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# Transcriptional profiling and metabolomic analysis of *Staphylococcus aureus* grown on autoclaved chicken breast

Joanne M. Dupre[a], William L. Johnson[b], Alexander V. Ulanov[c], Zhong Li[c], Brian J. Wilkinson[d], John E. Gustafson[a,b,*]

[a] Department of Biology, PO Box 30001, MSC 3AF, New Mexico State University, Las Cruces, NM, 88003, United States
[b] Department of Biochemistry and Molecular Biology, 246 Noble Research Center, Oklahoma State University, Stillwater, OK, 74078, United States
[c] Roy J. Carver Biotechnology Center, 407 S. Goodwin, University of Illinois at Urbana, Champaign, IL 61801, United States
[d] School of Biological Sciences, Illinois State University, Normal, IL 61790, USA

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ABSTRACT

Although *Staphylococcus aureus* is a major cause of food poisoning, little is known about its response to growth on food. Utilizing a transcriptional profiling and metabolomics approach, we compared *S. aureus* grown on autoclaved chicken breast (ACB) to Luria broth agar. ACB cultures demonstrated increased expression of genes associated with protein synthesis, cofactors, secondary metabolites, nitrogen and nucleotide metabolism, amino acid transport, and reduced expression of general stress, lipid metabolism, and virulence genes. The ACB culture also displayed characteristics of catabolite de-repression and anaerobic growth, and increased expression of arginine biosynthesis genes (*argFGH*) and an arginine/ornithine antiporter gene (*arcD*). *S. aureus* synthesizes arginine from proline and the ACB culture exhibited increased expression of proline transport genes (*opuBA*, *opuBB* and *putP*) and increased proline accumulation. Amino acid and sugar content in the ACB grown culture increased, and this was attributed to the consumption of ACB, transport of amino acids, and gluconeogenesis. Genes involved with biotin biosynthesis and uptake were upregulated and biotin is required for amino acid catabolism. Genes encoding urease and urease activity were upregulated in ACB cultures, while urea levels were reduced. This research provides fundamental information on the response of *S. aureus* growing on chicken meat that could find application in future attempts to reduce the growth of *S. aureus* in food.

## 1. Introduction

Staphylococcal food poisoning (SFP) caused by *Staphylococcus aureus* is a common foodborne illness, which is due to the production of heat stable staphylococcal enterotoxins (SE) in food that induce severe emetic symptoms (Gustafson and Wilkinson, 2005). Some of the reasons why *S. aureus* is a significant food borne pathogen include: the ability to grow in numerous foods; the possession of a cross-protective stress response system; the access to a pan-genome containing numerous SE genes (Gustafson and Wilkinson, 2005); high rates of human nasal nare and intestinal carriage and ease of airborne spread (Acton et al., 2008; Bischoff et al., 2006); and the ability to survive for long periods on fomites (Kramer et al., 2006).

Meat and poultry products are often implicated in SFP outbreaks (Le Loir et al., 2003). Staphylococci can colonize healthy poultry (Nagase et al., 2002), be isolated throughout poultry processing plants (Huys et al., 2005; Olivier et al., 1996), and SE-producing *S. aureus* strains have been isolated from retail chicken (Kitai et al., 2005a). Multiple antimicrobial-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), have also been isolated from retail chicken (Kitai et al., 2005b). *S. aureus* possesses a number of factors that allow it to thrive on meat produced for human consumption, including proteases that degrade external protein sources (Dubin, 2002) and multiple amino acid and oligopeptide transporters (Hiron et al., 2007; Yu et al., 2014). *S. aureus* can also catabolize at least ten amino acids as carbon and energy sources, and in the process generate essential metabolic intermediates (Halsey et al., 2017).

During catabolite repression in bacteria, the presence of a preferred carbon source causes the repression of genes and operons whose products are involved in the catabolism of less preferred carbon sources (Stulke and Hillen, 1999). Catabolite repression in Gram-positive organisms is under the control of the catabolite control protein (CcpA) in

* Corresponding author. Department of Biochemistry and Molecular Biology, Oklahoma State University, 246 Noble Research Center, Stillwater, OK, 74078-3035, United States.
*E-mail address:* john.gustafson@okstate.edu (J.E. Gustafson).

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a complex with the phosphorylated co-regulator Hpr (Deutscher et al., 1995). Some amino acid catabolic pathways (e.g. proline and arginine) are normally repressed in *S. aureus* by the CcpA-Hpr complex, and are only activated when the organism is growing with non-preferred carbon sources (Li et al., 2010; Nuxoll et al., 2012).

While the response of *S. aureus* to physical-shock, biofilm growth, or antimicrobial-challenge continue to be characterized (Anderson et al., 2006; Cassat et al., 2006; Delgado et al., 2008; Riordan et al., 2007), much less is known about the state of *S. aureus* cells growing on or within a food matrice. This is surprizing when one considers the impact this organism has on the food industry. We now report an autoclaved chicken breast (ACB) *S. aureus* growth model and the transcriptional and metabolic alterations that occur in *S. aureus* grown on ACB compared to growth on a standard laboratory media.

## 2. Materials and methods

### 2.1. Bacterial strains and growth conditions

All methicillin-susceptible and -resistant laboratory and clinical strains utilized in this study are described in Table S1. Strain SH1000, which is a NCTC 8325 derivative wherein a *rsbU* gene deletion was corrected (Horsburgh et al., 2002), was utilized for transcriptional profiling and metabolomic experiments. With the exception of MM43 and LP73, which were 82% identical based on pulsed field gel electrophoresis (PFGE) patterns of SmaI-digested chromosomal DNA, all other MRSA investigated are from distinct PFGE lineages (Delgado et al., 2007). For general liquid culture propagation, overnight cultures (18 h, 37 °C, 200 rpm) were grown in Luria broth (LB; Becton Dickenson, Sparks, MD, USA), diluted 1:100 in fresh LB, and working cultures were grown to mid-exponential phase growth ($OD_{580nm}$ = 0.5–0.6). These cultures were then diluted to an $OD_{580nm}$ = 0.01 for inoculations onto LB agar (LBA) or ACB.

### 2.2. Autoclaved chicken breast growth model, antimicrobial susceptibility determination, and urease production assay

Boneless, skinless chicken breasts (Pilgrim's Pride, Greeley, CO, USA) were purchased from a local supermarket and rinsed briefly with $ddH_2O$ to remove excess package broth. The pieces were then placed in a glass baking dish, covered with aluminum foil, autoclaved (15 min, 121 °C, 15 psi), and then allowed to cool to 25 °C before use.

For CFU enumeration on ACB, ACB was first sliced into individual 1 g pieces which were then placed into sterile 16 mm capped glass test tubes. The ACB pieces were then surface inoculated with 10 µl of the diluted LB cultures which was absorbed into the ACB piece. For comparison, 1 ml LBA in 16 mm sterile capped glass test tubes was inoculated as described above to equal the surface area of inoculated ACB, and the inoculum was allowed to absorb into the agar surface. These cultures were then placed in a 25 °C chilling incubator (Ectotherm, Solana Beach, CA, USA) and allowed to incubate for 18 and 48 h. At these time points during growth 9 ml of sterile PBS (pH 7.4) was added to each culture, which were then subsequently aggressively vortexed (1 min, full speed). The resulting resuspended cultures were then further diluted in PBS and CFUs per ml were determined.

Antimicrobial susceptibility profiles were determined following 24 h growth: MRSA strains were removed by swabbing from the ACB and LBA surfaces, resuspended in 0.85% saline, and antimicrobial disk (Remel, Lenexa, KS, USA) susceptibilities were determined according to standard CLSI guidelines (Clinical and Laboratory Standards Institute, 2005).

Urease assays were carried out utilizing Urea R broth (Difco, Sparks, MD, USA). Briefly, the cells from 18 h ACB and LBA surfaces were resuspended in PBS and adjusted to an $OD_{580nm}$ of 1.0. Fifty µl of these suspensions were then used to inoculate 3 ml of Urea R broth in triplicate. Urease activity was then determined by reading $OD_{560nm}$ following growth at 37 °C for 18 h.

### 2.3. Transcriptional profiling and qRT-PCR analysis

For micoarray and qRT-PCR analyses, 10 g ACB pieces were surface inoculated with 100 µl of diluted ($10^{-6}$) overnight SH1000 LB cultures and cultured in sterile 50 ml polypropylene tubes. For comparison, a 10 ml LBA slant prepared in a 50 ml polypropylene tube was inoculated as described above. At 18 and 48 h, 9 ml of sterile PBS was added to each culture surface, and the cells were aggressively vortexed (1 min, full speed). The resulting resuspended cells were then utilized for RNA isolation. Initially, all cell suspensions were centrifuged (1500 × *g*, 4 °C, 1 min) to sediment debris, and 5 ml of cell suspensions were diluted with PBS to reach a final $OD_{580nm}$ of 1.0. RNA for qRT-PCR and transcriptional profiling experiments was then isolated from these cell suspensions using a bead mill homogenization procedure as previously described (Riordan et al., 2007), following pretreatment of cell pellets with 5 ml of RNA protect (Qiagen Inc., Valencia, CA, USA).

qRT-PCR analysis was carried out on 18 h ACB and LBA cultures utilizing specific primers (Table S2). cDNAs for qRT-PCR analyses were synthesized from DNA*free* (Ambion, Austin, TX, USA) treated RNA using Moloney murine leukemia virus SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) as previously described (Riordan et al., 2007). Real time PCR amplication was performed with the iCycler iQ Real-Time PCR Detection System and iQ SYBR Green Supermix (BioRad Laboratories, Hercules, CA USA) on 3 cDNA biological replicates. The expression level of all samples analyzed by qRT-PCR were normalized to the 16S rRNA gene as an internal control, and the relative fold change in gene expression for each qRT-PCR sample was calculated using the $2_T^{-\Delta\Delta C}$ method (Livak and Schmittgen, 2001).

*S. aureus* DNA microarrays produced by the Pathogen Functional Genomics Resource Center (versions 4 and 5) were used to produce the transcriptional profiles of SH1000 following 18 and 48 h growth on ACB and LBA. cDNA samples were labeled with Cy3 or Cy5 postlabelling reactive dye following the manufacturer's suggestions (Amersham Biosciences, Amersham, Buckinghamshire, UK). Microarray experiments were then performed in duplicate, and fluorophore dyes were swapped to produce dual cDNA samples to minimize dye bias for each cDNA preparation analysed. Hybridized arrays were then scanned with a GenePix 4000B Microarray Scanner (Axon Instruments, Union City, CA, USA) and array TIFF images were analyzed using TIGR-Spotfinder (http://www.tigr.org/software) followed by data normalization with the LOWESS algorithm using TIGR- MIDAS (http://www.tigr.org/software) Significant changes were identified using Significance Analysis of Microarray Data software (Stanford University) which calculates fold changes of differentially expressed genes as positive or negative numbers. Genes that demonstrated ≥2 fold expression changes were considered significant. ORF IDs were mapped to the NCTC-8325 genome when possible, otherwise they were from one of the six *S. aureus* genomes represented on the microarray. Gene functional categories were tailored to facilitate the analysis of this study's results.

### 2.4. Metabolomics

Initially, 500 µl of diluted overnight LB cultures were used to inoculate the surface of 50 g ACB pieces and 50 ml LBA in 500 ml canning jars in triplicate. Following 18 h growth at 25 °C, the cells were resuspended by adding 25 ml of PBS and triturating with a pipette. The cell suspensions were then centrifuged (1500 × *g*, 4 °C, 1 min) to sediment debris and the cells were harvested by centrifugation (16,000 × *g*, 4 °C, for 10 min), washed with 5 ml of PBS, and repelleted. The cell pellets were then resuspended in 5 ml PBS, frozen in liquid nitrogen and lyophilized.

Cell sample extractions were then carried out essentially as described previously, (Roessner et al., 2000, 2001), with minor changes. Ten mg of lyophilized *S. aureus* cell pellets were pulverized in a mill (Qbiogene, Inc. Carlsbad, CA, USA) containing five 3 mm glass beads.

One and a half ml of 70% methanol was then added to the cell powder and the slurry was sonicated for 10 min with a 2510 Branson ultrasonic cleaner (Branson Ultrasonics Corp., Danbury, CT, USA) at 60 °C, followed by another extraction with water (1.5 ml) at room temperature. Both extracts were then combined and dried under vacuum. Prior to GC/MS analysis, the dried extracts were derivatized with 80 µl of methoxyamine hydrochloride (20 mg/ml) for 1 h at 40 °C and then with 80 µl of MSTFA at 65 °C for 40 min.

The GC/MS system utilized was an Agilent 6890N gas chromatograph, an Agilent 5973 mass selective detector, and a HP 7683B autosampler (Agilent Inc., Palo Alto, CA, USA). Gas chromatography was performed on a HP-5MS (30 m × 0.25 mm I.D. and 0.25 µm film thickness) capillary column (Agilent Inc.). The inlet and MS interface temperatures were 250 °C, and the ion source temperature was adjusted to 230 °C. An aliquot of 2 µl was injected with the split ratio of 5:1. The helium carrier gas was kept at a constant flow rate of 1.3 ml $min^{-1}$. The temperature program was an initial 5 min isothermal heating at 70 °C, followed by an oven temperature increase of 5 °C per min to 310 °C and a final 10 min at 310 °C. Mass spectra were recorded in the m/z 50–800 scanning range and the spectra of all peaks were compared with electron impact mass spectrum libraries NIST02 (National Institute of Standards and Technology, Gaithersburg, MD, USA), WILEY7n (Palisade Corporation, Ithica, NY, USA) and the custom library. To allow comparison between samples, all data were normalized to the internal standard (hentriacontanoic acid at 10 mg/ml) in each chromatogram and the dry weight of each sample. The chromatograms and mass spectra were evaluated using the HP Chemstation (Agilent) and AMDIS (NIST) programs. The retention time and mass spectra were implemented within the AMDIS method formats.

### 2.5. Statistical analysis

Mean $\log_{10}$ CFU per ml counts on LBA and ACB were tested for normality and compared by Student's t-test with Holm-Bonferroni correction using JMP Student Edition v10 (SAS Institute, Cary, NC, USA). Metabolite data sets containing 3 independent biological replicates per sample were statistically analyzed by Student's t-test using the algorithm incorporated into Microsoft Excel 2003 (Microsoft Corporation, Seattle, WA, USA). Differences were determined to be statistically significant by *t*-test at $P < 0.05$. Principal component analysis (PCA) was performed using XLSTAT V.2007.4. (Addinsoft, New York, NY, USA) on the transformed relative concentration per gram dry weight for the whole set of identified metabolites.

## 3. Results and discussion

### 3.1. Transcriptional profile of ACB grown S. aureus

All strains investigated grew well on both ACB and LBA (Table 1). Following 18 h growth, SH1000 and MM66 actually grew better on ACB than LBA. Kirby-Bauer disc susceptibility analysis indicated that all antimicrobial resistance phenotypes in the strains investigated (Table S1) were maintained following growth on ACB.

**Table 1**
Comparison of the growth of various *S. aureus* strains on LBA and ACB.

| | 18 h - LBA | 18 h - ACB | 48 h - LBA | 48 h - ACB |
|---|---|---|---|---|
| SH1000 | 8.51 ± 0.004 | 8.84 ± 0.009* | 9.37 ± 0.027 | 9.33 ± 0.063 |
| ATCC25923 | 8.58 ± 0.004 | 8.59 ± 0.013 | 9.71 ± 0.127 | 9.15 ± 0.018 |
| COL | 7.91 ± 0.068 | 8.33 ± 0.035 | 9.62 ± 0.038 | 9.29 ± 0.082 |
| LP73 | 9.32 ± 0.060 | 9.37 ± 0.090 | 9.91 ± 0.032 | 9.75 ± 0.062 |
| MM7 | 9.37 ± 0.035 | 9.40 ± 0.020 | 9.60 ± 0.126 | 10.16 ± 0.094 |
| MM25 | 9.31 ± 0.071 | 9.34 ± 0.068 | 9.58 ± 0.019 | 9.45 ± 0.019 |
| MM43 | 9.17 ± 0.089 | 9.47 ± 0.185 | 9.85 ± 0.073 | 10.14 ± 0.050 |
| MM66 | 8.62 ± 0.043 | 9.38 ± 0.058* | 9.50 ± 0.024 | 9.73 ± 0.040 |
| MV53 | 9.44 ± 0.059 | 9.28 ± 0.143 | 9.87 ± 0.087 | 9.43 ± 0.094 |

Numbers represent mean $\log_{10}$ CFU/g ± standard error.
*Significant change in $\log_{10}$ CFU/g between LBA and ACB cultures ($p \leq 0.002$ for all comparisons, n = 3).

A total of 550 ≥ 2-fold gene expression alterations involving 438 genes occurred following 18 and 48 h growth on ACB compared to growth on LBA (Table S3).

The highest number of altered gene expressions in the 18 h ACB culture belonged to the disrupted/hypothetical/unknown function category (54 genes) followed by the amino acid and protein metabolism (41 genes) (Table S4). These categories were followed by transport and virulence factors (both at 33 genes), cell envelope metabolism/resistances (32 genes), and central metabolism (30 genes) (Table S4).

The largest expression ratios favoring the upregulation of genes in major- and sub-categories following 18 h growth on ACB included: cofactors and secondary metabolites and cofactor and precursor transport (both at 6:1); amino acid transport (3.33:1); and nitrogen metabolism and nucleotide metabolism (both at 2.25:1) (Table S4). Gene expression ratios favoring the downregulation of genes in major- and sub-categories following 18 h growth on ACB included lipid metabolism (0.17:1), protein fate (0.29:1), DNA replication, recombination and repair (0.33:1), and virulence factors (0.43:1) (Table S4).

The highest number of altered genes in the 48 h culture belonged to amino acid and protein metabolism (57 genes) and the disrupted/hypothetical/unknown function category (53 genes) (Table S5). These categories were followed by transport and cell envelope metabolism/resistances (both at 37 genes), protein synthesis (33 genes), and central metabolism and virulence factors (both at 29 genes) (Table S5).

The largest gene expression ratios favoring the upregulation of genes in major- and sub-categories following 48 h growth on ACB included protein synthesis (15.5:1), nucleotide metabolism (7:1); cofactor and precursor transport (6:1); amino acid transport (4:1), and cofactors and secondary metabolites (3.5:1). Gene expression ratios favoring the downregulation of genes in major- and sub-categories following 48 h growth on ACB included stress response (0.17:1), lipid metabolism (0.28:1), and virulence factors (0.45:1) (Table S5).

### 3.2. Genes highly altered during growth on ACB compared to LBA

In line with the transcriptional array data, RT-qPCR confirmed the direction of altered gene expression for *argG*, *bioA*, *bioD*, *fadA*, and *fadB*, after 18 h growth on ACB (Table 2).

Arginine biosynthesis is under catabolite control (Nuxoll et al., 2012) and growth of *S. aureus* on ACB indicated that arginine accumulation was important. For instance, *argH, argG* and *argF*, were all upregulated in *S. aureus* grown on ACB at both time points investigated (Table 2). *arcD* and *arcR* are members of the arginine deiminase *arcABDCR* operon, which is required for the anaerobic catabolism of arginine (Zhu et al., 2007). *arcR* encodes a Crp/Fnr positive regulator that is required under anaerobic conditions for *arcABDCR* induction and the anaerobic catabolism of arginine (Makhlin et al., 2007). ArcR also binds to the promoters of a number of genes that play roles in anaerobiosis (Makhlin et al., 2007). *arcD* encodes an arginine/ornithine antiporter that accumulates arginine in the cell while expelling ornithine into the media (Zhu et al., 2007). Both *arcR* and *arcD* are upregulated at 18 h, yet differences in their expression were not detected at the 48 h ACB time point (Table 2). This finding also supports a need for increased arginine accumulation in the ACB grown culture, at the 18 h time point at least. Proline supports arginine biosynthesis in *S. aureus* and aspects of the transcriptional response of ACB grown cultures indicated a need for proline accumulation. *opuBB* and *opuBA,* which encode components of a proline/glycine betaine transport system (Horn et al., 2005); and *putP,* which encodes a high affinity proline permease (Wengender and Miller, 1995), were all upregulated in the ACB culture at both time points investigated (Table S3).

Urea is produced as proteins are degraded and it was expected that urease would be increased in *S. aureus* grown on ACB. The genes

**Table 2**
Forty genes demonstrating the highest altered expression in SH1000 following growth on ACB compared to LBA.

| Locus ID | Gene | Function | Fold change in microarray | | qRT-PCR $2^{-\Delta\Delta C}$ results |
|---|---|---|---|---|---|
| | | | 18 h | 48 h | 18 h |
| Up-regulated genes | | | | | |
| SAOUHSC_00097 | | putative purine nucleoside phosphorylase | 6.5 | 3.5 | |
| SAOUHSC_00189 | | pseudogene upstream of *pflA* | 5.7 | 5.8 | |
| SAOUHSC_00229 | *scdA* | cell wall metabolism protein | 9.2 | 5.6 | |
| SAOUHSC_00233 | *lrgB* | antiholin-like protein | 11.2 | 9.3 | |
| SAOUHSC_00253 | | hypothetical protein | 3.5 | 5.8 | |
| SAOUHSC_00299 | *yjiH* | YjiH family protein | 8.1 | 4.3 | |
| SAOUHSC_00608 | *adhP* | alcohol dehydrogenase | 5.2 | 5.0 | |
| SAOUHSC_00898 | *argH* | argininosuccinate lyase | 12.3 | 8.3 | |
| SAOUHSC_00899 | *argG* | argininosuccinate synthase | 26.8 | 8.0 | 26.0 |
| SAOUHSC_01128 | *argF* | ornithine carbamoyltransferase | 17.0 | 11.1 | |
| SAOUHSC_01450 | *steT* | serine/threonine exchanger transporter | 15.1 | 4.9 | |
| SAOUHSC_01953 | *epiA* | lantibiotic epidermin precursor | 10.9 | 4.7 | |
| SAOUHSC_02399 | *glmS* | glucosamine-fructose-6-phosphate aminotransferase | 6.2 | 5.2 | |
| SAOUHSC_02468 | *budB* | acetolactate synthase | 10.3 | 5.0 | |
| SAOUHSC_02656 | | hypothetical protein | 5.2 | 3.9 | |
| SAOUHSC_02711 | *queT* | putative pre-queuosine transporter QueT | 11.6 | 6.3 | |
| SAOUHSC_02712 | *bioW* | 6-carboxyhexanoate-CoA ligase | 19.9 | 10.0 | |
| SAOUHSC_02714 | *bioB* | biotin synthetase | 19.7 | 6.1 | |
| SAOUHSC_02715 | *bioA* | adenosylmethionine-8-amino-7-oxononanoate aminotransferase | 25.8 | 13.3 | 27.9 |
| SAOUHSC_02716 | *bioD* | dethiobiotin synthase | 22.7 | 22.0 | 49.6 |
| SAOUHSC_02830 | *ddh* | D-lactate dehydrogenase | 20.0 | 11.0 | |
| SAOUHSC_02866 | | MmpL efflux pump, putative RND superfamily | 10.0 | 2.2 | |
| SAOUHSC_02941 | *nrdG* | anaerobic ribonucleoside-triphosphate reductase activating protein | 10.0 | 7.1 | |
| SAOUHSC_02942 | *nrdD* | anaerobic ribonucleoside-triphosphate reductase | 17.3 | 18.5 | |
| SAOUHSC_02964 | *arcR* | Crp/FNR family transcriptional regulator | 11.5 | ND | |
| SAOUHSC_02967 | *arcD* | arginine/ornithine antiporter | 15.8 | ND | |
| Down-regulated genes | | | | | |
| SAOUHSC_00195 | *fadA* | acetyl-CoA acetyltransferase | −9.7 | −6.0 | 0.1 |
| SAOUHSC_00196 | *fadN* | 3-hydroxyacyl-CoA dehydrogenase | −9.2 | −7.3 | 0.1 |
| SAOUHSC_00198 | *caiC* | long-chain fatty acid CoA ligase | −5.5 | −5.3 | |
| SAOUHSC_00717 | | putative DM13 domain containing lipoprotein | −5.9 | −4.2 | |
| SAOUHSC_01110 | | fibrinogen binding-related protein | −7.1 | −4.7 | |
| SAOUHSC_01114 | *efb* | fibrinogen-binding protein precursor | −6.5 | −5.0 | |
| SAOUHSC_01115 | | fibrinogen-binding protein precursor-related protein | −6.6 | −5.0 | |
| SAOUHSC_01121 | *hla* | alpha-hemolysin precursor | −3.3 | −5.9 | |
| SAOUHSC_02161 | *mapW* | truncated map-w protein | −8.2 | −7.7 | |
| SAOUHSC_02709 | *hlgC* | gamma hemolysin, component C | −5.5 | −11.2 | |
| SAOUHSC_02820 | | putative lantibiotic ABC transporter | −3.3 | −5.3 | |
| SACOL0210 | | hypothetical protein | −5.8 | −5.6 | |
| SACOL2003 | *hlb* | phospholipase C | −5.9 | −3.1 | |
| SACOL2420 | | hypothetical protein | −6.5 | −11.1 | |

encoding urease (*ureABCDEFG*) are upregulated in the ACB culture in a time dependent fashion (Table S3). Urease activity was also increased in SH1000 and ATCC25923 ACB grown cultures (0.326 ± 0.088 and 0.409 ± 0.041, respectively) compared to LBA cultures (0.094 ± 0.008 and 0.140 ± 0.002, respectively, $n = 6$, $p < 0.05$).

Biotin is required for fatty acid biosynthesis, amino acid catabolism and gluconeogenesis (Beckett, 2007). Only *bioF* of the *S. aureus bioDABFWqueT* biotin biosynthesis operon was not upregulated in ACB grown culture (Table 2). In addition, the gene *bioY* which encodes a biotin transporter was upregulated at the 18 and 48 h ACB culture timepoints respectively (Table S3). Our results indicate that the amount of available biotin in ACB is not enough to support the nutritional requirements of *S. aureus* grown under these conditions.

In addition to *arcD* and *arcR*, other altered genes also support the hypothesis that the ACB culture is at least partially anaerobic. Genes encoding the fermentative enyzmes D-lactate dehydrogenase (*ddh*), acteolactate synthetase (*budB*) and alcohol dehydrogenase (*adhP*), were all highly upregulated during growth in ACB, as was a lactate permease (*lctP*) (Table 2 and S3). These genes were also upregulated in *S. aureus* during anaerobic growth (Fuchs et al., 2007). Class III anaerobic ribonucleotide reductases are responsible for the biosynthesis of deoxyribonucleotides during anaerobic growth and biofilm growth (Crespo et al., 2016; Masalha et al., 2001). Two genes located in an operon encoding an anaerobic ribonucleotide reductase (*nrdD*) and the anaerobic ribonucleotide reductase activase (*nrdG*) (Masalha et al., 2001) were upregulated in the ACB grown culture (Table 2).

During biofilm growth, *S. aureus* is thought to grow micro-aerobically or anaerobically and extracts arginine, glutamine, glycine, proline, serine, and threonine from the growth substrate (Beenken et al., 2004; Resch et al., 2005; Zhu et al., 2007). In biofilms there is also evidence that *S. aureus* catabolizes amino acids and releases ammonia (Zhu et al., 2007). It is probable that *S. aureus* growing on ACB mimics many aspects of biofilm grown *S. aureus*. The *lrgAB* operon plays a role in biofilm formation in *S. aureus* (Beltrame et al., 2015; Mann et al., 2009) and *lrgB* is highly upregulated in the ACB culture (Table 2). The main exopolysaccharide of *S. aureus* biofilms is composed of poly-*N*-acetylglucosamine that is synthesized by products of the *icaADBC* operon (Cramton et al., 1999; Mack et al., 1996). *icaR*, which is divergently transcribed from the *icaADBC* operon, encodes a TetR family transcriptional regulator that represses *icaADBC* activity (Conlon et al., 2002; Jefferson et al., 2003). *icaR* was downregulated at both time points investigated for the ACB culture (Table S3).

In line with the increased expression of amino acid transport genes in general, *steT*, which encodes a well-characterized serine/threonine exchanger transporter that weakly exchanges aromatic amino acids as well (Reig et al., 2007), was highly upregulated in the ACB culture at

both time points (Table 2).

With the general downregulation of genes involved with lipid metabolism, we note the downregulation of the genes *fadA*, *fadB* and *caiC* (Table 2), which are members of the operon predicted to be involved in the production of long chain fatty acids. This operon SAOUHSC_00199-*caiC*-SAOHSC_00197-*fadB-fadA* encodes: an acetyl-CoA transferase, CaiC, a long chain acyl-CoA ligase; a glutaryl-CoA dehydrogenase; FadB, a 3-hydroxyacetyl CoA dehydrogenase and FadA, an acetyl-Coa acyltransferase. Chicken meat contains fat (Husak et al., 2008) which is a source of fatty acids, and *S. aureus* can acquire exogenous fatty acids for fatty acid and phospholipid biosynthesis (Parsons et al., 2014a, 2014b). Instead of wasting energy producing long chain fatty acids, it is likely the organism was obtaining these fatty acids from the ACB.

The highly downregulated genes also included virulence factor genes (e.g. *efb*, *hlyI*, and *hlgC*) (Table 2) which is in line with global transcriptional profiling analysis that demonstrated a general reduction in virulence gene expression during growth on ACB (Tables S3, S4 and S5).

### 3.3. ACB metabolome

The relative concentration of 59 metabolites were altered in the 18 h ACB culture compared to LBA culture. Metabolites that demonstrated altered relative concentrations included: 22 amino acids; 13 amines and polyamines; 10 polar organic acids; and 14 sugars (Tables 3 and 4). Some metabolites were found in the same concentration in both ACB and LBA grown cells (e.g. glutamic acid: ACB, 4299.0 ± 47.2 and LBA, 4332.3 ± 473; phenylalanine: ACB, 96.0 ± 3.5 and LBA, 92.4 ± 6.4) while others were not detected in the ACB or LBA grown cultures (Tables 3 and 4).

It is probable that ACB proteins are degraded to individual amino acids by *S. aureus* proteases to be utilized as a carbon and energy source. Of the 11 altered protease genes in Table S3 however, 8 were downregulated and 3 were upregulated. Nonetheless, in line with the notion that *S. aureus* is utilizing amino acids extracted from ACB, 15 amino acids were present in higher concentrations in the ACB grown cells compared to the LBA grown culture (Table 3). *S. aureus* can biosynthesize proline from arginine (Townsend et al., 1996) and proline can be used to generate α-ketoglutarate which in turn supplies carbon to the TCA cycle and produces ATP (Halsey et al., 2017). Proline also acts as an osmoprotectant in *S. aureus* (Townsend and Wilkinson, 1992). Proline, proline-like and hydroxyproline levels were higher in the ACB culture, and hydroxyproline was not even detected in the LBA culture (Table 3). These data compliment the upregulation of proline uptake system genes in the ACB culture (Table S3). We propose that proline was accumulated via the upregulation of *putP*, *opuBA* and *opuBB,* in order to support arginine production or to act as an osmoprotectant. Arginine however was not detected in either the ACB or LBA grown culture metabolomes. The relative concentration of a number of potential intermediates for proline and arginine biosynthesis (asparagine, aspartic acid, ornithine and N-acetyl-glutamic acid) were also decreased in the ACB culture (Table 4). The relative concentrations of both serine and threonine were lower in the ACB culture (Table 4), and *steT* which encodes a serine/threonine exchanger transporter, was

**Table 3**
Metabolites increased ($p < 0.05$) in 18 h SH1000 ACB cultures, compared to LBA grown cultures.

| Metabolite class | Metabolite | Metabolite relative concentration per gram dry weight (mean ± SE) | | Fold increase ACB/LBA |
|---|---|---|---|---|
| | | ACB | LBA | |
| Amino acids | 2-aminobutyric acid | 105.1 ± 3.8 | ND | |
| | alanine | 536.1 ± 35.1 | 100.5 ± 3.8 | 5.3 |
| | B-alanine | 2021.1 ± 81.3 | 45.7 ± 0.5 | 44.2 |
| | cysteine | 5.3 ± 0.5 | 2.2 ± 0.1 | 2.4 |
| | glycine | 234.8 ± 14.9 | 3.9 ± 0.7 | 60.2 |
| | homoserine | 4.0 ± 0.2 | ND | |
| | hydroxyproline | 109.7 ± 12.3 | ND | |
| | isoleucine | 2.8 ± 0.5 | ND | |
| | leucine | 2783.4 ± 140.2 | 69.0 ± 6.3 | 40.3 |
| | lysine | 85.8 ± 2.4 | 33.7 ± 2.0 | 2.5 |
| | methionine | 254.4 ± 20.5 | 19.0 ± 3.0 | 13.4 |
| | proline | 793.8 ± 56.3 | 39.6 ± 6.2 | 20.0 |
| | proline-like | 194.2 ± 30.4 | 20.6 ± 1.4 | 9.4 |
| | tryptophan | 40.1 ± 0.7 | 1.9 ± 0.1 | 21.1 |
| | tyrosine | 24.2 ± 2.7 | 8.5 ± 1.0 | 2.8 |
| Amines & polyamines | adenine | 186.1 ± 11.4 | 27.5 ± 5.5 | 6.8 |
| | adenosine | 111.6 ± 10.9 | 54.8 ± 3.3 | 2.0 |
| | cadaverine | 77.3 ± 4.9 | ND | |
| | cytosine | 10.5 ± 1.0 | 5.3 ± 0.4 | 2.0 |
| | ethanolamine | 46.2 ± 2.2 | 14.1 ± 1.4 | 3.2 |
| | hydroxylamine | 58.6 ± 9.5 | 20.2 ± 2.0 | 2.9 |
| | spermidine | 150.3 ± 21.5 | 44.4 ± 5.4 | 3.4 |
| | tyramine | 205.1 ± 4.4 | 120.6 ± 8.4 | 1.7 |
| Polar organic acids | 2-hydroxyglutaric acid | 6.7 ± 0.4 | ND | |
| | citramalic acid | 15.3 ± 2.1 | ND | |
| | lactic acid | 146.9 ± 12.8 | 19.4 ± 1.5 | 7.6 |
| Sugars | 1-methyl-beta-D-galactopyranoside | 21.2 ± 2.0 | 6.0 ± 0.2 | 3.5 |
| | glucose | 2.6 ± 0.2 | ND | |
| | glucose-6-P | 55.9 ± 6.1 | ND | |
| | glycerol | 312.1 ± 30.9 | 37.0 ± 0.5 | 8.4 |
| | glycerol-3-P | 600.5 ± 43.5 | 125.3 ± 15.1 | 4.8 |
| | inositol-1-P | 172.7 ± 15.3 | 17.1 ± 2.1 | 10.1 |
| | ribitol | 3.7 ± 0.4 | 1.0 ± 0 | 3.7 |
| | ribose | 3.8 ± 0.2 | ND | |
| | sedoheptulose | 7.6 ± 0.4 | ND | |
| | sorbitol | 85.6 ± 10.2 | 20.1 ± 3.1 | 4.2 |
| | trehalose | 33.2 ± 4.3 | 15.4 ± 1.2 | 2.1 |

**Table 4**
Metabolites decreased ($p < 0.05$) in 18 h SH1000 ACB cultures, compared to LBA grown cultures.

| Metabolite class | Metabolite | Metabolite relative concentration per gram dry weight (mean ± SE) | | Fold decrease LBA/ACB |
|---|---|---|---|---|
| | | ACB | LBA | |
| Amino acids | asparagine | 12.7 ± 0.4 | 131.2 ± 19.6 | 10.3 |
| | aspartic acid | 2198.2 ± 265.6 | 5934.9 ± 79.2 | 2.7 |
| | glutamine | ND | 61.5 ± 2.3 | |
| | N-acetylglutamic acid | 148.8 ± 14.7 | 246.9 ± 23.2 | 1.7 |
| | ornithine | 81.9 ± 11.5 | 885.8 ± 36.2 | 10.8 |
| | serine | 5.8 ± 1.1 | 16.0 ± 1.1 | 2.9 |
| | threonine | 5.3 ± 0.1 | 7.1 ± 0.1 | 1.3 |
| Amines &polyamines | galactosamine | ND | 26.7 ± 1.2 | |
| | N-acetylglucosamine | 5.0 ± 0.5 | 8.3 ± 0.3 | 1.7 |
| | glucosamine | ND | 6.7 ± 0.5 | |
| | thymine | ND | 1.1 ± 0.1 | |
| | urea | 9.2 ± 0.6 | 18.0 ± 2.9 | 2.0 |
| Polar organic acids | 3-phosphoglycerate | 38.5 ± 5.0 | 304.5 ± 19.0 | 7.9 |
| | fumaric acid | 5.2 ± 0.1 | 12.0 ± 0.6 | 2.3 |
| | gamma-aminobutyric acid | ND | 21.4 ± 1.0 | |
| | gluconic acid | ND | 1.1 ± 0.1 | |
| | glycolic acid | 12.3 ± 1.4 | 24.5 ± 1.8 | 2.0 |
| | malonic acid | 5.4 ± 0.2 | 13.8 ± 0.8 | 2.6 |
| | threonic acid | 1.2 ± 0.2 | 4.4 ± 0.3 | 3.7 |
| Sugars | glycerol-2-phosphate | 3.7 ± 0.3 | 6.3 ± 0.6 | 1.7 |
| | mannitol | ND | 1.0 ± 0.2 | |
| | ribose-5-P | 3.8 ± 0.3 | 20.0 ± 2.9 | 5.3 |

upregulated during growth on ACB (Table 2).

Related to amino acid catabolism, we observed increased urease gene expression and activity in the ACB culture, and the relative urea concentration was down in the ACB culture compared to the LBA culture (Table 4).

Transcriptional profiling data demonstrated that the ACB culture displayed aspects of anaerobic growth and increased expression of genes encoding a D-lactate dehydrogenase and a lactate permease. In conjunction with this finding, the relative amount of lactic acid observed in the ACB grown culture was greater than observed in the LBA culture (Table 3).

The increased relative amount of sugars observed in the ACB culture (Table 3), especially glucose and glucose-6-phosphate, indicates that gluconeogenesis is active in SH1000 growing on ACB. Glucose and glucose-6-phosphate were not detected in the LBA culture (Table 3).

Spermidine promotes biofilm formation in *Bacillus subtilis* (Hobley et al., 2017), and the relative concentration of spermidine was higher in the ACB grown culture. Cadaverine is a foul smelling biogenic amine produced during the putrefaction and protein hydolysis of animal tissue (Kim et al., 2015). It has been reported that *S. aureus* has the ability to produce cadaverine from lysine utilizing a lysine decarboxylase (Kuley et al., 2012), and cadaverine was identified only in the ACB grown culture (Table 3).

A gene encoding a glycerol 3-phosphate transporter (*glpT*) was upregulated at both time points analyzed for the ACB culture (Table S3) and the relative glycerol-3-phosphate concentration was higher in the ACB grown culture (Table 3).

## 4. Conclusions

Transcriptional profiling revealed that when compared to growth on LBA, growth of *S. aureus* on ACB leads to increased expression of genes involved with: protein synthesis, cofactor and secondary metabolite production and transport; nitrogen and nucleotide metabolism; and amino acid transport. Our results also demonstrated that the ACB culture reduced the expression of genes associated with general stress, lipid metabolism, and virulence. Overall metabolomics data demonstrated that the relative concentration of amino acids and sugars increased in the ACB grown culture. In addition, our data supports the hypothesis that the ACB culture demonstrated an increased need for arginine, proline and biotin. The limited availability of sugars on ACB probably stimulated sugar accumulation in the ACB grown culture via increased gluconeogenesis. Since preferred carbon and energy sources (Li et al., 2010) are not present in ACB, or are present in low quantities, the ACB culture displays characteristics of being catabolite de-repressed which in turn supports increased amino acid transport into the cell, and perhaps amino acid biosynthesis, which are then catabolized to produce essential carbon intermediates and ATP. All these gene expression and metabolome alterations are occurring during a growth state where specific genes involved with anaerobiosis and biofilm formation were upregulated. The gene regulation alterations associated with anaerobic and biofilm growth probably result from the fact that the surface of ACB was heterogeneous and therefore challenges *S. aureus* with unique oxygen levels and growth niches, compared to the laboratory LBA.

Collectively this study provides information on how a Gram-positive food borne pathogen responds to growth on chicken meat, which could be used by the food industry to reduce the impact of food born intoxication caused by *S. aureus*.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.01.004.

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