Transcriptome profiling of mastitis-specialized Staphylococcus aureus reveals the impact of low-oxygenation on the regulation of unique pathways after internalization into bovine mammary alveolar cell-T (MAC-T)

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Abstract

Background Mastitis-specialized lineages of Staphylococcus aureus are important pathogens in the dairy industry. The molecular mechanisms underlying host- and organ-specialization in these lineages are still not fully understood. Recent findings suggested that differential expression of genes may have contributed to the evolution of strains with enhanced virulence. However, studies on gene expressions under key intra-mammary conditions are quite limited for mastitis S. aureus. The purpose of the study was to investigate the influence of low oxygen levels on the transcriptome profiles of bovine mastitis S. aureus, using high-throughput whole genome qRT-PCR. Results Results showed that under normal oxygenation, a mastitis-isolate expressed subsets of genes for adaptation, environmental-sensing, and binding including merR, sigB, vraS, yycG/yycF, araC, and tetR. In addition, coupling of fermentative metabolism to virulence was indicated by accumulated transcripts for catabolite control protein A (ccpA) and pentose-monophosphate operon and depleted transcripts for tricarboxylic acid cycle. Furthermore, sarU mediated agr activation was evidenced by transcripts for toxins, adaptation, and in-vivo viability factors as staphopains and gntR operon. On the other hand, reduced oxygenation increased transcription of fibrinogen-binding genes, isd- operon, and sdrH showing aggressive adherence phenotype. While normal oxygenation produced gene activities for quick and aggressive responses, low-oxygenation induced phenotypes for persistence, binding, and metabolic inactivity. Conclusion Significant differences in the transcriptional profiles were observed for mammary alveolar cell-T (MAC-T) internalized S. aureus under low oxygen levels compared to that at normal levels. This indicated that low oxygen is an important key mammary factor
that influence transcriptome profiles of intra-mammary-specific phenotypes of S. aureus. These findings will help in understanding the effect of oxygen on the differentiation and evolution of intramammary S. aureus.

Introduction

Mastitis is a most costly disease for the dairy industry. It affects milk production, animal welfare, and food safety. For example, mastitis costed $662 Canadian dollars per milking cow per year for a typical Canadian dairy farm [1]. Another study conducted in Finnish dairy has identified *Staphylococcus aureus* as the most important pathogen for culling dairy cows [2].

*Staphylococcus aureus* is one of the most important contagious mastitis pathogens in dairy cattle. Until 1995, a single common clone (electrophoretic type, ET3) was responsible for a majority of bovine mastitis cases [3]. Since then, more virulent subclonal populations were identified that showed hypersusceptibility to acquisition of resistance [4,5]. While it is known that acquired resistance increases epidemicity, it does not significantly alter the inherent adaptive evolution of virulence per se. In addition, the predominant strains of this species that cause bovine mastitis in different geographic regions continue to be intra-mammary specialized and methicillin-sensitive *S. aureus* (MSSA) [6].

Despite enormous efforts, the regulatory process that control the rapid evolution and emergence of specialized *S. aureus* strains in different host tissues is still elusive. Fitness in host tissues and organs through processes of cellular differentiation and/or acquisition of lineage-specific trait(s) has been suggested [7,8]. However, less is known about how the host factors, such as low oxygen levels during mastitis, induce specific regulatory pathways in the pathogen, which will lead
to selection of mastitis-specific *S. aureus* in the mammary gland. Using *Burkholderia dolosa* Lieberman et al., (2011) [9] were able to identify oxygen-dependent adaptive evolutions affecting important pathogenic traits during human lung infections. In the un-inflamed bovine mammary gland, oxygen levels are around 23 mmHg. However, in *S. aureus* infected mammary gland, that level has been found further reduced to only 1.3 mmHg [10]. Therefore, the objectives of this study were to investigate the whole-genome transcriptional profiling of an mammary-specialized *S. aureus* isolate after *in vitro* internalization into mammary epithelial cells under normal and low oxygen levels.

**Materials and Methods**

**Bacterial strains and culture preparation**

In these experiments, we used a *S. aureus* isolate from a dominant mastitis clone. This *S. aureus* isolate was identified as Published Field Gel Electrophoresis (PFGE) type A (A7 and A3) corresponding to clfA subtype Q, identical to the sequenced RF122 isolate [11]. It was used in mammary alveolar cell-T (MAC-T) invasions under normal (incubator with 5% CO₂) and reduced oxygen (5% O₂) conditions. The reduced oxygen condition was maintained in a Modular Incubator Chamber as explained below. The isolate was stored at -80°C without subjecting to extensive subculturating to avoid genetic changes. Innoculum cultures were prepared according to Bayles et al., (1998) [12] with some modifications. Overnight cultures (grown in the invasion medium, which was the cell culture growth medium without antibiotics and Fetal Bovine Serum (FBS), as defined below) were centrifuged and the pellet was washed once with sterile phosphate-buffered saline (pH 7.2) and resuspended in 10 ml of the invasion medium to give a density of 10^{10} CFU ml^{-1}. Serial dilutions
of this were prepared and 1 ml/well of the $10^2$ dilution was used to inoculate MAC-T cell monolayers at the multiplicity of infections of 100 (MOI 100).

**Cell culture**

A bovine mammary epithelial cell line designated MAC-T [13] was used. The growth medium contained 44.5% Dulbecco’s Modified Eagle Medium (DMEM)(Gibco BRL), 44.5% Roswell Park Memorial Institute (RPMI) Medium 1640 (1X) with L-glutamine (Sigma-Aldrich Ltd, Oakville, Ontario, Canada), 10% FBS (Invitrogen Canada Inc., Burlington, Ontario, Canada), and 1% of 100x concentrated antibiotic/antimycotic solution (Invitrogen Inc.). It was then sterilized by 0.22 µm filters. Cells were seeded at $1 \times 10^6$ cells/well and grown for 2-3 days at 37°C with 5% CO$_2$ before being used for the experiments.

For normal oxygenation, cells were cultured in the incubator with 5% CO$_2$. To ensure adequate amount of oxygen flux into cells, culture plates with wide-surface, flat-bottom, and shallow wells were used that were inoculated with a thin layer of media (1ml) with frequent swirling of plates. These measures have been found necessary, as the oxygen does not readily diffuse into culture media [14]. For reduced oxygenation, the Modular Incubator Chamber model 101 (Billups-Rothenberg, Inc, California, USA) was used to create lower limits of microaerophilic conditions. To achieve low oxygen level, 5% oxygen in the Modular Incubator Chamber (37.5 mm Hg) was used. For each experiment in these chambers, two 6-well tissue culture plates were simultaneously incubated under the same condition; one with co-cultures and the other with free bacteria without host cells to act as control. The experiment was repeated three times (3x) each under normal and reduced conditions. This design aimed to mimick the intra-mammary oxygen levels during
mastitis.

**Invasion Assay:** The assay was carried according to Shompole et al. (2003) [15]. Approximately 16 h prior to experiment the cell growth medium was replaced with 1 ml of the invasion medium. The morning of the experiment, the medium was removed and monolayers were washed once with the invasion medium. The MAC-T cell plates were then inoculated with *S. aureus* in the invasion medium at the MOI 100 and incubated at 37°C under normal oxygenation or reduced oxygenation. Plates containing only free bacteria in the invasion medium without host cells were used as the control for both oxygenation conditions. After 1 h, supernatants of the cocultures were removed and monolayers washed three times with the invasion medium containing lysostaphin (10 μg ml⁻¹; Sigma) to kill extracellular bacteria [16, 17]. Plates were then re-washed with the invasion medium before incubating in the fresh invasion medium containing 100μg /ml gentamicin (Invitrogen Inc.) for 8 h. Supernatants were then removed and discarded. MAC-T cell monolayers were carefully and quickly washed with sterile distilled water, scraped with disposable sterile scrapers (Fisher Scientific, Ottawa, Ontario, Canada), and quickly stored at -80°C in 5-10 volumes of RNAlater as described by the manufacturer (Applied Biosystems, Ambion Inc., Streetsville, Ontario, Canada), before RNA extractions. Extractions were done according to the Ambion RiboPure Bacteria kit (Ambion Inc.) at the PFGRC, J. Craig Venter Institute (JCVI), Maryland, USA.

**Whole-genome transcriptional profiling:**
We compared gene expression in *S. aureus* recovered from a MAC-T intracellular environment under normal oxygen tension with gene expression in free-living *S. aureus* under the same condition. Similarly we compared gene expression in *S. aureus* recovered from a MAC-T intracellular environment under low oxygen tension...
with that of free-living *S. aureus* under the same condition.

The whole-genome qPCR was conducted on twelve samples under four treatments: internalized *S. aureus* and free *S. aureus* under normal or reduced oxygen conditions, with three samples for each treatment. Primer pairs covering 5182 open reading frames on three genomes (COL, Newman, and RF122), were designed (JCVI, Maryland, USA). We needed 13.5 of 384-well plates/run to cover 5182 ORFs. Duplicate wells from triplicate samples from 4 experiments = 13.5 x 2 x 3 x 4 = 324 plates. The large number of these open reading frames were for hypothetical proteins based on theoretical information or for ‘unknown’ proteins due to lack of enough information on them. Primers were diluted to 1.25 uM and the final concentration was 0.125 uM. (i) cDNA synthesis and purification: cDNA was synthesize by taking 2 µg of total RNA, mixing with a final concentration of 0.5 µM of dNTP mix and random hexamers using SuperScript III (Invitrogen Inc.). cDNA was purified using Qiagen minElute column (Qiagen), according to the manufacturer protocol. (ii) High-throughput qRT-PCR Purified cDNA was diluted 1:10 in DEPC water. Nine ml of diluted cDNA and 15ml of Roche 2x SYBR Green master were mixed. Eight µl of cDNA/mastermix was aliquoted into 384-well plates. Primers were added to cDNA master mix to a final volume of 10µl. Plates were sealed with qRT-PCR tape and stored in the -80 until use. Plates were run in a Roche LifghtCycler480 (LC480) RealTime PCR system 384-plate format for gene-expression, interfaced with robotics to create an automated high-throughput. qRT-PCR cycles were as follow: Step 1: 5 minutes at 95°C. Step 2: 95°C for 10 secs, 60°C for 10 secs, 72°C for 10 secs. Cycle 65 times. Step 3: melt curve analysis on all wells.

**Normalization and data analysis** the data from the qRT-PCR was first normalized on average crossing-poing (Cp) values by plates by subtracting the mean Cp value
of the plate from each Cp value on the plate. The known *S. aureus* housekeeping
genes (*gmk, gyrA, rplD, rpoB* and 16s rDNA sequence) were used to normalize data
from different plates. Each gene (represented by the open reading frame) was
evaluated in duplicate and the duplicates were averaged before normalization. For
the purpose of expression profiling for each gene, the difference between the
internalized *S. aureus* and free *S. aureus* under either normal or reduced oxygen
conditions was calculated. The difference was considered significant only if there
was 4-fold difference in expression levels.

The normalized expression data generated by the high-throughput LC480 system
was run in the Biological Role Query Tool (BioQT) linked to the server at the JCVI.
BioQT retrieves and presents annotation information from several databases (CMR
Cellular Roles, Gene Ontology (GO) Terms, Protein Families (PFam), TIGRFams,
Kyoto Encyclopedia for Genes and Genomes (KEGG) Pathways, Enzyme Commission
information (EC) numbers, and KEGG Orthologs for lists of protein accessions or
gene locus IDs for all organisms currently entered into JCVI’s Comprehensive
Microbial Resource (CMR) or into a JCVI small genome database. BioQT reports
summaries of cellular roles and also includes a pie chart that represents the role
distribution.

**Results**

**Transcriptome profiles of mastitis *S. aureus* isolate internalized in
mammary cells at normal O₂ levels**

Transcript levels of genes that were either up- or down-regulated in response to
normal oxygen condition in incubator were measured. In the up-regulated category,
elevated levels of transcripts were produced in transport and binding proteins such
as detoxification and adaptation genes mmpL efflux pumps like norG, pnbA (para-nitrobenzyl esterase), betA and betB (betaine hydrolysis), and camS as well as in cellular processes. Increased transcriptional activities were also seen in specific subsets of regulatory and pathogenesis genes such as agr signaling molecules, sarU, vraSR, merR, araC, gntR, tetR, sspB2, superantigens, and proteases (Figure 1 and Table 1). Interestingly, transcript levels for genes in glycolysis, fermentation, and anaerobic pathways were higher and these genes included fructose-bisphosphatase, L-lactate dehydrogenase (ldh1), and fructose-1,6-bisphosphate aldolase (fdAB). Genes for aldehyde dehydrogenase (aldA2), bifunctional acetaldehyde-CoA/alcohol dehydrogenase, alpha-acetolactate decarboxylase (budA2), pyruvate-formate-lyase-activating enzyme (pflA, pflB), formate acetyltransferase genes, and phenol soluble modulins were also expressed. Significant increases also occurred in transcript levels for cell envelope, energy, DNA metabolisms, and other regulatory functions. On the other hand, transcripts for major biosynthetic and central intermediary metabolism processes were down-regulated. These included L-lactate dehydrogenase (ldh2) locus (SACOL261) and PTS components IIABC (locus SACOL2552), fibronectin binding protein (FnBP) (SAB1289c) and nuoF (NADH dehydrogenase subunit 5) (Figure 2 and Table 2).

**Transcriptome profiles of mastitis S. aureus internalized into mammary cells at reduced oxygen condition.**

Under reduced oxygen, up-regulated and down-regulated genes were also observed. As shown in Figure 3, the majority of up-regulated gene activities were for surface and envelope proteins involved in transport, binding, and cellular processes. For example, transcript levels of cation-induced transport processes i.e., sodium-, iron-, and ammonium-mediated transport, as well as surface proteins such as fibrinogen
binding related proteins, iron-regulated surface determinant (Isd) protein genes isdA, isdC, isdH, and serine aspirate dipeptide repeat protein genes (sdrH) were elevated. In addition, genes for adaptation proteins such as phenol soluble modulins, aerolysins, superantigen were also transcribed (Table 3). The down regulated genes under the reduced oxygen condition included some components of glycolytic and fermentative pathways such as gntR, fructose and sucrose metabolic genes, and many hypothetical genes (21.4%) (Table 4). In addition, genes related to purines, pyrimidines, nucleotides, and nucleosides were mostly suppressed (Figure 4).

Discussion

In this study, we have identified specific genes and pathways that were expressed or suppressed in response to in vitro MAC-T internalization of S. aureus into mammary epithelial cells under both normal oxygenation and low oxygenation. Mammary gland is a complex environment where oxygen is significantly low measuring about 23 mmHg in intact glands. This level is further reduced in S. aureus infected glands to only 1.3 mmHg [10]. Unfortunately, there is a shortage of publications dealing with effects of oxygen on S. aureus evolutions in the mammary. We hypothesized that S. aureus may respond to the low oxygen environment by regulating transcription of certain genes or gene networks.

Under the normal oxygenation, internalization of S. aureus into mammary epithelial cells increased transcript levels of both agr and sarU. Biphasic induction of Agr regulated transcripts during invasion has been reported previously [15]. The gene sarU is an activator of agr expression and is a member of the SarA protein family [18, 19]. The agr locus can acts as a molecular switch to turn on transcription of
toxin genes while repressing synthesis of cell wall protein postexponentially in *S. aureus*; whereas, *SarA*, a homologue to *SarU*, enhances postexponential expression of α, β and δ toxins both directly and indirectly via up-regulation of *agr* [18,19]. Thus, it is quite possible that *sarU* can function like *sarA* to enhance production of toxins which induce cell lysis.

Internization of *S. aureus* into mammary epithelial cells during the normal oxygen level also increased a co-ordinated transcriptional activity of the cell-wall sentinels, the *vraS*, *pnbA* for beta-lactams hydrolysis, *tetR* regulators, and *betA* and *betB* for conversion of choline to betaine which is a well known potent osmoregulator in *S. aureus* [20]. Induction of these transcriptional profiles strongly implied intracellular adaptive and protective responses to cell wall stress stimulations. Moreover, expressions of *sspB* and *yyG/yycI* (renamed *WalK/WalR*) have been found to be associated with the invasive phase of infection, virulence (*sdrD, ebpS*), viability, and wall metabolism [21].

Increased transcripts for glycolysis, pentose monophosphate pathway (PMP) operon (*gntR, norB*), and fermentation genes (*ldh*) and TCA cycle suppression were produced in internalized *S. aureus* under the normal oxygen level. Production of NADPH through the PMP is a well known mechanism for reductive biosynthesis of nucleic acids, glycolytic intermediates, and more importantly for evasion and bypassing of pathways that produce oxidative damage. Furthermore, the concurrent increase in the transcript levels of catabolite control protein A (*CcpA*) suggested that it is possible to be responsible for induction of anaerobic metabolism. *CcpA* is a glucose-responsive member of the LacI/GalR family of transcriptional regulators [22]. *CcpA* also modulates transcription of exotoxins, such as α-hemolysin and toxic shock syndrome toxin 1, protein A (*SpA*), and capsule formation in a glucose-responsive
Thus, the coupling of virulence to anaerobic metabolism found in this study implied the importance of organ-specific oxygen levels in overall regulation and \textit{ccpA} as the potential candidate for the control. Similarly, consistent with the earlier finding [25] phenol soluble modulins were also transcribed in this study under the normal oxygen level. Expressions of the \textit{araC} and \textit{merR} leading to \textit{sigmaB} activation suggested coupling of stress, metal ions, antimicrobial resistance, and carbon metabolism to virulence, in a fascinating co-regulatory process [26, 27]. At the reduced oxygen level, (Figure 3, Table 3) fibrinogen binding, iron-regulated surface determinant genes \textit{isdA}, \textit{isdC}, and \textit{isdH}, and serine aspirate dipeptide repeat (\textit{sdrH}) were expressed. This result is consistent with their induction by iron depletion [28]. Interestingly, the unique suppression of genes for metabolism of purines, pyrimidines, nucleotides, and nucleotides would reflect limited activities in bacteria, since these molecules are well-known sources of energy or substrates for other metabolisms. The ATP or GTP are used for protein synthesis, while UTP are needed for activating glucose and galactose. Similarly, CTP is essential for lipid metabolism, while coenzyme A, or nucleotides are required for the synthesis of nucleic acids. This finding is supported by significant downregulation in metabolic pathways (Figure 4, Table 4).

**Conclusion**

We have conducted transcriptome analysis of internalized mastitis- 	extit{Staphylococcus aureus} isolate at low and normal oxygen tension. Data indicated that at low oxygen, unique set of transcriptome profiles were produced compared to normal oxygen. Transcripts of fermentation and PMP pathways even at normal oxygen level, catabolite control repression, and virulence were strikingly co-accumulated. While
increased transcripts for adaptation, detoxification, and environmental sensing were observed at normal oxygen level, upregulation of iron- and fibrinogen-binding genes and downregulation of metabolism was evident at the reduced oxygen. Future annotation of the large number of hypothetical proteins would certainly show more insights into the basic mechanisms underlying persistence of *S. aureus* in the mammary glands.

**Abbreviations**

qRT-PCR: Quantitative Real Time PCR; MOI: multiplicity of infections; FBS: Fetal Bovine Serum; Cp: Crossing Point; BioQT: Biological Role Query Tool; TCA: tricarboxylic acid cycle; CcpA: catabolite control protein A; MAC-T: Mammary alveolar cell-T; MSSA: methicillin sensitive *S. aureus*; MRSA: methicillin resistant *S. aureus*; PFGE: pulsed field gel electrophoresis; MOI: multiplicity of infection; DMEM: Dulbecco’s Modified Eagle Medium; RPMI: Roswell Park Memorial Institute; FBS: Fetal Bovine Serum.

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and material**

All relevant data are within this paper. Additional tables of full data are also provided.

**Competing interests**
The authors declare that they have no competing interests.

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**Authors' contributions**

KBS conceptualized, designed, carried out experiments, interpreted results, wrote the paper, and edited manuscript. XZ supported research and helped in overall research and editions. KBS, MBJ, and RM performed research; MBJ, SP, and XZ, contributed reagents/analytic tools, and edited manuscript; KBS, MBJ, analyzed data. All authors approved manuscript for publication.

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Tables

Table 1. Upregulated genes in mastitis *S. aureus*

internalized into MAC-T cell line at normal $O_2$ condition
| Main Role\(^a\) | MC\(^b\) | Sub-role | SC\(^c\) | Product\(^d\) | Locus |
|-----------------|--------|----------|--------|-------------|-------|
| Cellular processes | 24 | Detoxification | 1 | pnbA | SACOL24 |
| Cellular processes | 24 | Adaptations to atypical conditions | 2 | betA | SACOL24 |
| Cellular processes | 24 | Adaptations to atypical conditions | 2 | betB | SACOL24 |
| Cellular processes | 24 | Pathogenesis | 15 | agrA | SACOL24 |
| Cellular processes | 24 | Pathogenesis | 15 | agrD | SACOL24 |
| Cellular processes | 24 | Pathogenesis | 15 | sarU | SACOL24 |
| Cellular processes | 24 | Pathogenesis | 15 | sspB2 | SACOL24 |
| Cellular processes | 24 | Pathogenesis | 15 | hld | SACOL24 |
| Cellular processes | 24 | Pathogenesis | 15 | hilY | SACOL24 |
| Cellular processes | 24 | Other | 15 | aur/leukocidin | SACOL24 |
| Cellular processes | 24 | Carbohydrate, organic alcohols, acids | 2 | PTS, IIBC | SACOL24 |
| Cellular processes | 24 | Other | 2 | camS | SACOL24 |
| Cellular processes | 24 | Toxin production and resistance | 15 | vraS | SACOL24 |
| Cellular processes | 24 | Toxin production and resistance | 15 | superantigen | SACOL24 |
| Cellular processes | 24 | Toxin production and resistance | 15 | sarU | SACOL24 |
| Cellular processes | 24 | Toxin production and resistance | 15 | Leukocidin | SACOL24 |
| Transport & binding | 24 | Carbohydrate, organic alcohols, acids | 2 | PTS, IIBC | SACOL24 |
| Energy metabolism | 14 | Glycolysis/gluconeogenesis | 4 | ldh1 | SACOL24 |
| Energy metabolism | 14 | Glycolysis/gluconeogenesis | 4 | fdaB | SACOL24 |
| Energy metabolism | 14 | Anaerobic | 4 | pflA | SACOL24 |
| Energy metabolism | 14 | Anaerobic | 4 | pflB | SACOL24 |
| Energy metabolism | 14 | Anaerobic | 4 | ldh1 | SACOL24 |
| Energy metabolism | 14 | Electron transport | 1 | acpD | SACOL24 |
| Regulatory functions | 14 | DNA interactions | 8 | merR | SACOL24 |
| Regulatory functions | 14 | DNA interactions | 8 | araC | SACOL24 |
| Regulatory functions | 14 | DNA interactions | 8 | sarU | SACOL24 |
| Regulatory functions | 14 | DNA interactions | 8 | gntR | SACOL24 |
| Regulatory functions | 14 | DNA interactions | 8 | tetR | SACOL24 |
| Regulatory functions | 14 | Other | 4 | agrA | SACOL24 |
| Regulatory functions | 14 | Other | 4 | agrD | SACOL24 |
| Regulatory functions | 14 | Other | 15 | sarU | SACOL24 |
| Regulatory functions | 14 | Protein interactions | 5 | yycG | SACOL24 |
| Unknown function | 13 | General | 10 | yycl | SACOL24 |

\(^a\)Main Biological Role; \(^b\)Main role-count; \(^c\)Sub-role count; 
\(^d\)Gene product; \(^e\)S. aureus strain used as reference
Table 2. Downregulated genes is mastitis *S. aureus* internalized into MAC-T cell line at normal $O_2$ condition

| Main Role* | MC | Sub-role | SC | Product§ | Locus | Organism description ||
|------------|----|----------|----|----------|-------|----------------------|
| Cell envelope | 18 | Other | 7 | SAB0392 | S.aureusRF122 |
| Cell envelope | 18 | Other | 7 | SAB2220c | S.aureusRF122 |
| Cell envelope | 3 | Other | 3 | SAB1660c | S.aureusRF122 |
| Cell envelope | 22 | Other | 8 | SACOL0193 | S.aureus COL |
| Cell envelope | 18 | Other | 7 | SAB0396 | S.aureusRF122 |
| Cell envelope | 18 | Other | 7 | SAB2529c | S.aureusRF122 |
| Cell envelope | 18 | Other | 7 | SACOL2443 | S.aureus COL |
| Cell envelope | 18 | Other | 11 | SAB0901 | S.aureusRF122 |
| Energy metabolism | 4 | Glycolysis | 2 | ldh2 | SACOL2618 | S.aureus COL |
| Energy metabolism | 4 | Glycolysis | 2 | ldh2 | SACOL2618 | S.aureus COL |
| Energy metabolism | 4 | Anaerobic | 2 | ldh2 | SACOL2618 | S.aureus COL |
| Energy metabolism | 4 | Anaerobic | 2 | ldh2 | SACOL2618 | S.aureus COL |
| Energy metabolism | 4 | Electron transport | 1 | nuoF | SACOL0494 | S.aureus COL |
| Regulatory | 3 | Other | 2 | fnbp‖ | SAB1289c | S.aureusRF122 |
| Signal transduction | 2 | PTS | 2 | | SAB0782 | S.aureusRF122 |
| Signal transduction | 2 | PTS | 2 | | SACOL2552 | S.aureus COL |
| Protein fate | 1 | PTS | 1 | | SAB2566 | S.aureusRF122 |

*Main Biological Role; *Main role-count; *Sub-role count; *Gene product; *S. aureus strain used as reference.

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Table 3 Upregulated genes in mastitis *S. aureus* internalized into MAC-T cell line at reduced $O_2$ condition
| Main Role            | MC | Sub-role      | SC | Product§ | Locus          |
|----------------------|----|--------------|----|----------|----------------|
| Cellular processes   | 13 | Pathogenesis | 11 | hly      | SACOL1173      |
| Cellular processes   | 13 | Pathogenesis | 11 | superantigen | SACOL1180  |
| Cellular processes   | 13 | Pathogenesis | 11 | sspB     | SACOL1970      |
| Cellular processes   | 13 | Pathogenesis | 11 | Leukocidin | SACOL2004      |
| Cellular processes   | 13 | Pathogenesis | 11 | Aero,leukocidin | SACOL2006 |
| Cellular processes   | 13 | Pathogenesis | 11 | hid      | SACOL2022      |
| Unknown              | 6  | General      | 4  | PSM      | SACOL1187      |
| Unknown              | 6  | General      | 4  | fg-bindng** | SACOL1220   |
| Cell envelope        | 5  | Other        | 5  | isdA     | SACOL1140      |
| Cell envelope        | 5  | Other        | 5  | isdC     | SACOL1141      |
| Cell envelope        | 5  | Other        | 5  | sdH      | SACOL2019      |
| Transport&binding    | 4  | Cations, Fe-compound | 4 | Na-binding | SACOL2011    |
| Transport&binding    | 4  | Cations, Fe-compound | 4 | NH4+transport | SACOL2031  |
| Energy metabolism    | 3  | TCA cycle    | 2  | sdhC     | SACOL1158      |
| Energy metabolism    | 3  | Ami acids amines | 1 | arcC1    | SACOL1182      |
| Hypothetical         | 3  | Conserved    | 3  | zapA,    | SACOL1151      |
| Protein fate         | 1  | degradation  | 1  | sspB     | SACOL1970      |

aMain Biological Role; bMain role-count; cSub-role count; dGene product; eS.aureus strain used as reference **Fibrinogen binding protein

Table 4 Downregulated genes in mastitis S. aureus

internalized into MAC-T cell line at reduced O₂ condition

| Main Role            | MC | Sub-role      | SC | Product§ | Locus          |
|----------------------|----|--------------|----|----------|----------------|
| Hypothetical         | 9  | Conserved    | 9  | hypothetical | SACOL1987   |
| Hypothetical         | 9  | Conserved    | 9  | hypothetical | SACOL1991   |
| Hypothetical         | 9  | Conserved    | 9  | hypothetical | SACOL1992   |
| Purine, pyridine     | 6  | synthesis    | 6  | pyrC     | SACOL1213      |
| Purine, pyridine     | 6  | synthesis    | 6  | carA     | SACOL1214      |
| Purine, pyridine     | 6  | synthesis    | 6  | carB     | SACOL1215      |
| Purine, pyridine     | 6  | synthesis    | 6  | pyrF     | SACOL1216      |
| Purine, pyridine     | 6  | synthesis    | 6  | pyrE     | SACOL1217      |
| Unknown function     | 5  | General      | 3  | fg-binding** | SACOL1164  |
| Unknown function     | 5  | General      | 3  | nraZ     | SACOL1191      |
| Cellular processes   | 3  | Cell division | 3 | ftsA      | SACOL1198      |
| Cellular processes   | 3  | Cell division | 3 | ftsZ      | SACOL1199      |
| DNA metabolism       | 3  | DNA repair   | 3  | FtsO     | SACOL1150      |
| Energy metabolism    | 2  | Sugars       | 1  | fructokinase | SACOL2028   |
| Regulatory           | 2  | DNA interaction | 1 | gntR     | SACOL1997      |
| Regulatory           | 2  | RNA interaction | 1 | pyrR     | SACOL1210      |

aMain Biological Role; bMain role-count; cSub-role count; dGene product; eS.aureus strain used as reference **Fibrinogen binding protein

Additional File Legends

Additional Files Tables 1 to 4: uploaded independently

Additional Files Table1.Upregulated genes in mastitis S. aureus internalized into
MAC-T cell line at normal $O_2$ condition

Additional Files Table 2. Downregulated genes is mastitis $S.\ aureus$ internalized into MAC-T cell line at normal $O_2$ condition

Additional Files Table 3. Upregulated genes in mastitis $S.\ aureus$ internalized into MAC-T cell line at reduced $O_2$ condition

Additional Files Table 4. Downregulated genes in mastitis $S.\ aureus$ internalized into MAC-T cell line at reduced $O_2$ condition

Figures

![Distribution of Role Categories](image)

Figure 1

BioQT output: Biological roles of upregulated genes in mastitis $S.\ aureus$ internali
Figure 2

BioQT output: Biological roles of downregulated genes in mastitis S. aureus internalized in MAC-T cell line at normal O2 level

Figure 3

BioQT output: Biological roles of upregulated in mastitis S. aureus internalized in
Figure 4

BioQT output: Biological roles of downregulated genes in mastitis S.aureus intern

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

12 Saidkb, Additional Table 2, NormalO2Down.doc
11 Saidkb, Additional Table 1, NormalO2Up.doc
13 Saidkb, Additional Table 3, ReducedO2Up.doc
14 Saidkb, Additional Table 4, ReducedO2Down.doc