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Supplemental Information

Strains of *Staphylococcus aureus* that Colonize and Infect Skin Harbor Mutations in Metabolic Genes

Karen P. Acker, Tania Wong Fok Lung, Emily West, Joshua Craft, Apurva Narechania, Hannah Smith, Kelsey O'Brien, Ahmed M. Moustafa, Christine Lauren, Paul J. Planet, and Alice Prince

Table S1. Primers used for PCR amplification. Related to Figure 1.

Locus	Primer Sequence (5'-3')	Amplicon size	Source
spa	AACATAGAATTCGCGCAACACGATGAAG CCAATACTCGAGTAGTTCGCGACGACG	**1.3 kb**	**This study**
sea	TTGGAAACGGTTAAAACGAA GAACCTTCCCATCAAAAACA	**120 bp**	**Johnson et al., 1991**
seb	TCGCATCAAACTGACAAACG GCAGGTACTCTATAAGTGCC	**478 bp**	**Johnson et al., 1991**
sek	GGAGAAAAGGCAATGAA TAGTGCCGTTATGTCCA	**516 bp**	**Bania et al., 2016**
Seq	GGAATTACGTTGGCGAA AACTCTCTGCTTGACCA	**330 bp**	**Bania et al., 2016**
sek* and *seq	TAGCATATGCTGATGTAGG AAATATCGACATCCAAATGG	**1.5 kb**	**This study**
IS256	CTAATGGAAAATCAACGAACAG GAATAATCTTTTCTCTTCTGCG	**955 bp**	**This study**
PVL	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAASTGTATTGGATAGCAAAAGC	**433 bp**	**This study**
speG	GCCTTATGAATCCTTAACGGAAC TCTGTTTTAAATCCTTGTGACTCG	**498 bp**	**This study**

Table S2. Genome sequence analysis. Related to Figure 1.

Strain	Strain	Coverage on reference	Reference positions touched	de novo contigs >200 nucs	N50	Total assembly size	accession
PE001	6-201	129.5097133	0.955708401	53	148454	2796914	SAMN10689348
PE002	16-660	308.4103519	0.929297595	49	120637	2712274	SAMN10689358
PE003	23-977	226.33487	0.910541732	57	115112	2828524	SAMN10689363
PE004	24-859	190.2588002	0.915070234	416	12627	2695998	SAMN10689365
PE005	31-921	146.1693183	0.939041705	68	103413	2782563	SAMN10689370
PE006	40-808	177.5429384	0.918942955	56	105735	2796571	SAMN10689380
PE007	44-398	206.621994	0.901813315	74	115135	2757172	SAMN10689385
PE008	48-634	390.3148304	0.918614717	71	84800	2880647	SAMN10689389
PE009	43-121	351.3036432	0.956912404	72	86961	2736934	SAMN10689383
PE010	64-515	166.089783	0.916041721	70	77982	2726169	SAMN10689408

Table S3. Primers used for qRT-PCR. Related to Figure 2.

Gene	Primer sequences (5' – 3')
gltA	Forward: GATCTAGCTGAAAACGCGCA Reverse: CTGTCATTGGATGCACGTGA
acnA	Forward: GCAAACATGGCACCAGAGTA Reverse: TTTGGTCCTGAAAGCGATGC
Icd	Forward: CCAGCTGAGCATGATGTTGT Reverse: GGAGCTGTACCATGTGTTGC
sucA	Forward: GCCGTGTTACATGATGAGCA Reverse: CACCATATTGCGCTTCCCAA
sucB	Forward: TGCTATCATCGGCGAAGGTA Reverse: TGACACCATTTTCACGAGCA
sucC	Forward: TAGAGGTAAAGCAGGCGGAG Reverse: CCCTTCTTCAGACGCCATCA
sucD	Forward: GGACCACGTATGCCAGAGAA Reverse: CTGTAAGCCACCAGTACCCA
sdhB	Forward: GGACCACGTATGCCAGAGAA Reverse: CTGTAAGCCACCAGTACCCA
sdhA	Forward: TGTATGGTGGCGACTTCCTT Reverse: TTGTTGTCCAGTTGTTGCCC
fumC	Forward: ATGCTTGACCGTTGCGAAAT Reverse: AGCGCCTTCAATGTTCCATG
menD	Forward: ATCGTTTCAACTGCACTGGG Reverse: AAATACCGCCACCATCGTTG
hemA	Forward: GCCAGGGTTAAGCGAAAGAG Reverse: CTTCGCTTGCTCATGAGGAC
hemB	Forward: TTGCTGAAATTCGTCGTGGA Reverse: TCATCATGTCGCACCCTTCT
16S rRNA	Forward: GCGCTGCATTAGCTAGTTGGT Reverse: GGCCGATCACCCTCTCA

Figure S1.

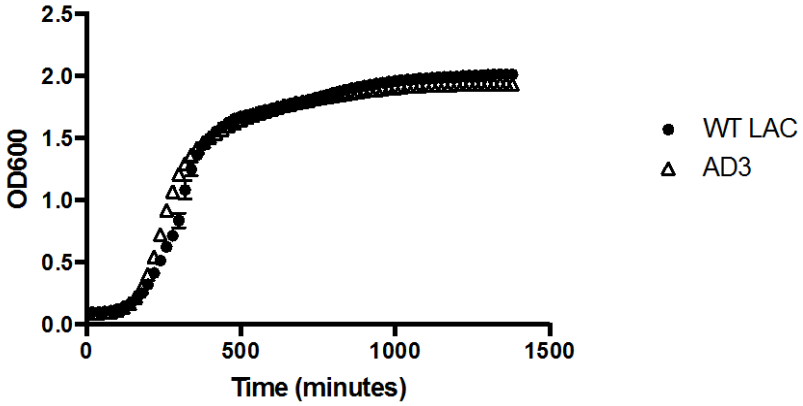

Figure S1. Growth curves of AD3 and WT LAC growth curve grown aerobically in LB. Related to Figure 4 .

Transparent Methods

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Alice Prince (asp7@cumc.columbia.edu).

Experimental Model and Subject Details

Human

Isolates of *S. aureus* were obtained from male and female patients aged 0 to 18 years with AD who presented to Columbia University Medical Center Department of Dermatology. To screen patients for *S. aureus,* a dry, sterile swab (rayon with Amies transport medium, HealthLink) was used to culture from the anterior nares, perianal skin, and one or more skin areas with clinically active AD, clinical evidence of infection, or eroded skin. Swabs were sent to the institution's microbiology lab for routine processing and testing. Severity of AD was quantified by EASI score (Hanifin et al., 2001). Exclusion criteria for screening included age greater than 18 years, inconclusive diagnosis of atopic dermatitis, evidence of acute systemic illness, current use of systemic antibiotics, fungal or viral skin infection, and any individual who declined to participate. Inclusion criteria for microbiological evaluation included laboratory detection of *S. aureus* on culture. All data were collected following approval from Columbia University Institutional Review Board (AAAI5956). Informed consent was obtained from all subjects.

Animal

Male and female mice C57BL/6J mice were purchased from the Jackson Laboratory and were housed under standard conditions at Columbia University Medical Center in New York, NY. All animal experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the NIH, the Animal Welfare Act, and U.S. federal law. The Institutional Animal Care and Use Committee of Columbia University approved the protocol (AAAR5412). None of the mice used in our experiments were previously used for other procedures. Six- to 8-week-old healthy sex-matched mice were inoculated intradermally on the back with $1x10^7$ CFUs *S. aureus* WT LAC or clinical isolates in 100 mL PBS. PBS alone was used as a control. For the memory experiments, mice were initially infected with PBS, WT LAC, or clinical isolate AD3 and re-challenged with WT LAC on day 28 after initial infection. Dermonecrosis areas were measured daily up to 10 days. Punch biopsies (5 mm)

were taken at the site of dermonecrosis and homogenized through 40-mm filters for recovery of *S. aureus*, immune cells by flow cytometry, or supernatant for cytokine analysis.

Cell lines

Human primary keratinocytes (HEKn) were purchased from Gibco and grown in DermaLife K complete medium (Lifeline Cell Technology) supplemented with growth factors (DermaLife K LifeFactors kit, Lifeline Cell Technology), 1% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Corning) in a humidified incubator at 37°C with 5% CO2 and were used up to passage 8. HEKn cells were plated 24hrs prior to infection without antibiotics to achieve 3x10^5 cells/ml for Seahorse analysis or 4x10^5 cells/ml for all other experiments.

Bacterial strains

S. aureus strains were grown at 37°C with shaking in Luria-Bertani broth (LB, BD). Bacterial inocula were estimated based on OD_{600} and verified by retrospective plating on LB agar plates to determine colony forming units (CFU). *S. aureus* recovered from mouse skin biopsies were serially diluted and plated on BBL CHROMagar Staph aureus plates and enumerated by counting CFUs.

Method Details

Gene detection

Spa types were obtained by PCR amplification and sequencing of the variable 16S ribosomal rRNA gene region, and assigned according to the Ridom web server (http://www.spaserver.ridom.de). Isolates were assigned a clonal complex using whole genome sequencing (see below). PCR amplification was performed to screen for genes encoding staphylococcus protein A (*spa*), staphylococcal enterotoxin A (*sea*), VB (*seb*), VK (*sek*), VQ (*seq*), presence of both *sek* and *seq*, exotoxin G (*speG*), *IS256*, and Panton-Valentine leucocidin (*PVL*) using an Applied Biosystems Veriti 96-Well Thermal Cycler (Life Technologies). Primer sets are available in Table S1. Genomic DNA was prepared from liquid culture following digestion with 50µM Tris-HCL-10 µM EDTA, 0.3 mg/ml lysostaphin, and 8 units/µL mutanolysin for 1 hour at 37 ºC. DNA was extracted using a DNA Blood and Tissue kit (Qiagen) or Wizard Genomic DNA Purification Kit (Promega). PCR was performed in a 50-µL (for *spa*) or 20-µL (for all other gene targets) reaction mixture at the following amplification for 30 cycles: 30 sec-1 m of denaturation at 95°C, 1

m of annealing at 55°C, 2-4 m of extension at 72°C. All Genomic preparations were outsourced for sequencing of *spa* (Genewiz, Inc.).

Whole genome sequencing

Whole genome sequencing was performed on ten *S. aureus* samples cultured from the skin of patients with high and low EASI scores. DNA was quantified using the Qubit dsDNA High Sensitivity Assay Kit and Qubit Fluorometer (Invitrogen, Life Technologies). Indexed paired-ended libraries were prepared from purified DNA using Nextera XT DNA Sample Preparation Kit according to the manufacturing directions though during PCR clean-up the samples were resuspended in 32.5 µL Resuspending Buffer and only 30 µL of the sample was transferred to the Clean Amplified NTA Plate. The resulting libraries were quantified using the Qubit dsDNA High Sensitivity DNA Kit. Samples were sequenced using pooled paired-end 250 bp runs on the MiSeq Benchtop Sequencer (Illumina Inc.) and data were generated in fastq files for subsequent analysis.

Genome sequence analysis

The paired-end tag reads were assembled *de novo* using ABySS 1.3.2 software and the assembly parameter k=47 (Simpson et al., 2009) (Table S2). Genomes were annotated and compared to known *S. aureus* sequences using the Rapid Annotation using Subsystem Technology (RAST) server (Aziz et al., 2008). Sequences were investigated by employing the protein-specific Basic Local Alignment Search Tool (BLASTP) within GenBank (Altschul et al., 1997) and were considered present if sequence identity was ≥95% with reference gene sequence. Genomes were assigned sequence types (ST) and clonal complexes (CC) by the *S. aureus* MLST typing scheme (available at: https://pubmlst.org/saureus/) (Jolley and Maiden, 2010) using the MLST typing perl script v. 2.16.1 for contigs (available at: https://github.com/tseemann/mlst) (database updated 12/12/2018).

Phylogenetic Analysis

We produced two kinds of phylogenetic matrices for comparison, (*i*) a nucleotide reference-based SNP matrix (NUC-SNP), (*ii*) an amino acid-based, concatenated, orthologous, open-reading-frame matrix (AA-ORF). For the NUC-SNP matrix, SNP calling was performed using SAMtools and MUMmer software on previously sequenced whole gene alignments against the reference genome of USA300 strain TCH1516 (Kurtz et al., 2004; Li et al., 2009). We excluded SNPs that were not biallelic, listed as “heterozygous”, or

had a per base Q score greater than or equal to 20. For preassembled genomes available from public databases, we used whole-genome alignment with reference to the *S. aureus* TCH1516 genome using the show-snps utility of NUCmer (http://mummer.sourceforge.net). Phylogenetic matrices were created by combining results of both SNP calling techniques above. We excluded all regions from the reference genome that have been annotated as mobile genetic elements as previously described (Planet et al., 2015), and we also applied a mask that excluded repetitive sequences from the reference genome that were more than 80% identical at the nucleotide level to another portion of the genome over at least 100 bp in length. For the AA-ORF matrix we determined orthologous gene sets from assembled *S. aureus* isolate genomes using a modified version of the OrthologID pipeline (Chiu et al., 2006) that uses OrthoMCL for gene family clustering (Li et al., 2003).

Maximum likelihood (ML) phylogenies were constructed with the POSIX-threads version of RAxML v8.0.19 (Stamatakis, 2006). For NUC-SNP data we used an ascertainment bias correction and a general time-reversible (GTR) substitution model accounting for among-site rate heterogeneity using the Γ distribution and four rate categories (ASC_GTRGAMMA model) for 100 individual searches with maximum parsimony random-addition starting trees. For the AA-ORF matrix we used the PROT_GAMMA_JTT model and the same search parameters. Node support was evaluated with 1000 nonparametric bootstrap pseudoreplicates filtering the optimal ML tree through the bootstrap trees, so that the shown node support values indicate the percent proportion of bootstrap trees that contained a given internode branch. The genome of MSHR1132, a *Staphylococus argenteus* strain, was used as an outgroup to root the tree. Phylogenetic matrices and tree files are available as supplementary materials (Data S1, Data S2, Data S3, Data S4).

Metabolic gene sequence analysis

The ten proteomes of the clinical isolates were used with the WhatsGNU tool basic function (https://github.com/ahmedmagds/WhatsGNU) where each protein was given a GNU (Gene Novelty Unit) score that was the number of times an exact match was found in the *S. aureus* pan proteome database. GNU scores for key components of the TCA cycle, the glycolytic pathway, and terminal components of the electron transport chain were used to produce a heatmap using pheatmap package in R. The GNU

scores of all the proteins in each isolate were then used to produce a histogram in GraphPad Prism v.7. Proteins less than 50 amino acids were excluded from the histograms.

Infection of human primary keratinocytes

HEKn cells were pretreated with 10ug/ml fibronectin 1 hour prior to infection with or without 0.1M 2-deoxyglucose (2-DG) as indicated. HEKn cells were infected with bacteria at an MOI of 50 and incubated at 37°C with 5% CO2, followed by addition of lysostaphin 10ug/ml at 4 hours and cell detachment with Tryple Express at 24 hours. Supernatants were collected and used for cytotoxicity assays, fumarate measurement, or sent to Eve Technologies (Calgary, Canada) for cytokine analysis. Cytotoxicity was determined by LDH assay using multiply frozen and thawed keratinocytes as a positive control. Fumarate was measured from HEKn supernatant at 5 hours of infection using the Fumarate Assay Kit according to the manufacturer's instructions.

Extracellular flux analysis

Glycolysis was measured by Seahorse technology performed according to the XF Glycolysis Stress Test Kit User Guide. HEKn cells were seeded at 30,000 cells/well in a Seahorse XF24 well plate and incubated at 37°C with 5% CO2. A sensor cartridge was calibrated as per the manufacturers' instructions overnight at 37°C without CO2. On the day of infection, the primary keratinocytes were washed once and media was replaced with XF base medium supplemented with 2 mM glutamine and 10 µg/ml fibronectin 1 hour prior to infection. The cells were infected at an MOI of 50 and incubated at 37°C without CO2 for 3 hours. The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using a Seahorse XF24 analyzer. Each measurement cycle consisted of a mixing time of 3 minutes and a data acquisition period of 3 minutes (12 data points). The following reagents were added sequentially at 20-minute intervals: glucose at a final concentration of 10 mM to stimulate glycolysis, oligomycin at 1 µM to suppress oxidative phosphorylation, and 2-DG at 50 mM to inhibit glycolysis. The metabolic activity of the bacteria was determined by adding the same amount of bacteria in XF24 well plates without HEKn cells.

Flow cytometry

Red blood cells were lysed from mouse skin homogenates and immune cells were recovered by centrifugation at 700G at 4°C and washed with FACS buffer (10% FBS and 0.1% sodium azide in PBS).

Cells were stained for 30 min at 4°C in presence of counting beads (Bangs Laboratories) and labeled with a combination of BV605-labelled anti-CD11c, BV421-lalbeled CD86, BV510- labelled CD103, PE-CF594–labelled anti-Ly6C, PerCP-Cy5.5–labelled anti-Ly6G, BV650-labelled anti-NK-1.1, AF700-labelled CD45, APC/Cy7-labeled MHCII, PE-labelled CD207, AF594-labelled anti-CD11b, and Fc block. Analysis of immune cell populations was conducted using BD LSR II (BD Biosciences). Cells in the skin were classified as follows:

Langerhans cells CD45+ CD11b+ CD11c+ MHCII+ CD207+,

Langerhans dendritic cells (DCs) CD45+ CD11b+ CD11c+ MHCII+ CD207+ CD103+,

interstitial macrophages CD45+ Ly6C− CD11b+ CD11c+ MHCII+,

Ly6C- monocytes CD45+ Ly6C− CD11b+ CD11c+ MHCII+,

CD11b+ DCs CD45+ Ly6C+ CD11b+ MHCII+ CD11c+,

neutrophils (PMNs) CD45+ Ly6C+ CD11b+ MHCIILy6G+.

All flow cytometry data were analyzed on FlowJo version 10.

Western blots and Antibodies

HEKn cell lysates were frozen in RIPA (1 mM Tris-HCl, pH7.5, 15 mM NaCl, 0.5 mM 447 EDTA, 0.01% SDS, 0.1% Triton X-100, 0.1% deoxycholate) containing 1x HALT protease inhibitor cocktail (ThermoFisher Scientific). Protein concentrations were quantified using the Precision Red advanced protein kit (Cytoskeleton Inc.) and samples were standardised to a concentration of 100 µg. Blots were run using Bolt mini gels (Invitrogen) and transferred to PVDF membranes (Invitrogen) using the iBlot machine (LifeTechnologies). Antibodies for Hif1α, P-S6, and β-actin were used according to the manufacturer's instructions. Secondary antibodies conjugated to horseradish peroxidase HRP were diluted 1:5000. Images were visualized using a digital chemiluminescent detection imager (ProteinSimple) and ImageJ.

RNA isolation from bacterial cells for qRT-PCR

S. aureus strains were grown overnight and re-inoculated 1:100 in LB at 37°C to an OD_{600} of 1. Bacteria were pelleted, resuspended, and incubated in cell wall lysis mixture (18ug/ml lysostaphin, 8U/ml mutanolysin, 50mg/ml lysozyme in 50uM TRIS-HCl 10uM EDTA pH 7.5) for 30 minutes at 37°C, followed by addition of TRK lysis buffer (Omega Bio-tek). After 10 minutes at room temperature, 70% ethanol was

added and samples were were transferred to E.Z.N.A RNA isolation columns. RNA was isolated following the manufacturer's instructions and treated with DNase using the DNA-free DNA removal kit (Invitrogen). cDNA was synthesized using the high-capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative reverse transcription-PCR (qRT-PCR) was performed using Power SYBR green PCR master mix (Applied Biosystems) in a StepOne Plus thermal cycler (Applied Biosystems). Primers used are listed in Table S3. Data were analyzed using the $\Delta\Delta$CT method.

Quantification and Statistical Analysis

See figure legends for statistical details. Samples without normal distribution were analyzed using the nonparametric Mann-Whitney test. Samples with normal distribution were analyzed with two-tailed Student's t test and one- or two-way ANOVA followed by Dunnet's multiple comparisons test. $p < 0.05$ between groups was considered significant. Outliers were determined by Grubb's test and removed. Statistical analysis was performed using GraphPad Prism Version 7.00 (GraphPad). Data are presented as single points with lines representing mean values or as bar graphs with mean ± SEM.

Data and Software Availability

The sequencing data from this study are available in the NCBI Biosample database under Bioproject number PRJNA520898.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-Hif1α	Cayman Chemical	Cat#10006421
Rabbit monoclonal anti-phospho-S6 ribosomal protein	Cell Signaling	Cat#4858
Mouse monoclonal anti-β-actin	Sigma Aldrich	Cat#A5316
BV605-labeled anti-mouse CD11c antibody	Biolegend	Cat#117333; RRID:AB_11204262
BV421-labeled anti-mouse CD86 antibody	Biolegend	Cat#105031; RRID:AB_10898329
BV510-labeled anti-mouse CD103 antibody	Biolegend	Cat#121423; RRID:AB_2562713
PE-CF594–labeled anti-mouse Ly6C antibody	BD Horizon	Cat#562728
PerCP-Cy5.5–labeled anti-mouse Ly6G antibody	Biolegend	Cat#127615; RRID:AB_1877272
BV650-labeled anti-mouse NK-1.1 antibody	Biolegend	Cat#108735; RRID:AB_11147949
AF700-labeled anti-mouse CD45 antibody	Biolegend	Cat#103127; RRID:AB_493714
APC/Cy7-labeled anti-mouse MHCII antibody	Biolegend	Cat#107627; RRID:AB_1659252
PE-labeled anti-mouse CD207 antibody	Biolegend	Cat#144203; RRID:AB_2561498
AF594-labeled anti-mouse CD11b antibody	Biolegend	Cat#101254; RRID:AB_2563231
Anti-mouse CD16/32 antibody	Biolegend	Cat#101319; RRID:AB_1574973
Goat anti-rabbit-HRP	Abcam	Cat#ab205718
Bacterial and Virus Strains		
Staphylococcus aureus USA300 WT LAC	Anthony Richardson, University of Pittsburgh, USA	N/A

Staphylococcus aureus USA300 WT JE2	Michael Otto, National Institute of Allergy and Infectious Diseases USA	N/A
Staphylococcus aureus USA300 WT JE2 *fumC* transposon mutant	Nebraska Transposon Mutant Library	N/A
Staphylococcus aureus AD#1	Isolate from patient	N/A
Staphylococcus aureus AD#2	Isolate from patient	N/A
Staphylococcus aureus AD#3	Isolate from patient	N/A
Staphylococcus aureus AD#4	Isolate from patient	N/A
Staphylococcus aureus AD#5	Isolate from patient	N/A
Staphylococcus aureus AD#6	Isolate from patient	N/A
Staphylococcus aureus AD#7	Isolate from patient	N/A
Staphylococcus aureus AD#8	Isolate from patient	N/A
Staphylococcus aureus AD#9	Isolate from patient	N/A
Staphylococcus aureus AD#10	Isolate from patient	N/A
Chemicals, Peptides, and Recombinant Proteins		
Human fibronectin	Corning	Cat#354008
2-Deoxyglucose	Sigma-Aldrich	Cat#D6134
Lysostaphin	Sigma-Aldrich	Cat#L7386
Power SYBR green PCR master mix	Applied Biosystems	Cat# 4367659
Mutanolysin	Sigma-Aldrich	Cat# M9901
Critical Commercial Assays		
LDH Cytotoxicity Assay	Roche	Cat#11644793001
Fumarate Assay Kit	Abcam	Cat# ab102516
Seahorse XF Glycolysis Stress Test Kit	Agilent	Cat# 103020-100
Qubit dsDNA High Sensitivity Assay Kit	Invitrogen	Cat# Q32851
Nextera XT DNA Library Preparation Kit	Illumina	Cat# FC-131-1024
E.Z.N.A Total RNA Isolation kit	Omega Bio-tek	Cat# R6834-02

Deposited Data		
Experimental Models: Cell Lines		
Human primary keratinocytes, neonatal (HEKn)	Gibco	Cat#C0015C
Experimental Models: Organisms/Strains		
C57BL/6J	Jackson Laboratories	Stock No. 000664
Oligonucleotides		
Primers, see Table S***		
Software and Algorithms		
Seahorse XFe24 analyzer	Agilent	N/A
FlowJo	FlowJo	http://flowjo.com
RAST server		http://rast.theseed.org/FIG/rast.cgi
ABySS 1.3.2		http://www.bcgsc.ca/platform/bioinfo/software/abyss
SAMtools		http://samtools.sourceforge.net/

MUMmer		http://mummer.sourceforge.net/
Prism7	Graphpad	http://www.graphpad.com
Other		