferrichrome-iron receptor
0785	4.3	5.3E-44	276.7	1045.8	Phosphohistidine phosphatase SixA
0798	6	9.5E-26	17.2	87.9	Endo-beta-N-acetylglucosaminidase F2
0799	6	8.7E-14	6.2	31.9	Endo-beta-N-acetylglucosaminidase F2
0825	4.4	3.6E-28	95.7	333.8	acetyltransferase (putative)
0844	6.2	2.1E-93	104	505.7	Bifunctional protein: zinc-containing alcohol dehydrogenase; quinone oxidoreductase (NADPH:quinone reductase) (EC 1.1.1.-); Similar to arginate lyase
0845	4.6	3.7E-57	201.6	791.9	hypothetical protein

0860	71	1E-263	40.3	2604.3	putative iron-regulated membrane protein
0861	76.3	3.1E-43	0.7	59.4	hypothetical protein
0862	26.4	3E-106	165.6	4077.2	Ferrichrome-iron receptor
0866	7.7	0.00016	1.4	8.4	hypothetical protein
0867	40	4.2E-25	3.4	95.8	Peptide methionine sulfoxide reductase MsrA (EC 1.8.4.11) / Peptide methionine sulfoxide reductase MsrB (EC 1.8.4.12)
0868	16.2	4.6E-11	3	39.4	Arginine/ornithine antiporter ArcD
0877	6.6	1.7E-45	178.3	983.3	Myosin-crossreactive antigen
0879	9.3	3.9E-79	38.7	296.6	hypothetical protein-signal peptide and transmembrane prediction
0880	12.5	1E-194	240.2	2786.9	FIG01092758: hypothetical protein
0920	9.7	2E-115	70.6	571.6	hypothetical protein
0925	5.2	6E-101	245.1	1126.7	Glutaryl-CoA dehydrogenase (EC 1.3.99.7)
0934	267.7	0	43.8	13480.3	putative outer membrane receptor
0935	133	3E-270	67.2	9496.1	hypothetical protein
0936	103.8	4E-274	51.9	5231.2	hypothetical protein
0937	43	3E-222	79.7	3265.7	Cytochrome c551 peroxidase (EC 1.11.1.5)
0938	16.9	1E-198	127.9	1887.9	hypothetical protein
0939	42.4	3.9E-05	0	4.6	hypothetical protein
0974	5.4	2.2E-09	4	19.8	hypothetical protein
0975	7.7	3E-06	1.5	13.1	8-amino-7-oxononanoate synthase (EC 2.3.1.47)
0981	4.2	3.9E-44	205.6	707.2	Rrf2 family transcriptional regulator
1007	4.1	1.3E-12	17.7	59.3	hypothetical protein
1008	5.2	2E-103	162.7	688	ABC transporter ATP-binding protein
1009	4.6	9.6E-18	15.7	59.2	hypothetical protein
1011	7	1E-120	142.5	821.2	hypothetical protein
1013	4	3.1E-17	37.7	119.6	hypothetical protein
1015	7.7	3.1E-67	29.9	188.2	CAAX amino terminal protease family
1018	40	1E-142	38.2	1375.8	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
1050	4.1	7.1E-16	32.1	102.5	hypothetical protein

1092	7.9	3.2E-33	487.1	3968.1	Cytochrome c oxidase subunit CcoN (EC 1.9.3.1) / Cytochrome c oxidase subunit CcoO (EC 1.9.3.1)
1134	4.4	0.00035	2.6	8.9	hypothetical protein
1139	5.1	0.00183	1.6	5.8	Potassium-transporting ATPase C chain (EC 3.6.3.12) (TC 3.A.3.7.1)
1151	4.2	7.9E-32	176.2	598.8	Zinc uptake regulation protein ZUR
1152	5.9	4.4E-49	75.2	363.5	hypothetical protein
1161	4.6	2E-139	525.2	2306.4	Cell division protein FtsH (EC 3.4.24.-)
1201	5.1	1.5E-73	165.3	679.5	hypothetical protein
1204	5.6	1.1E-27	21	88.8	Membrane metalloprotease
1223	4.2	1.3E-48	2118.4	12286.3	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
1274	4.9	6E-122	361.9	1653.1	Hypothetical nudix hydrolase YeaB
1276	6.1	4.2E-85	93.3	449.4	Cardiolipin synthetase (EC 2.7.8.-)
1306	16.3	1E-192	347.2	5965.1	hypothetical protein
1353	5	7.1E-29	159.3	642.4	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
1416	11.9	3E-132	106.4	1025.8	Methionine ABC transporter substrate-binding protein
1417	10.5	5E-66	45.8	351.6	Methionine ABC transporter permease protein
1418	12.3	6E-137	71.9	755.4	Methionine ABC transporter ATP-binding protein
1437	6.1	2.3E-79	339.8	1960.4	Ferric siderophore transport system, periplasmic binding protein TonB
1628	7.5	3E-120	167.3	1045.6	hypothetical protein
1644	9.5	2E-122	138.2	1117.8	Membrane-flanked domain
1645	4.4	2.9E-35	93.9	322.7	hypothetical protein
1683	4.1	1.1E-25	71.6	239.2	DNA-binding response regulator, LuxR family
1684	5.9	3.8E-29	45.9	220.1	hypothetical protein
1685	5.1	3E-24	35.9	144.4	hypothetical protein
1686	6.8	8.2E-74	97.7	524.4	hypothetical protein
1687	9.1	2E-137	197.9	1622.1	hypothetical protein
1688	7	1.1E-88	60.5	343.3	hypothetical protein

1689	6	3.5E-70	81.5	392.8	Membrane-bound lytic murein transglycosylase D precursor (EC 3.2.1.-)
1691	5.1	1.9E-65	216.1	957.5	hypothetical protein
1692	6.2	9.3E-67	79.9	395.8	hypothetical protein
1693	4.6	9.5E-64	171.4	639.2	hypothetical protein
1694	5.7	1.1E-69	162.6	785.7	hypothetical protein
1695	4.9	3E-118	282.4	1227.2	hypothetical protein
1696	5.7	3E-131	785.7	4778.3	FIG01101450: hypothetical protein
1697	13.8	1E-210	171.1	2134.9	ClpB protein
1698	28.3	3.4E-91	9.4	218.7	hypothetical protein
1699	5.3	8.7E-34	41.5	166.4	VgrG protein
1700	4.1	3.4E-28	76.5	244.3	hypothetical protein
1709	20.5	7.7E-66	15.8	251.4	hypothetical protein
1710	5.9	2.6E-44	53.5	242.4	VgrG protein
1711	5.6	2.6E-59	84.6	376.4	Conserved domain protein
1713	4.2	6.4E-27	48.3	156.5	hypothetical protein
1714	4.7	2E-18	16.3	65	hypothetical protein
1743	12.9	4E-100	55.7	609.8	hypothetical protein
1745	5.1	9.8E-23	44.7	180.2	hypothetical protein
1787	6.8	4.7E-39	33.6	178.2	Probable glutathione S-transferase-related transmembrane protein (EC 2.5.1.18)
1791	4.6	0.00047	2.2	8.4	hypothetical protein
1796	5.2	2E-48	86.1	351.3	hypothetical proteintransmembrane prediction
1831	7.8	6.4E-95	285.7	2013.2	Cold shock protein CspG
1832	11.5	5.2E-74	27.7	243.3	RNA-binding protein
1855	10.3	1E-75	32.9	274.3	hypothetical protein
1871	4.4	1.7E-93	1020.7	5026.7	Putative esterase
1872	113.1	5E-278	30.7	3181.6	putative outer membrane receptor
1873	29.6	2E-198	110.8	2959.3	FIG00405034: hypothetical protein
1874	4.5	7.5E-90	2210.3	14202.6	hypothetical protein
1875	18.7	2E-138	76.5	1249.4	Cytochrome c551 peroxidase (EC 1.11.1.5)
1887	7.9	7.3E-26	12.9	71.8	hypothetical protein
1895	6	7E-130	923.7	6557.6	Catalase (EC 1.11.1.6) / Peroxidase (EC 1.11.1.7)
1896	268	2.4E-20	0.2	30.6	hypothetical protein

1897	282.5	2E-134	5.3	1091.9	putative iron-regulated membrane protein
1898	278.7	7E-230	35.8	10632.7	TonB-dependent siderophore receptor
1899	16	2E-117	47.6	587.8	Transcriptional regulator, AraC family
1900	56.2	1E-168	15.2	704.7	Transcriptional regulator, AraC family
1901	98.7	3.9E-53	4.7	408.3	iron-chelator utilization protein
1902	73.2	2.6E-33	6.9	415	Multi antimicrobial extrusion protein (Na ⁽⁺⁾ /drug antiporter), MATE family of MDR efflux pumps
1903	45.5	9.3E-13	16.8	670.1	Ferrichrome-iron receptor
1904	16.2	7.7E-16	19.6	243.3	PepSY-associated TM helix domain protein
1960	5.1	2E-117	626.4	3351.4	1,4-alpha-glucan (glycogen) branching enzyme, GH-13-type (EC 2.4.1.18)
1961	4	2.7E-56	413.8	1554.7	Glycogen synthase, ADP-glucose transglucosylase (EC 2.4.1.21)
1962	5.1	4E-122	501.7	2441.6	Glucose-1-phosphate adenylyltransferase (EC 2.7.7.27)
1974	5.6	5.2E-56	110.8	479.4	PQQ-dependent oxidoreductase, gdhB family
2005	25	6E-183	670.2	19350.1	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins
2068	9.6	2.7E-70	165.5	1371.7	hypothetical protein
2079	4.3	2.1E-12	15.3	48.8	hypothetical protein
2080	7.3	7.8E-39	21.8	126.3	hypothetical protein
2100	22.3	6E-272	249.9	5553.3	Aldehyde dehydrogenase B (EC 1.2.1.22)
2101	21.5	7E-250	213.7	4466.3	Probable L-lysine-epsilon aminotransferase (EC 2.6.1.36) (L-lysine aminotransferase) (Lysine 6-aminotransferase)
2102	10.1	4.4E-98	840.9	10708.2	hypothetical protein
2103	6.8	3.6E-13	4.9	27.8	hypothetical protein
2104	68.2	8E-119	7.7	470.7	hypothetical protein
2105	72.8	4.7E-93	3.4	199.8	hypothetical protein
2114	4.2	2.4E-11	9.4	34.2	hypothetical protein
2128	7.3	2.6E-36	63.8	371	hypothetical protein
2129	10.1	2.2E-96	71.4	566.9	hypothetical protein
2138	4	6.9E-32	113.7	356.1	Formate dehydrogenase chain D (EC 1.2.1.2)

2214	5.5	2E-26	68.2	312.3	AmpG protein, beta-lactamase induction signal transducer
2215	6.1	1.9E-07	3.3	16.8	hypothetical protein
2229	13.7	6E-211	2294.5	39658.8	Butyryl-CoA dehydrogenase (EC 1.3.99.2)
2230	10.9	3E-157	1385.7	19139	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)
2231	9.8	6E-133	3050.6	38548.6	Enoyl-CoA hydratase [isoleucine degradation] (EC 4.2.1.17) / 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35)
2232	11.2	3E-153	363.5	4078.2	Transcriptional regulator, MarR family
2238	6.3	8.7E-08	3.1	15.1	hypothetical protein
2257	19.5	3E-229	385.5	8542.6	Aspartokinase (EC 2.7.2.4) / Homoserine dehydrogenase (EC 1.1.1.3)
2258	14.2	9E-134	180.6	2292.9	Homoserine kinase (EC 2.7.1.39)
2259	11.9	1E-100	248.6	2771.2	Threonine synthase (EC 4.2.3.1)
2298	1027.5	0	25.8	25433.8	Siderophore biosynthesis L-2,4-diaminobutyrate decarboxylase
2299	1657.2	0	14.9	22531.3	Siderophore biosynthesis protein, monooxygenase
2300	1746.7	0	3.4	8426.6	Desferrioxamine E biosynthesis protein DesC @ Siderophore synthetase small component, acetyltransferase
2301	1403.9	0	33.1	64527	Desferrioxamine E biosynthesis protein DesD @ Siderophore synthetase superfamily, group C @ Siderophore synthetase component, ligase
2303	4.6	2.5E-12	11.9	41	probable ABC transporter ATP-binding protein
2305	4.8	1.3E-10	12.2	41.2	hypothetical protein
2313	8.5	7.2E-63	38.7	257.7	hypothetical protein
2330	6.2	3.1E-42	76.2	363.9	VgrG protein
2334	5.3	4E-116	416.6	2130	putative two-component system sensor protein histidine kinase
2336	5.9	6E-112	191.5	964.6	Acriflavin resistance plasma membrane protein
2337	6.3	2E-161	383.6	2295.6	UspA
2338	9.6	4E-184	3004.3	35625.6	hypothetical protein
2346	5.4	9E-128	414.4	2106.8	putative Cytochrome bd2, subunit I

2347	4.3	3.4E-32	210.7	768.8	Cytochrome d ubiquinol oxidase subunit II (EC 1.10.3.-)
2390	6.1	8.3E-39	49.9	241.5	hypothetical protein
2391	8.4	2.3E-20	7.6	51.2	hypothetical protein
2392	17	4E-187	138.9	2080.2	Predicted signal-transduction protein containing cAMP-binding and CBS domains
2472	5.8	8.4E-43	72.6	338.4	hypothetical protein
2476	6.4	3.7E-45	45.6	229.9	hypothetical protein
2513	5.8	9.1E-72	89.1	406.5	hypothetical protein
2515	8.5	1.6E-33	15.2	90.5	Single-stranded DNA-binding protein
2522	4.7	2.9E-55	244.6	1001.7	Pyruvate oxidase [ubiquinone, cytochrome] (EC 1.2.2.2)
2539	4.2	1.2E-68	452.3	1810.7	PhnB protein; putative DNA binding 3-demethylubiquinone-9 3-methyltransferase domain protein
2552	5.8	1.8E-70	88	404.9	Ammonium transporter
2561	8.8	4E-174	347.6	2997.6	hypothetical protein
2562	6.8	2.6E-92	101.2	547.6	hypothetical protein
2571	9.5	5E-154	431.2	4202.5	3-isopropylmalate dehydrogenase (EC 1.1.1.85)
2572	9.5	6E-172	283.2	2480.3	3-isopropylmalate dehydratase small subunit (EC 4.2.1.33)
2573	10.2	3E-167	470.9	4955.4	3-isopropylmalate dehydratase large subunit (EC 4.2.1.33)
2574	9.3	8E-145	427.8	4019.8	2-isopropylmalate synthase (EC 2.3.3.13)
2588	5.2	8.3E-55	124.3	509.6	Cell division inhibitor
2594	32.1	3E-100	195.6	6384.2	RNA-binding protein
2645	9.7	1E-70	65.4	508.3	hypothetical protein
2653	4.8	3.5E-32	71.3	268.1	PhnB protein; putative DNA binding 3-demethylubiquinone-9 3-methyltransferase domain protein
2654	6.3	3.4E-24	14.4	78.5	alpha/beta hydrolase fold
2655	5.6	7.6E-29	23.2	99.7	hypothetical protein
2683	5.6	7.6E-53	137.8	595.3	TonB family protein / TonB-dependent receptor
2684	4.3	3.4E-31	73.3	232.6	putative outer membrane protein, probably involved in nutrient binding
2692	18.6	1.2E-47	9.9	154.4	Citrate transporter
2693	29.3	1.1E-43	4.2	99.3	hypothetical protein

2699	25.3	2E-146	31.2	664.8	hypothetical protein
2700	7.6	2.2E-44	25.1	140.1	hypothetical protein
2711	6.1	7E-106	180.5	928.9	Acetyl-coenzyme A synthetase (EC 6.2.1.1)
2725	23.8	8E-240	125.8	2724.2	Branched-chain alpha-keto acid dehydrogenase, E1 component, alpha subunit (EC 1.2.4.4) / Branched-chain alpha-keto acid dehydrogenase, E1 component, beta subunit (EC 1.2.4.4)
2729	4.7	1.4E-31	41.4	157.3	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)
2730	4.1	1.8E-41	146.2	468.9	Fumarate/succinate/L-aspartate dehydrogenases
2739	4.9	1.6E-18	18.7	72.4	hypothetical protein
2741	6	7E-142	435.6	2451.8	Outer membrane protein A precursor
2742	6.7	1E-167	488.5	3277.7	Manganese transport protein MntH
2750	4.3	7.6E-09	13.9	48.4	hypothetical protein
2751	6.9	1E-13	7.2	35.2	hypothetical protein
2753	5.7	9.8E-21	13.9	64.3	hypothetical protein
2754	4.6	3.1E-08	6	21.7	hypothetical protein
2755	9.6	5.6E-10	2.7	20.2	hypothetical protein
2776	4.6	5.8E-14	12.4	41.8	protein of unknown function DUF1211
2780	6.3	0.00014	1.5	7.2	Peptide methionine sulfoxide reductase MsrB (EC 1.8.4.12)
2813	6.4	1E-20	13.1	69.2	Alpha-L-fucosidase (EC 3.2.1.51)
2833	4.4	3.6E-31	57.7	200.3	FIG016027: protein of unknown function YeaO
2887	6.1	1.1E-06	8.9	46.4	Cold shock protein CspA
2888	8.6	1.7E-32	11.5	84.7	ATP-dependent RNA helicase RhlE
2908	4.8	7.5E-09	5.7	21.4	hypothetical protein
2934	21.8	3E-148	60.5	1104.6	protein of unknown function
2935	85.8	1E-298	113.7	10263.2	Hemin transport protein HmuS
2936	143.7	2E-302	23.3	2960.7	Methyltransferase (EC 2.1.1.-)
2937	211.1	0	29.7	6087.2	ABC-type hemin transport system, ATPase component
2938	237.9	0	30.4	8240.9	Hemin ABC transporter, permease protein
2939	319.2	0	49.4	16731	Heme ABC transporter, cell surface heme and hemoprotein receptor HmuT

2971	4.7	1.4E-18	157.4	616.4	Mannosyltransferase OCH1 and related enzymes
3001	6.4	1.8E-25	76.2	393.9	hypothetical protein
3012	8.2	1.4E-84	51.6	336.1	hypothetical protein
3020	52	8E-228	36.4	1574.4	putative iron-regulated membrane protein
3021	22.9	1E-221	157.8	3325	TonB-dependent receptor, putative
3022	25.3	4E-142	54.7	1137.1	hypothetical protein
3023	35.1	1E-292	109.9	3589.3	TonB-dependent receptor
3047	6.1	1E-115	400.6	2283.8	Jumonji domain containing 5
3065	19	8E-108	317.6	6601.5	conserved hypothetical protein
3066	18.5	3.3E-94	879.5	19471.4	Alcohol dehydrogenase (EC 1.1.1.1)
3067	19.8	3E-109	1597.4	37158.6	Aldehyde dehydrogenase (EC 1.2.1.3)
					Endo-beta-N-acetylglucosaminidase F1 precursor (EC 3.2.1.96)
3083	9.2	8.6E-21	7.4	58.1	(Mannosyl-glycoprotein endo-beta-N-acetyl-glucosaminidase F1) (Di-N-acetylchitobiosyl beta-N-acetylglucosaminidase F1) (Endoglycosidase F1)
3105	9.7	3.3E-55	28.4	222.2	hypothetical protein
3211	5.2	5.3E-29	51.2	201.6	hypothetical protein
					Siroheme synthase / Precorrin-2 oxidase (EC 1.3.1.76) /
3241	18.9	2E-205	78.1	1284.7	Sirohydrochlorin ferrochelatase (EC 4.99.1.4)
3242	48.3	2E-237	30.9	1285.9	TonB-dependent receptor
					Sulfite reductase [NADPH]
3243	40.6	4E-247	60.2	2246.2	hemoprotein beta-component (EC 1.8.1.2)
					Sulfite reductase [NADPH]
3244	56.6	1E-252	70.6	3721.9	flavoprotein alpha-component (EC 1.8.1.2)
					Siroheme synthase / Precorrin-2 oxidase (EC 1.3.1.76) /
3245	60.4	4E-246	36.8	1965.9	Sirohydrochlorin ferrochelatase (EC 4.99.1.4) /
					Uroporphyrinogen-III methyltransferase (EC 2.1.1.107)
3246	56.7	2E-279	75.9	4010.2	Cysteine synthase (EC 2.5.1.47)
3247	45.7	3E-271	57.5	2314.9	Serine acetyltransferase (EC 2.3.1.30)

3248	52.1	9E-293	88.8	4434.9	Sulfate adenylyltransferase subunit 1 (EC 2.7.7.4)
3249	42.1	6E-222	62.2	2267.9	Sulfate adenylyltransferase subunit 2 (EC 2.7.7.4)
3250	32.3	4E-159	39.5	1102.4	Phosphoadenylyl-sulfate reductase [thioredoxin] (EC 1.8.4.8) / Adenylyl-sulfate reductase [thioredoxin] (EC 1.8.4.10)
3319	5.8	3.9E-05	1.8	10.1	hypothetical protein
3351	11.7	3E-136	136	1356.6	Hydrolases of the alpha/beta superfamily
3402	4.2	1.2E-35	180.8	614.2	hypothetical protein
3407	29.7	1E-146	142.9	4053.8	Threonine dehydratase biosynthetic (EC 4.3.1.19)
3408	26.5	2E-180	492.2	15373.9	Ketol-acid reductoisomerase (EC 1.1.1.86)
3409	35.9	1E-274	98	3317.2	Acetolactate synthase small subunit (EC 2.2.1.6)
3410	40.4	0	277.8	13606.8	Acetolactate synthase large subunit (EC 2.2.1.6)
3411	29.6	1E-300	295.9	9833.1	Dihydroxy-acid dehydratase (EC 4.2.1.9)
3418	4.2	5.1E-36	102.4	336.1	hypothetical protein
3438	4.1	1.7E-27	83.4	265.6	hypothetical protein
3442	7.9	8E-103	82.4	508.7	Phenylacetic acid degradation protein PaaY
3443	7.1	1E-158	232.4	1466.2	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)
3444	7.6	2E-131	148.6	952.1	Phenylacetic acid degradation protein PaaD, thioesterase
3445	7.8	3E-181	290.6	2077.1	3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157); 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35)
3446	10	2E-196	322.1	3118.4	Enoyl-CoA hydratase (EC 4.2.1.17)
3447	25.2	7E-142	29.1	632.3	Phenylacetate-CoA oxygenase, PaaJ subunit
3448	26.8	1E-164	43.3	979.9	Bem46 protein
3449	21.7	1E-224	128.8	2498.2	Phenylacetate-CoA oxygenase, PaaI subunit
3450	19.9	3E-129	40.1	672.2	Phenylacetate-CoA oxygenase, PaaH subunit
3451.1	12.5	1.5E-59	13.2	134.4	hypothetical protein
3451.2	15.4	2.7E-77	16.2	207.8	hypothetical protein

3452	16.2	2E-237	268.3	4306.7	Phenylacetate-CoA oxygenase, PaaG subunit
3453	10.1	1E-109	99.9	856.1	hypothetical protein
3454	57.4	7E-283	98.2	5454.7	Phenylacetate-CoA oxygenase/reductase, PaaK subunit
3455	52.2	9E-281	53.8	2571	Phenylacetate-coenzyme A ligase (EC 6.2.1.30)
3460	7.8	3.7E-09	2.3	16.1	hypothetical protein
3461	16.9	5.6E-20	2.1	34.9	hypothetical protein
3465	15.6	4E-148	220.4	3297.2	FIG00994095: hypothetical protein
3466	11.4	8E-128	305.2	3380.7	2,4-dienoyl-CoA reductase, mitochondrial precursor (EC 1.3.1.34)
3484	4.5	2E-10	8.8	35.8	hypothetical protein
3521	4.3	1.4E-46	263.7	971.9	hypothetical protein
3576	9.5	1.7E-28	25.2	185.6	putative alpha-dextrin endo-1, 6-alpha-glucosidase
3577	7.8	1.9E-30	24.7	156.7	Cytosol aminopeptidase PepA (EC 3.4.11.1)
3578	11	3.6E-73	23.4	201.2	FIG01092412: hypothetical protein
3579	4.1	2E-14	26	81.3	FIG01092988: hypothetical protein
3580	12.4	2E-66	21.9	205.6	FIG01080692: hypothetical protein
3581	14.3	1.9E-69	14.4	160.9	Enzymatic protein of unknown function
3591	8.4	2.1E-19	5.8	39.9	Predicted L-lactate dehydrogenase, hypothetical protein subunit SO1518
3592	7.7	5.6E-43	19	114.1	Predicted L-lactate dehydrogenase, Iron-sulfur cluster-binding subunit YkgF
3593	22.3	4E-26	4.3	62.4	Predicted L-lactate dehydrogenase, Fe-S oxidoreductase subunit YkgE
3616	4.1	3.1E-40	162.9	525.9	hypothetical protein
3633	4.3	4.9E-36	84.6	285.8	hypothetical protein
3664	5.5	7.3E-58	1341.1	9676.7	Ankyrin 1
3716	5.5	0.00298	2.2	11.4	hypothetical protein
3785	6.5	9.2E-83	251.5	1461.3	hypothetical protein
3786	4.7	9.4E-44	119.1	422.7	putative outer membrane protein, probably involved in nutrient binding

3787	14.9	1E-131	144.7	1905.9	TonB family protein / TonB-dependent receptor
3888	4.2	1E-33	108.2	354.8	hypothetical protein
3943	4.7	3E-116	369.4	1609.3	hypothetical protein
3987	4.3	9.2E-64	232.1	865.5	hypothetical protein
4020	9.8	1.1E-38	729.7	8612.8	Aerobactin siderophore receptor IutA
4030	5	4E-44	169.2	690	hypothetical protein
4031	6.1	5.2E-14	6.7	33.1	hypothetical protein
4055	6.5	1E-116	660.9	4585.6	hypothetical protein
4056	5.7	9E-137	462.9	2574.3	hypothetical protein

Table S7 Genes downregulated at least 4-fold in mouse blood treated NUHP1 cells. a

Locus Tag (BD94_)	Fold change	P-value	Normalized means_CK	Normalized means_blood	Feature ID
0020	-11.1	8.1E-08	13.9	0.9	hypothetical protein
0033	-4.3	0	2570.7	613.1	hypothetical protein
0039	-4.1	1.6E-51	1141.8	255.2	hypothetical protein
0064	-4.1	0	2065.5	488.1	FIG00654445: hypothetical protein
0096	-4.7	1.7E-19	138.7	22.8	Uncharacterized protein HI1736
0125	-4.5	5.3E-22	162.9	27.4	hypothetical protein
0158	-15.2	0	16252.9	1396	Putative uncharacterized protein TTHA1760
0159	-15.9	0	8516.8	649.7	FIG00649706: hypothetical protein
0160	-15	5E-227	5362.7	380.2	ABC-type Fe ³⁺ transport system protein; Molybdenum transport protein, putative
0161	-15.9	0	19685.2	1593.9	Molybdopterin oxidoreductase (EC 1.2.7.-)
0162	-16.6	0	42412.9	3476.8	Molybdopterin oxidoreductase, iron-sulfur binding subunit (EC 1.2.7.-)
0163	-21.5	0	21025.9	1167.4	Molybdopterin oxidoreductase subunit, predicted; chaperone protein HtpG
0231	-6.6	1.5E-29	153.7	17.8	FIG00495995: hypothetical protein
0242	-4.7	8.3E-58	763.8	136.2	Beta-lactamase (EC 3.5.2.6)
0250	-4.2	0	13342.3	4809.4	SSU ribosomal protein S2p (SAe)
0252	-4	0	13392.6	5089.7	Translation elongation factor Ts
0279	-4	8.2E-13	420.4	83.4	FIG01092102: hypothetical protein
0316	-12.3	2E-209	4259.8	363.4	Succinate dehydrogenase cytochrome b subunit
0317	-8.8	0	11019	1637.4	Succinate dehydrogenase flavoprotein subunit (EC 1.3.99.1)
0318	-8.9	0	4944.2	603.3	Succinate dehydrogenase iron-sulfur protein (EC 1.3.99.1)
0339	-7.9	1.6E-31	1285.9	147.2	hypothetical protein
0366	-6.7	0	11830.8	2395.5	LSU ribosomal protein L11p (L12e)
0367	-6.9	0	19221.4	3817.2	LSU ribosomal protein L1p (L10Ae)
0368	-7.9	0	15713.7	2762.4	LSU ribosomal protein L10p (P0)
0369	-8.1	0	10047.9	1649.9	LSU ribosomal protein L7/L12 (P1/P2)
0416	-4	0	18057.8	6944.2	Cell division trigger factor (EC 5.2.1.8)

0425	-9.7	0.0038	4.3	0.2	hypothetical protein
0520	-7.6	6.7E-70	465.8	49.8	Cobalt-zinc-cadmium resistance protein CzcA; Cation efflux system protein CusA
0521	-11.9	1.4E-44	243.2	15.6	Cobalt-zinc-cadmium resistance protein CzcA; Cation efflux system protein CusA
0522	-6.4	8.6E-37	164.4	19.5	Probable Co/Zn/Cd efflux system membrane fusion protein
0549	-4.6	1.4E-09	4103.4	1064.7	Heat shock protein 60 family co-chaperone GroES
0679	-23.7	7.4E-21	179.7	5.9	conserved hypothetical protein
0774	-16	3.9E-84	409.1	20.3	hypothetical protein
0789	-4.5	0	6225	1675.8	hypothetical protein
0800	-4	0	3694.4	1046.3	NADH-ubiquinone oxidoreductase chain M (EC 1.6.5.3)
0801	-4.1	0	3474.9	974.3	NADH-ubiquinone oxidoreductase chain M (EC 1.6.5.3)
0802	-4.1	0	4324.9	1219.1	NADH-ubiquinone oxidoreductase chain L (EC 1.6.5.3)
0803	-4.7	6.9E-84	826.5	151.8	NADH-ubiquinone oxidoreductase chain K (EC 1.6.5.3)
0804	-4.5	3E-125	1550.4	326.6	NADH-ubiquinone oxidoreductase chain J (EC 1.6.5.3)
0805	-4.1	5E-126	1712.9	410.7	NADH-ubiquinone oxidoreductase chain I (EC 1.6.5.3)
0807	-4	0	3254.2	892.3	NADH-ubiquinone oxidoreductase chain G (EC 1.6.5.3)
0808	-4.4	0	2975.2	721.1	putative outermembrane protein
0810	-4.6	1.2E-68	1247.6	248.2	NADH-ubiquinone oxidoreductase chain E (EC 1.6.5.3)
0811	-4.2	0	4105.9	1107.6	NADH-ubiquinone oxidoreductase chain D (EC 1.6.5.3)
0812	-4.7	8E-144	1741.9	358.2	NADH-ubiquinone oxidoreductase chain C (EC 1.6.5.3)
0813	-4.8	2E-128	1453.8	283.3	NADH-ubiquinone oxidoreductase chain B (EC 1.6.5.3)
0814	-5.6	3E-124	1223.8	196.1	NADH ubiquinone oxidoreductase chain A (EC 1.6.5.3)
0820	-8.7	5E-173	4181.8	511.4	ATP synthase F0 sector subunit c
0822	-4.1	0	3098.7	818.7	ATP synthase delta chain (EC 3.6.3.14)
0849	-5.6	8.4E-05	10.3	1.3	hypothetical protein
0891	-7.1	1E-116	2556.4	357.7	hypothetical protein
0894	-28.4	4.6E-63	2373.6	81.2	RND efflux system, outer membrane lipoprotein, NodT family

0895	-21.8	9.6E-62	4599.4	216.7	RND efflux system, inner membrane transporter CmeB
0896	-18	5E-110	1976.6	103	efflux transporter, RND family, MFP subunit
1026	-4.5	2.2E-39	368.6	66.6	hypothetical protein
1027	-5.1	9.2E-06	15.2	2.2	hypothetical protein
1062	-4.1	0	1850.7	442.3	Alkylphosphonate utilization operon protein PhnA
1132	-8.7	5.2E-44	1712.6	183.7	Lipoprotein
1169	-4.2	2.1E-25	183.8	33.7	hypothetical protein
1191	-6.5	1.2E-16	92.6	10.7	acetyltransferase, GNAT family
1234	-7.1	5.8E-05	565.1	68.3	hypothetical protein
1235	-46.9	2.8E-13	1297.2	25.7	TonB-dependent receptor
1238	-5.9	0	4850.8	930.5	hypothetical protein
1255	-4.7	2.8E-07	22.9	4.2	hypothetical protein
1288	-17.8	6.3E-13	21.6	0.7	hypothetical protein
1289	-4.5	6.4E-27	202	35	hypothetical protein
1293	-5.9	0	12041.6	2788.3	unknown
1295	-4.9	8E-142	2332.1	467.7	Acid phosphatase (EC 3.1.3.2)
1303	-5.8	1.8E-75	571.6	81.7	Protein AraJ precursor
1329	-4.1	1.7E-20	126.9	23.9	Imidazole glycerol phosphate synthase amidotransferase subunit (EC 2.4.2.-)
1330	-5.2	8.1E-48	398.2	62	Histidinol-phosphatase (EC 3.1.3.15) / Imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19)
1331	-4.4	1.2E-25	404.9	72.9	Histidinol-phosphate aminotransferase (EC 2.6.1.9)
1332	-4.4	6.3E-34	272.1	48.9	Histidinol dehydrogenase (EC 1.1.1.23)
1335	-7.1	2E-165	2426.6	337.1	3-oxoacyl-[acyl-carrier-protein] synthase, KASII (EC 2.3.1.179)
1349	-8.6	2.2E-85	405.8	38.2	hypothetical protein
1350	-17.6	6E-107	347.1	15.6	hypothetical protein
1351	-12.8	1E-159	1037.3	72.9	hypothetical protein
1364	-5.6	9E-153	1674.8	286.4	cytosolic long-chain acyl-CoA thioester hydrolase family protein
1369	-12.7	1.3E-74	2420.7	180.7	Proteinase inhibitor I11, ecotin precursor
1432	-4	0	3991.6	1113.5	Probable low-affinity inorganic phosphate transporter
1433	-4.7	0	4723.8	1171.4	Phosphate transport regulator (distant homolog of PhoU)

1482	-5.3	9.4E-25	100.4	14.3	AraC family regulatory protein
1600	-5.8	7.2E-30	129.6	16.6	AraC family regulatory protein
1609	-7.4	0	3963.5	565.9	hypothetical protein
1673	-4	3.6E-42	678.9	141.4	hypothetical protein
1759	-6.4	2.2E-35	143.8	17.3	beta-lactamase domain protein
1760	-7.3	4.9E-33	133.6	13.9	Permeases of the major facilitator superfamily
1777	-4.1	4.1E-43	669.1	135.3	Adenosine deaminase (EC 3.5.4.4)
1913	-4.8	4.7E-26	277.7	44.4	Hydrolase, haloacid dehalogenase-like family
1973	-12.6	3E-119	3185	253.6	hypothetical protein
2042	-15.5	1.8E-98	1105.2	62.9	hypothetical protein
2176	-28	1.4E-11	912.1	29.6	Putative outer membrane protein, probably involved in nutrient binding
2177	-9.1	8.8E-08	261.3	23.7	hypothetical protein
2280	-15.1	4E-110	731.8	42.8	hypothetical protein
2374	-5.3	1.8E-58	556.7	86.1	protein of unknown function DUF306, Meta and HsIJ
2427	-59.8	2E-169	21990.1	414.4	hypothetical protein
2428	-120.7	1E-196	31923.8	279.2	hypothetical protein
2430	-15.3	2.3E-70	2345.1	145.7	hypothetical protein
2475	-5	0	10157.9	2800.5	LSU ribosomal protein L9p
2477	-14.6	0	9504.7	787.6	hypothetical protein
2495	-10.6	1.8E-64	372	28.4	hypothetical protein
2500	-6.3	2.9E-11	53.7	6.9	RagB/SusD domain protein
2533	-8.7	1.2E-74	2351	263.2	Predicted sucrose-specific TonB-dependent receptor
2534	-10	3.8E-30	1452.1	135	Trehalase (EC 3.2.1.28)
2535	-4.4	1.1E-28	321.1	57.8	Predicted trehalose permease, MFS family, FucP subfamily
2553	-5.1	2.5E-64	863.4	147.6	Permease of the drug/metabolite transporter (DMT) superfamily
2620	-4.6	0	3509.9	848.4	Probable acyl-ACP desaturase, Stearoyl-ACP desaturase (EC 1.14.19.2)
2640	-5.3	1E-128	1499.7	265.8	Vitellogenin II precursor
2697	-7.2	5.9E-11	44.1	4.8	TonB-dependent receptor, putative
2698	-6.8	5.2E-30	346.4	40.3	Drug resistance transporter EmrB/QacA subfamily
2704	-5.2	0	4388.5	941.8	Succinoglycan biosynthesis protein
2834	-534.9	0	4139.7	8.4	Mg(2+) transport ATPase protein C

2835	-405	2E-221	19779.3	51.7	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)
2836	-989.8	0	15707.6	17.9	Probable Co/Zn/Cd efflux system membrane fusion protein
2837	-573.4	3E-217	38638.2	69.1	Cobalt-zinc-cadmium resistance protein CzcA; Cation efflux system protein CusA
2838	-370.4	0	13565.7	42.9	Heavy metal RND efflux outer membrane protein, CzcC family
2872	-20.6	3.4E-63	5923.5	312.6	hypothetical protein
2891	-12.9	8E-213	6050.5	519	Aspartokinase (EC 2.7.2.4)
2912	-13.6	2.4E-65	2540.9	178.1	hypothetical protein
2967	-6.4	8.7E-79	613.5	80	hypothetical protein
2983	-6.1	2.2E-57	305.4	39.7	Methionine transporter MetT
2984	-6	2.2E-23	85.7	10.6	hypothetical protein
3074	-4.5	1E-53	1456.1	306.3	putative lipoprotein
3186	-4.3	9.5E-09	43.9	7.4	DNA repair protein RadC
3227	-15.3	1.4E-55	2131.4	129.8	hypothetical protein
3238	-6.3	3.6E-34	174.6	21.4	hypothetical protein
3334	-41.9	1.5E-16	2970.3	70.7	Oar protein
3400	-27.3	1E-150	1827.3	62.2	hypothetical protein
3403	-10.2	0	2899.2	284	hypothetical protein
3404	-6.8	0	3654.4	586.5	SusC, outer membrane protein involved in starch binding
3512	-5.6	3E-14	48.2	6.5	hypothetical protein
3513	-6.7	2.6E-29	93.3	10.9	hypothetical protein
3514	-6.6	4.7E-29	113.3	13.2	Probable cytochrome-c peroxidase (EC 1.11.1.5)
3515	-6.4	2.6E-27	114.7	13.2	hypothetical protein
3516	-37.3	2E-137	854.9	19.9	hypothetical protein
3601	-4.3	1.7E-44	617.8	117.7	O-acetylhomoserine sulfhydrylase (EC 2.5.1.49) / O-succinylhomoserine sulfhydrylase (EC 2.5.1.48)
3602	-5.6	6E-105	856.9	132.7	Aspartokinase (EC 2.7.2.4) / Homoserine dehydrogenase (EC 1.1.1.3)
3603	-8.7	1.9E-46	186.3	15.7	Aspartokinase (EC 2.7.2.4) / Homoserine dehydrogenase (EC 1.1.1.3)
3617	-4	0	2347.6	589.8	FIG000859: hypothetical protein YebC
3621	-4.1	2.2E-64	962.2	210.7	peptidase M48, Ste24p

3641	-5.6	0	17923.3	4638.9	LSU ribosomal protein L15p (L27Ae)
3642	-7.6	0	4862.1	708.3	LSU ribosomal protein L30p (L7e)
3643	-7.1	0	14655.8	2937.7	SSU ribosomal protein S5p (S2e)
3644	-7.1	0	11590	2161	LSU ribosomal protein L18p (L5e)
3645	-7.4	0	20691.2	3600.9	LSU ribosomal protein L6p (L9e)
3646	-7.5	0	13112.5	2438.5	SSU ribosomal protein S8p (S15Ae)
3647	-6.9	0	3782.2	592.6	SSU ribosomal protein S14p (S29e) @ SSU ribosomal protein S14p (S29e), zinc-independent
3648	-6.8	0	20370	3998.8	LSU ribosomal protein L5p (L11e)
3649	-6.6	0	11143.6	2285.5	LSU ribosomal protein L24p (L26e)
3650	-6.9	0	14221.8	2921.4	LSU ribosomal protein L14p (L23e)
3651	-6.6	0	7325.6	1367.7	SSU ribosomal protein S17p (S11e)
3652	-6.4	0	2858.4	460.1	LSU ribosomal protein L29p (L35e)
3653	-6.3	0	10202.6	2148.1	LSU ribosomal protein L16p (L10e)
3654	-5.9	0	32325.2	7069.4	SSU ribosomal protein S3p (S3e)
3655	-5.9	0	13789.9	3379.1	LSU ribosomal protein L22p (L17e)
3656	-5.7	0	9380.4	2191.4	SSU ribosomal protein S19p (S15e)
3657	-4.9	0	24646.8	6840.6	LSU ribosomal protein L2p (L8e)
3658	-4.7	0	5087	1260.1	LSU ribosomal protein L23p (L23Ae)
3659	-4.3	0	16070.2	5667.1	LSU ribosomal protein L4p (L1e)
3676	-4	0	9652.8	3303.6	SSU ribosomal protein S10p (S20e)
3688	-29.5	0	23003.1	893.2	hypothetical protein
3702	-4.1	5.7E-10	32.8	6.3	hypothetical protein
3758	-8.9	0.00697	4.2	0.2	hypothetical protein
3783	-10.5	0	6893.3	793.4	putative outer membrane protein, probably involved in nutrient binding
3784	-7.2	0	11108.6	2124.4	SusC, outer membrane protein involved in starch binding
3809	-4.4	0	25148.6	8493.9	FIG01289191: hypothetical protein
3843	-4	1.1E-25	222.4	43.9	Thiamin-regulated outer membrane receptor Omr1
3848	-5.5	0	23229.7	5447.8	Aconitate hydratase (EC 4.2.1.3)
3862	-4.3	9.4E-50	752.6	150.4	Acetylornithine deacetylase (EC 3.5.1.16)

3863	-6.7	1.4E-93	473.2	57	Acetylglutamate kinase (EC 2.7.2.8)
3864	-14.1	2E-141	755.2	47.2	Ornithine carbamoyltransferase (EC 2.1.3.3)
3865	-12	1E-130	847.8	62.7	Acetylornithine aminotransferase (EC 2.6.1.11)
3866	-8.8	6E-102	808.1	79.6	N-acetyl-gamma-glutamyl-phosphate reductase (EC 1.2.1.38)
3867	-7	5E-114	1178.3	150.2	Argininosuccinate synthase (EC 6.3.4.5)
3868	-4.2	8.4E-21	147.4	27.5	FIG00651573: hypothetical protein
3873	-5.4	1E-109	1098.7	182.8	Type cbb3 cytochrome oxidase biogenesis protein CcoS, involved in heme b insertion
3874	-6.5	0	18819.8	4098.7	Cytochrome c oxidase subunit CcoN (EC 1.9.3.1) / Cytochrome c oxidase subunit CcoO (EC 1.9.3.1)
3875	-5.5	5.5E-75	793.3	123.6	hypothetical protein
3876	-6.6	0	7300.2	1398.2	Cytochrome c oxidase subunit CcoP (EC 1.9.3.1)
3877	-5.5	1E-132	2953.5	552.1	Type cbb3 cytochrome oxidase biogenesis protein CcoG, involved in Cu oxidation
3878	-5.6	2E-100	695.4	105.2	hypothetical protein
3879	-5.4	1E-122	1092.1	181.7	membrane-bounded cytochrome biogenesis DsbD/cycZ-like domain
3887	-5.5	0	3352.7	655.6	ATP-dependent protease La (EC 3.4.21.53) Type I
3889	-4.8	2.2E-17	70.1	11	putative lipoprotein
3901	-4.5	1.2E-17	74.1	12.7	Peptide chain release factor homolog
3904	-4.3	2.1E-21	268.9	49.6	hypothetical protein
3911	-21.3	8E-268	5249.7	256.2	Fumarate hydratase class I, aerobic (EC 4.2.1.2)
3955	-6.5	0	15652.6	3515.2	LSU ribosomal protein L25p
3995	-7.4	3E-147	2550.8	338.6	Glutamyl-tRNA reductase (EC 1.2.1.70)
3996	-7.2	8E-112	1641.2	217.5	Porphobilinogen deaminase (EC 2.5.1.61)
3997	-6.5	2E-101	883	117.7	Uroporphyrinogen-III synthase, divergent, Flavobacterial type (EC 4.2.1.75)
3998	-5.1	2E-137	1702.4	325.4	Uroporphyrinogen III decarboxylase (EC 4.1.1.37)
4026	-5.9	1E-18	57.1	7.2	hypothetical protein
4027	-8.4	1.8E-36	136.1	12.2	hypothetical protein
4029	-4	4.8E-13	70.4	12.5	hypothetical protein