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dCollection

Thesis for the Degree of Master of Science

Genome and Transcriptome Analyses of *Staphylococcus aureus* FORC_062 Isolated from Human Blood

혈액에서 분리한 *Staphylococcus aureus* FORC_062에 대한 유전체 및 전사체 분석

February, 2019

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Department of Agricultural Biotechnology
College of Agriculture and Life Sciences
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석사학위논문

Genome and Transcriptome Analyses of *Staphylococcus aureus* FORC_062 Isolated from Human Blood

지도교수 최 상 호

이 논문을 석사학위논문으로 제출함

2019년 2월

서울대학교 대학원

농생명공학부

조 안 나

조안나의 석사학위논문을 인준함

2019년 2월

위원장 서 진 호 (인)

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Abstract

Staphylococcus aureus is a Gram-positive, facultative anaerobe and causes a range of diseases from skin ailments to life threating diseases such as sepsis. It is becoming tougher to treat infection of *S. aureus*, as this species has developed resistance to various types of antibiotics rapidly. Especially, methicillin resistance of *S. aureus* in South Korea is more than 67%, which is higher than other developed countries. In this study, to characterize *S. aureus* FORC_062 isolated from blood of domestic patient, the genome and transcriptome of this strain were analyzed.

The genome of *S. aureus* FORC_062 was sequenced by PacBio. The genome consists of a circular chromosome of 2,905,353 bp with a GC content of 32.92%. The chromosome contains 2,727 open reading frames, 60 rRNAs, and 16 tRNA genes. Genes encoding many virulence factors were found by BLAST such as α-, β-, γ-hemolysin, leukotoxin, toxic shock syndrome toxin. To evaluate the cytotoxicity of this strain, lactate dehydrogenase (LDH) release assay were conducted. The result showed that FORC_062 has higher cytotoxicity than other clinical isolates. Average nucleotide identity (ANI) analysis of the genome with 19 other whole genomes of *S. aureus* showed that FORC_062 is the most closely related to N315, a clinical isolate of a Japanese patient. Comparative genome analysis of FORC_062 and N315 revealed that FORC_062 has additional genes; (1) tetracycline resistance *tetM* and (2) type II toxin-antitoxin system MazEF. Previous study demonstrated that the MazEF toxin-antitoxin system alters the β-lactam susceptibility of *S. aureus* (Christopher F.

Schuster *et al.*, 2015). *In silico* prediction was confirmed by Kirby-Bauer Disk Susceptibility Test.

Consumption of raw chicken breast is increasing rapidly and a large portion of raw chicken meat distributed on market in South Korea is infected with *S. aureus*. Therefore, raw chicken breast was selected for the model food. RNA sequencing was conducted to figure out the transcriptional response when the strain FORC_062 was exposed to model food. The results showed that genes related to amino acid transporter, deamination, glucose metabolism and lipid degradation were up-regulated, while genes related to amino acid biosynthesis and other carbohydrate metabolism except for glucose were down-regulated. In addition, genes related to various virulence factors such as enterotoxin, adhesion and VII secretion system were up-regulated. The results indicated that *S. aureus* may use the chicken as a reservoir to survival and increase its pathogenicity.

All results described above demonstrated that *S. aureus* FORC_062 is possibly pathogenic to human and has high resistance to antibiotics. Furthermore, this strain may use the chicken breast as a reservoir to survive and increase its pathogenicity. Further research is imperative to understand this important pathogen and to prevent future outbreaks in South Korea.

Key words: *Staphylococcus aureus*, raw chicken breast, genome analysis, transcriptome analysis;

Student Number: 2017-21311

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I. INTRODUCTION

Staphylococcus aureus is an opportunistic microorganism and causes skin ailments to life-threating disease such as sepsis. The importance of studying *S. aureus* comes from yearly increasing infections and antibiotics resistance. Especially, MRSA (methicillin resistance *Staphylococcus aureus*) in South Korea is more than 67%, which is a very high statistic compared to other developed countries (Kim *et al.*, 2016). Besides, acquisition of resistance to new type antibiotics are constantly emerging (Chambers and DeLeo, 2009).

The regular occurrence of MRSA combined with the popularity of poultry meat especially raw chicken breast is proving to be a threat to food safety and public health. According to the investigation from Animal and Plant Quarantine Agency in South Korea, 40.3% chicken meat distributed on market is infected by MRSA. Therefore, understanding the behavior of *S. aureus* when this pathogen is exposed to the raw chicken breast may help us to figure out the survival and pathogenesis of *S. aureus* in raw chicken meat.

There are a lot of advantage of bioinformatics techniques in their application to public safety and food safety area. Genomics is effective in characterizing and detecting pathogens, and transcriptomics is useful to understand the adaption and response of pathogens to a specific environment (Bergholz *et al*., 2014). Although research on *S. aureus* is being carried out constantly, whole genome sequences of *S.*

aureus isolated from South Korea patient are rare. As the MRSA ratio of South Korea is much higher than other developed countries, it is necessary to accumulate more genome data of the species and understand their unique feature. Along with whole genome sequencing, RNA sequencing will be an effective approach to characterize the pathogen's behavior when it exposed to the model food.

S. aureus FORC_062 was isolated from a patient in Samsung hospital and Ministry of Drug and Food Safety in South Korea. In this study, the genome of FORC_062 was completely sequenced and analyzed, also, its transcriptome was sequenced to understand its response to raw chicken breast. It is expected that the results of this study will further our understanding of *S. aureus* and help to prevent outbreak in the future.

II. MATERIALS AND METHODS

Bacterial strains, and growth condition

Table 1 shows the *Staphylococcus aureus* strains, which were used in this study. The strains were both aerobically incubated at 37°C for 12 h in Tryptic Soy medium.

Genomic DNA extraction and identification

DNeasy Blwood & Tissue Kit (QIAGEN, Valencia, California, USA) was used according to the manufacturers' protocol to extract genomic DNA. The 16S rRNA gene was amplified from the extracted genomic DNA and sequenced by an automated ABI3730XL capillary DNA sequencer (Applied Biosystems, Foster City, CA, USA) for taxonomic identification (Ku *et al*., 2014).

Virulence gene-specific PCR

Table 2 shows the primers of the genes (*tst, femA, mecA, eap, nuc, sec*) that were used for virulence gene-specific PCR. After resolving the amplified DNA fragments in 1.5% agarose gel, the electrophoresis results were visualized by using Geldoc™ EZ Image (Bio-Rad, Richmond, California, USA).

LDH cytotoxicity assay

The cytotoxicity of the *S. aureus* strains was indirectly estimated by measuring the cytoplasmic lactate dehydrogenase (LDH) activity. LDH is released from the human cell when the plasma membrane is damaged. LDH release assay was conducted using INT-407 human epithelial cells (ATCC® CCL-6™). For the assay, INT-407 cells were grown with bacterial culture in 96-well tissue culture plates (Nunc, Roskilde, Denmark) with a cell density of 2.0 x 10^4 per well. The cell monolayers were incubated for 1 day, infected with bacteria at a multiplicity of infection (MOI) of 10, 30 and 50. The positive control (completely lysed) is the well added 2% Triton X-100 and the negative control (non-lysed) is the well added the assay media instead of the bacterial culture. The experiments were repeated with biologically duplicated samples and triplicate wells were run for each sample.

Transmission electron microscope

FORC_062 was negatively stained with phosphotungstic acid (PTA) for a few seconds and then washed two to three times. The organism was observed using LIBRA 120 (Carl Zeiss, Oberkochen, Germany) transmission electron microscope (TEM) at 120 kV.

Genome sequencing and annotation

Whole genome sequencing and assembly were performed at LabGenomics Incorporation (Seoul, South Korea). Dual platforms of Illumina MiSeq (Illumina, San Diego, California, USA), and PacBio (Pacific Biosciences, Menlo Park, California, USA) were used. The raw sequence reads from Illumina MiSeq were assembled with CLC Genomics workbench 7.5.1 (CLC Bio, Aarhus, Denmark), and those from PacBio were assembled with PacBio SMRT Analysis 2.3.0 (Pacific Biosciences). ORFs were predicted and annotated using GeneMarkS (Besemer, Lomsadze, and Borodovsky, 2001) and rapid annotations using subsytems technology (RAST) (Aziz *et al*., 2008). The ribosomal binding sites (RBSs) were predicted using RBS finder (J. Craig Venter Institute, Rockville, Maryland, USA). The existing annotations were modified after cross-checking with results from InterProScan 5 (Jones *et al*., 2014) and GAMOLA (Altermann and Klaenhammer, 2003).

The end product was submitted to GenBank. Using virulence factor database (VFDB; http://www.mgc.ac/VFs/) as reference, putative virulence factors of FORC_062 were found by basic local alignment search tool (BLAST). The circular genome maps of the chromosome was visualized by using cluster of orthologous group (COG)-based WebMGA and GenVision (DNASTAR, Madison, Wisconsin, USA).

For the identification of Insertion Sequence region, database of ISfinder (Siguier P., *et al.*, 2006) was used as reference.

Phylogenetic tree analysis and comparative genome analysis

Phylogenetic tree analysis was conducted by using 16S rRNA sequences of FORC_062 and other species within the *Staphylococcus* genus. MEGA7 was used (Kumar, Strecher, and Tamura, 2016), while neighbor joining method was employed and the number of bootstrap replicates used was 1000. To characterize the genetic relatedness amongst *Staphylococcus aureus* strains, average nucleotide identity (ANI) analysis was conducted. The completed genome sequences of 20 strains (*S. aureus* COL, FORC_001, FORC_012, FORC_026, FORC_027, FORC_039, JH1, JH9, MRSA252, MRSA476, Mu3, Mu50, MW2, N315, NCTC8325, Newman, FR122, USA300_FPR3757, USA300_TCH1516 and FORC_062) were used for the analysis. The ANI values were calculated using Jspecies (Richter and Móra, 2009). The result was visualized with R. As *S. aureus* N315 was determined to be the most closely related to FORC_062, comparative genome analysis between FORC_062 and N3515 was carried out. For the comparative analysis, Artemis comparison tool (ACT) was used (Carver *et al*., 2005).

Kirby-Bauer disk susceptibility test

The susceptibility of *S. aureus* FORC_062 to various antibiotics was identified through the Kirby-Bauer disk susceptibility test. The diameters of clear zone formed by each antibiotic were interpreted as susceptible, intermediate, or resistant according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) as described by Bauer et al. Lawrence et al. (Lawrence *et al.*, 1972; Bauer *et al*., 1966)

with Mueller-Hinton agar (Difco). Briefly, bacteria were prepared by transferring single colonies incubated 12 hours over from tryptic soy agar into 0.85% saline water, followed by measure turbidity of FORC_062 inoculum using turbidimeter (Mettler Toledo) according to McFarland standards 0.5 (Absorbance OD_{600}; 0.063) (Mcfarland *et al*., 1907). 1ml inoculum of FORC_062 was added to Mueller-Hinton agar plate, and the 10 antimicrobial disks (ampicillin, amoxyclin, cefazolin, cefotaxime, ceftazidime, ciprofloxacin, erythromycin, linezolid, tetracycline, sulfamethoxazole) (Oxoid, UK) were placed on the same agar plate on which *S. aureus* FORC_062 cells were spread. To compare the antibiotics susceptibility between *S. aureus* FORC_062 and N315, the same experiment was conducted for N315. *S. aureus* ATCC 29213 served as a quality control strain in the disk susceptibility test. The plates were incubated for 18 hours over to confirm antibiotic resistance of the bacteria.

RNA extraction

FORC_062 was first prepared so that it can be added to the control and the experimental conditions: FORC_062 was grown to mid-log phase (OD_{600}; 0.7), and centrifuged at 10,000 x g for 1 min. After removing the supernatant, the pellet was washed with 1x phosphate buffered saline (PBS), and the resulting solution was centrifuged at 10,000 x g for 1 min (Alexa Price-Whelan *et al*., 2013). The wash and centrifugation step was repeated two or three times. Lastly, the pellet was re-suspended with M9 minimal medium containing histidine 0.004% (w/v) (Burke M. E., & Pattee P. A., 1972) and casamino acid 0.1% (w/v) (Lincoln R. A., *et al.*, 1995).

Prepared FORC_062 was incubated at 37°C for 2 h and 4 h in M9 minimal medium either with or without raw chicken meat. After incubation, the culture was centrifuged at 5,000 x g at 4°C for 10 min. The pellet was re-suspended with 5 ml of diethylpyrocarbonate (DEPC) treated cold 1x PBS and the solution was quickly mixed with 10 ml of RNAprotect® Bacteria Reagent (RBR) (QIAGEN). Total RNAs were extracted from the RBR-treated samples under 4°C using RNeasy Mini Kit (QIAGEN) according to the manufacturers' protocol. To prevent DNA contamination, TURBO DNase (AMbion, Austin, Texas, USA) was used. The quality of RNAs were confirmed by LabGenomics Incorporation, with Agilent 2100 Bioanalyzer and Agilent RNA 6000 Nano reagents (Agilent Technologies, Waldbronn, Germany)

Strand-specific cDNA library construction and RNA sequencing

Strand-specific complementary DNA (cDNA) library construction and RNA sequencing were performed by LabGenomics Incorporation. rRNAs were depleted by using Ribo-Zero™ rRNA Removal Kit (Epicentre, Madison, Wisconsin, USA). Enriched mRNA and TruSeq Stranded mRNA Sample Preparation kit (Illumina) was used to construct cDNA library. The quality check of cDNA libraries was performed by LabGenomics Incorporation, with Agilent 2100 Bioanalyzer and Agilent DNA 1000 reagents (Agilent Technologies). Strand-specific paired-ended 100 nucleotide reads from each cDNA library were obtained using Illumina HiSeq2500. The experiment was conducted with biological triplication.

Transcriptomic data analysis

The reads obtained from RNA sequencing were mapped to the reference genome of FORC_062 by using CLC Genomics Workbench 5.5.1 (CLC Bio). The GenBank (http://www.ncbi.nlm.nih.gov) accession numbers of the reference genome are CP022582 (Chromosome). The relative transcript abundance was measured by the reads per kilobase (kb) of transcript per million mapped sequence read (RPKM) (Mortazavi *et al*., 2008). The genes were considered to be differentially expressed in sample when the fold change was ≥ 2 and *P*-value was < 0.05. The volcano plot and the heat map were created to visualize the RNA sequencing result using CLC Genomics Workbench 5.5.1 (CLC Bio) and Gitools (Biomedical Research Park, Barcelona, Spain; Perez-Llamas and Lopez-Bigas, 2011).

RNA purification and transcript analysis

Total RNA from FORC_062 was isolated using RNeasy Mini Kit (QIAGEN). The culture condition was the same as above (See the 'RNA extraction' part). cDNA was synthesized using iScripit™ cDNA Synthesis Kit (Bio-Rad). The quantitative real-time PCR (qRT-PCR) of the synthesized cDNA was conducted using iQ™ SYBR® Green Supermix (Bio-rad) and the Chromo 4 Real-Time PCR detection system (Bio-rad) as described previously (Lim and Choi, 2014). The genes and their specific primers used are listed in Table 2. The relative expression levels of the specific transcripts were calculated by using the 16S rRNA expression level as the internal reference for normalization. The experiment was conducted with biological

duplicates and for each biological sample, technical triplicate wells were used.

Growth kinetics of FORC_062

The growth condition and bacteria preparation step was the same as described above (See the 'RNA extraction' part), except that the incubation times were 0 h, 2 h, 4 h and 6h. During incubation at each time points, the incubated culture was collected, and serially diluted by 1X PBS and plated on tryptic soy agar. The plates were incubated in 37°C for 12 h and the colonies were counted. The experiments were run with biological duplicates and for each biological sample, technical triplicates were used.

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or Source
Bacterial strains		
Staphylococcus aureus		
FORC_062	Wild type, clinical isolate	Ministry of Food and Drug Safety
ATCC 29213	Positive control strain for cytotoxicity test and antimicrobial susceptibility test	Laboratory collection
F2615	Wild type, clinical isolate	Ministry of Food and Drug Safety
F2695	Wild type, clinical isolate	Ministry of Food and Drug Safety
F2726	Wild type, clinical isolate	Ministry of Food and Drug Safety
N315	Wild type, clinical isolate	Laboratory collection
NCCP 14769	Positive control strain for PCR detection of *tst, femA, mecA, sec*	Laboratory collection
Newman	Positive control strain for PCR detection of *nuc, eap*	Laboratory collection
RN4220	Positive control strain for cytotoxicity test	Laboratory collection

Table 2. Oligonucleotides used in this study

Oligonucleotide name	Oligonucleotide sequence (5` → 3`)	Purpose	Predicted size (bp)
For virulence gene-specific PCR screening			
eap_F	TACTAACGAAGCATCTGCC	[a] PCR analysis of *eap*	230
eap_R	TTAAATCGATATCACTAATACCTC		
*femA*_F	AAAAAAGCACATAACAAGCG	[b] PCR analysis of *femA*	132
*femA*_R	GATAAAGAAGAAACCAGCAG		
*mecA*_F	ACTGCTATCCACCCTCAAAC	[b] PCR analysis of *mecA*	163
*mecA*_R	CTGGTGAAGTTGTAATCTGG		
*nuc*_F	GCGATTGATGGTGATACGGTT	[a] PCR analysis of *nuc*	267
*nuc*_R	AGCCAAGCCTTGACGAACTAAAGC		
*sec*_F	AGATGAAGTAGTTGATGTGTATGG	[b] PCR analysis of *sec*	451
*sec*_R	CACACTTTTAGAATCAACCG		
*tst*_F	ACCCCTGTTCCCTTATCATC	[b] PCR analysis of *tst*	326
*tst*_R	TTTTCAGTATTTGTAACGCC		

Oligonucleotide name	Oligonucleotide sequence (5` → 3`)	Purpose	Predicted size (bp)
[c] **For qRT-PCR**			
FORC62_0228_qRT_F	CGT ATG ACG GTC GTC CAG AG	qRT-PCR of FORC62_0228	176
FORC62_0228_qRT_R	TGT ATC ACC GCG AGG GAT TG		
FORC62_0274_qRT_F	AGCGAACTGGGAAGGTCAAG	qRT-PCR of FORC62_0274	135
FORC62_0274_qRT_R	TCTTGAACGGCATCAGCAGT		
FORC62_0275_qRT_F	TGCGACAGGTCGAGCATTAG	qRT-PCR of FORC62_0275	141
FORC62_0275_qRT_R	GCTTGGTTTTGTCTGTCGCC		
FORC62_0554_qRT_F	CGA CAG CCC CAA GTG AGA AT	qRT-PCR of FORC62_0554	83
FORC62_0554_qRT_R	ATC TGC AGT TGC AGT TTG CG		
FORC62_0582_qRT_F	AGAAGCCTTAGAGAGCGGGA	qRT-PCR of FORC62_0582	144
FORC62_0582_qRT_R	ATCGTTACTGCTAGCGGCTC		
FORC62_0584_qRT_F	GGCGTCATCACCACACTTTG	qRT-PCR of FORC62_0584	154
FORC62_0584_qRT_R	ACGCACCATCTTTTGCACAC		
FORC62_0690_qRT_F	GTT AGC AAT TGC GAC AGC GA	qRT-PCR of FORC62_0690	198
FORC62_0690_qRT_R	AAG CCT AAA GCA ATT GCG CC		

FORC62_0764_qRT_F	AAA CAC GCG GCG AAA AAC TT	qRT-PCR of FORC62_0764	190
FORC62_0764_qRT_R	TCA CGA TGC GCA GTA CCA AA		
FORC62_1403_qRT_F	GTGCAAATGCCGTGTCCATT	qRT-PCR of FORC62_1403	155
FORC62_1403_qRT_R	TGCACCAGCTTGTTTAGCAAC		
FORC62_1404_qRT_F	ATG TTA CAG CGG CCA GTC AA	qRT-PCR of FORC62_1404	118
FORC62_1404_qRT_R	TGA TTT GGA ACA GCA GAC GC		
FORC62_1470_qRT_F	GCTGCTGCAATCCCTGAAAC	qRT-PCR of FORC62_1470	84
FORC62_1470_qRT_R	CCAACCTGATAGGAAGCCCC		
FORC62_1471_qRT_F	TGA TGC CAG AAA CAG CAC CA	qRT-PCR of FORC62_1471	179
FORC62_1471_qRT_R	TCC TTG ACC AGC CAT GAC AA		
FORC62_2031_qRT_R	GGC GAT GTA GGT CCA GGA AA	qRT-PCR of FORC62_2031	100
FORC62_2031_qRT_R	AGC GGT GTG ATT CTG GTG AA		
FORC62_2173_qRT_F	GGTTTCATCGCGGCCATTTT	qRT-PCR of FORC62_2173	164
FORC62_2173_qRT_R	GTTGCTGTTGCTGCGATGAT		
FORC62_0006_qRT_F	TTG GTA CAG GAA TCG GTG GC	qRT-PCR of FORC62_0006 (gyrase B)	86
FORC62_0006_qRT_R	TCC ATC CAC ATC GGC ATC AG		

[a] The oligonucleotides were designed using *S. aureus* Newman genome sequence (GenBank™ accession numbers AP009351).

[b] The oligonucleotides were designed using *S. aureus* NCCP 14769 genome sequence (GenBank™ accession numbers CP022582).

[c] The oligonucleotides were designed using *S. aureus* FORC_062 genome sequence (GenBank™ accession numbers CP022582).

III. RESULTS

Virulence gene-specific PCR screening

To identify the virulent properties of FORC_062 among other human blood isolated *S. aureus* strains, major virulence genes of *S. aureus* were amplified by using PCR. The target genes and their expected sized were listed in table 2. As a result of PCR for FORC_062, six major virulence genes were found (Fig. 1).

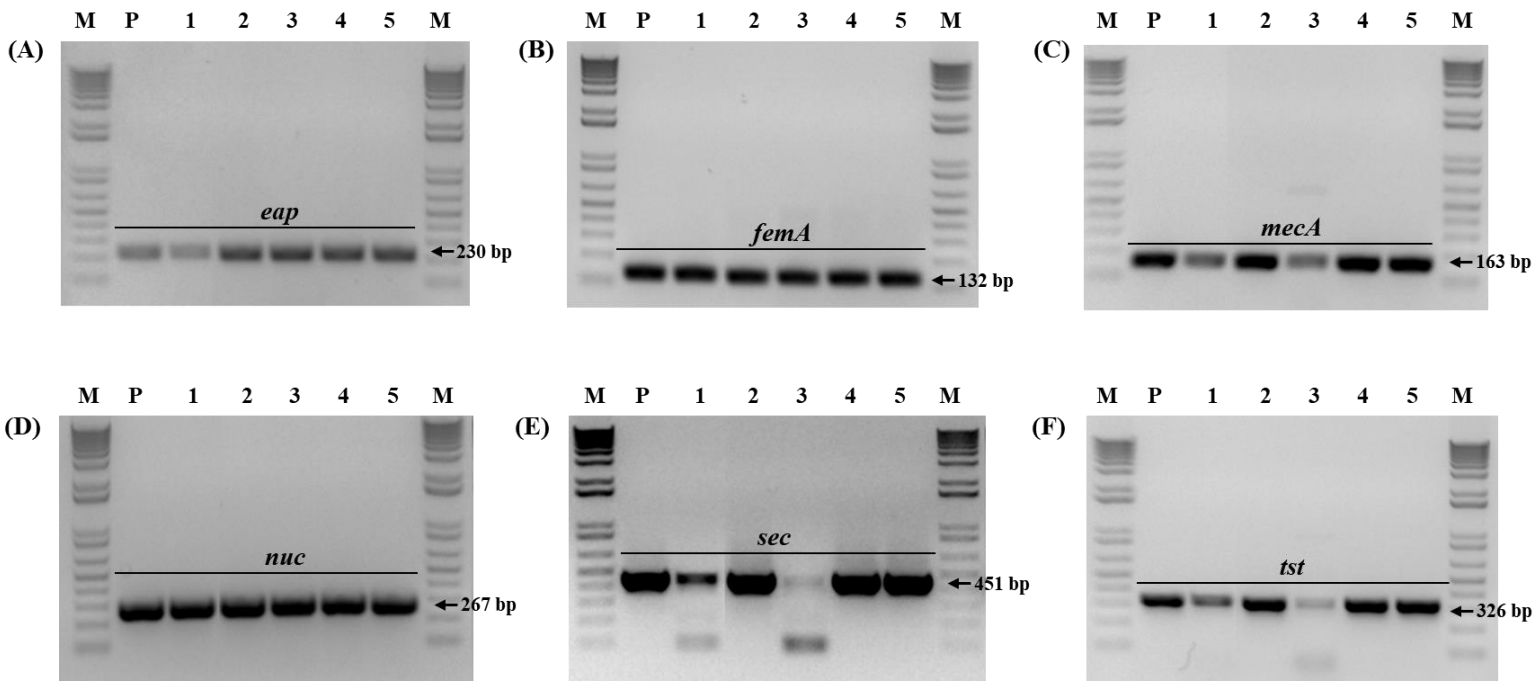

M P 1 2 3 4 5 M
(A)
eap
230 bp
(B)
femA
132 bp
(C)
mecA
163 bp
(D)
nuc
267 bp
(E)
sec
451 bp
(F)
tst
326 bp

Fig 1. Virulence gene-specific PCR screening of *S. aureus* strains. 6 virulence genes of *S. aureus* were amplified using PCR. Each band sizes are specified as part of the figure. *eap*, extracellular adherence protein encoding gene; *femA*, aminoacyltransferase encoding gene; *mecA*, methicillin-resistant related gene; *nuc*, thermonuclease encoding gene; *sec*, enterotoxin C encoding gene; *tst*, toxic shock syndrome toxin 1 encoding gene. Molecular weight marker, lane M; Positive control strain, lane P; *S. aureus* RN4220, lane 1; *S. aureus* F2695, lane 2; *S. aureus* F2726, lane 3; *S. aureus* F2726, lane 4; *S. aureus* FORC_062 (F2742), lane 5.

Cytotoxicity analysis of *S. aureus* strains

As FORC_062 is a clinical isolate, the experiment which evaluates the virulence of this strain with other *S. aureus* strains was need. Therefore, LDH release assay was performed using INT-407 cell lines. The INT-407 cells were infected with this strain, other clinical isolated *S. aureus* strains (F2695, F2726, F2615) and control *S. aureus* strains (ATCC29213, RN4220) at various multiplicity of infections (MOIs) for 4h. The result showed that FORC_062 may be more virulent than other clinical strains and as virulent as positive control strains ATCC29213.

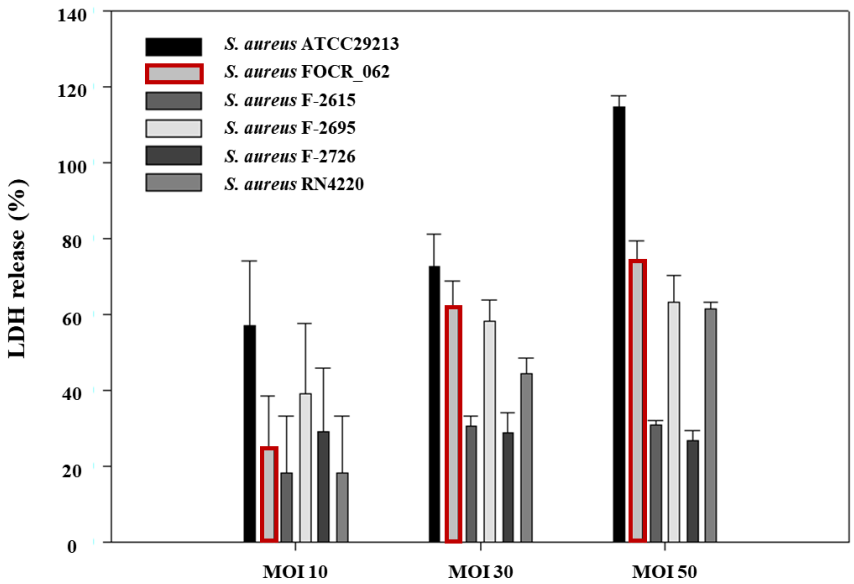


Fig 2. Cytotoxicity assay of *S. aureus* FORC_062. INT-407 cells were infected with *S. aureus* strains at various MOIs for 4 h. The cytotoxicity was determined by the LDH release activity. The total LDH is the amount of LDH released from the cells which were lysed by 2% triton X-100. The experiments were repeated with biologically duplicated samples and triplicate wells were run for each sample. The error bars represented the standard deviations (SD).

Transmission electron microscopy (TEM) image of *S. aureus* FORC_062

The conformation of FORC_062 was identified through transmission electron microscope (TEM). FORC_62 is round-shaped bacterium. (Fig. 3).

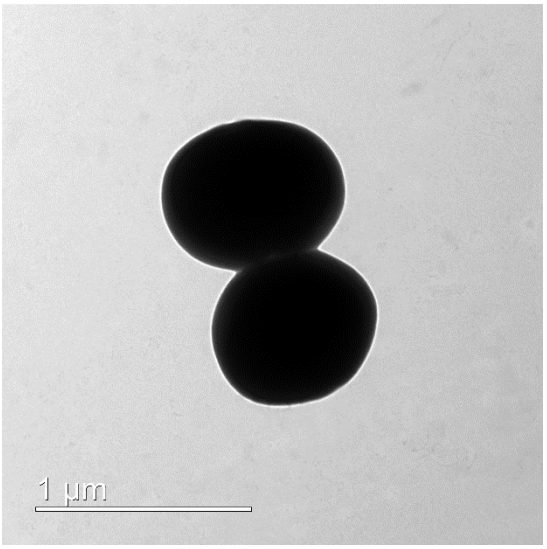


Fig 3. Transmission electron microscopy image of *S. aureus* FORC_062.

The cells were negatively stained with PTA. Bars, 1.0 µm.

Genome properties of *S. aureus* FORC_062

The whole genome sequencing properties of *S. aureus* FORC_062 were summarized in Table 3. The genome consists of a circular chromosome of 2,905,353 bp with a GC content of 32.92%, containing 2,721 predicted ORFs, 60 tRNA genes and 16 rRNA genes. Among the predicted ORFs, 2,293 ORFs (84.27%) were predicted to encode the functional protein and 428 ORFs (15.73%) were expected to encode hypothetical proteins. The genome annotation information including a chromosome has been deposited under the GenBank accession number, CP022582. All the results are summarized in Table 4. Based on the bioinformatics analysis of the chromosome, circular genome map was drawn (Fig. 4) and genes with the specialized function such as putative virulence factors were noted.

Table 3. Summary of *S. aureus* FORC_062 genome sequencing

Property	Term
Finishing quality	Finished
Libraries used	PacBio SMRTbell™ library (> 10 kb)
Sequencing platform	PacBio RS II
Assembler	CLC Genomics Workbench 7.5.1
	PacBio SMRT Analysis 2.3.0
Gene calling method	GeneMarkS, RAST server
Average genome coverage	453 X
Contig length (bp)	2,905,353
Contig No.	1
Scaffold No.	1
N50	2,905,353
Locus tag	FORC62
GenBank accession No.	CP022582
GenBank release date	2018-07-24
BioProject No.	PRJNA395485
Source material identifier	FORC_062

Table 4. Chromosomal properties of *S. aureus* FORC_062

Property	Term
Sample	*S. aureus* FORC_062
Classification	Chromosome
GenBank accession No.	CP022582
Genome size (bp)	2,905,353
Protein coding genes	2,721
Annotated genes	2,293
Hypothetical genes	352
tRNA	60
rRNA	16

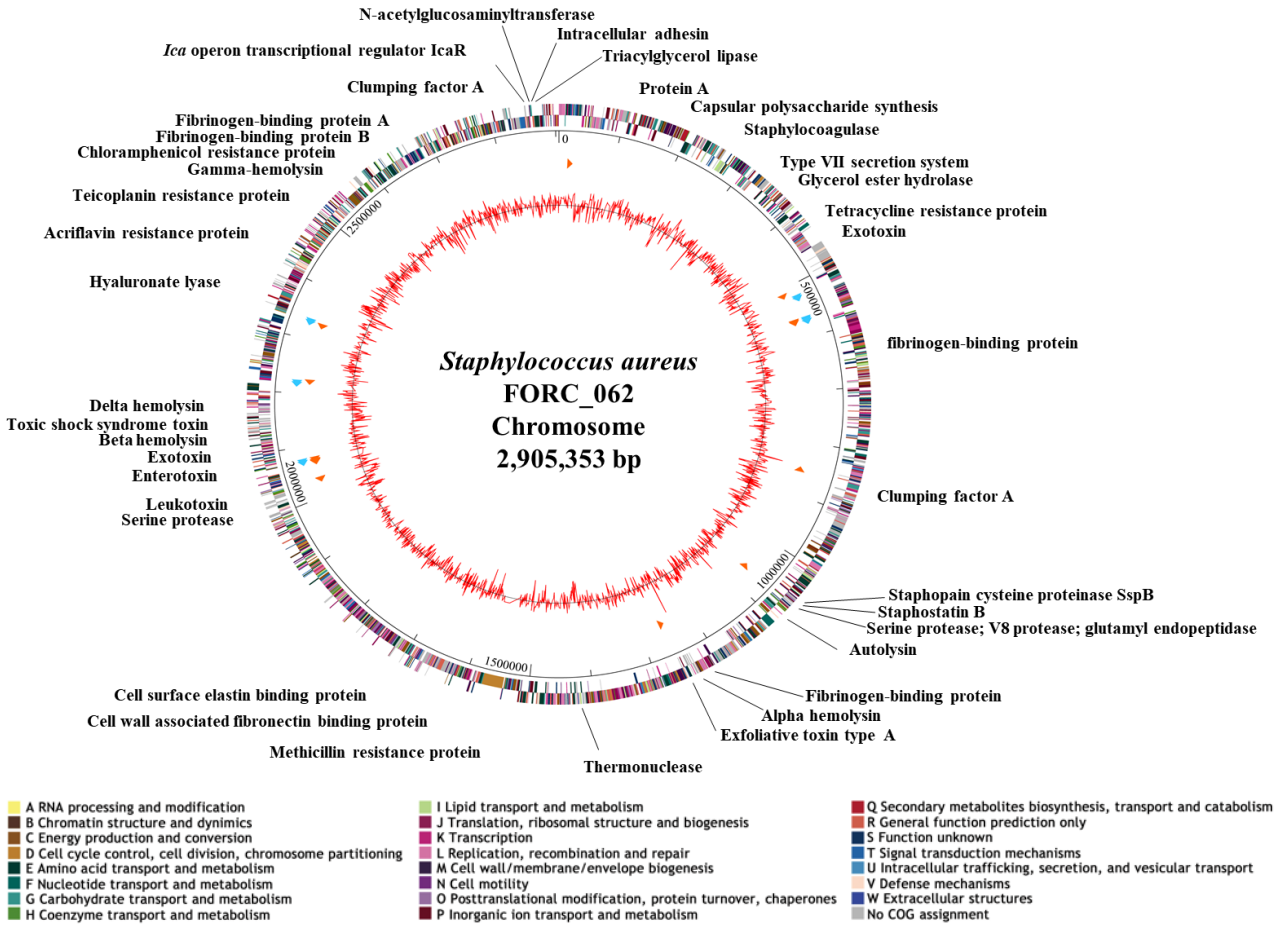

N-acetylglucosaminyltransferase
Intracellular adhesin
Ica operon transcriptional regulator IcaR
Triacylglycerol lipase
Clumping factor A
Protein A
Capsular polysaccharide synthesis
Fibrinogen-binding protein A
Staphylocoagulase
Fibrinogen-binding protein B
Chloramphenicol resistance protein
Type VII secretion system
Gamma-hemolysin
Glycerol ester hydrolase
Teicoplanin resistance protein
Tetracycline resistance protein
Acriflavin resistance protein
Exotoxin
Hyaluronate lyase
fibrinogen-binding protein
Staphylococcus aureus
FORC_062
Chromosome
2,905,353 bp
Delta hemolysin
Toxic shock syndrome toxin
Beta hemolysin
Exotoxin
Enterotoxin
Clumping factor A
Leukotoxin
Serine protease
Staphopain cysteine proteinase SspB
Staphostatin B
Serine protease; V8 protease; glutamyl endopeptidase
Autolysin
Fibrinogen-binding protein
Alpha hemolysin
Exfoliative toxin type A
Cell surface elastin binding protein
Cell wall associated fibronectin binding protein
Methicillin resistance protein
Thermonuclease
0
500000
1000000
1500000
2000000
2500000
A RNA processing and modification
B Chromatin structure and dynimics
C Energy production and conversion
D Cell cycle control, cell division, chromosome partitioning
E Amino acid transport and metabolism
F Nucleotide transport and metabolism
G Carbohydrate transport and metabolism
H Coenzyme transport and metabolism
I Lipid transport and metabolism
J Translation, ribosomal structure and biogenesis
K Transcription
L Replication, recombination and repair
M Cell wall/membrane/envelope biogenesis
N Cell motility
O Posttranslational modification, protein turnover, chaperones
P Inorganic ion transport and metabolism
Q Secondary metabolites biosynthesis, transport and catabolism
R General function prediction only
S Function unknown
T Signal transduction mechanisms
U Intracellular trafficking, secretion, and vesicular transport
V Defense mechanisms
W Extracellular structures
No COG assignment

Fig 4. Genome map of *S. aureus* FORC_062. The outer circle indicates the locations of all annotated open reading frames (ORFs), and the inner circle with the red peaks indicates GC content. Between these circles, sky blue and orange arrows indicate the rRNA operons and the tRNAs, respectively. All annotated ORFs are colored differently according to the clusters of orthologous groups (COG) assignments as indicated and for more information on each category, refer to the legend below.

Pathogenesis and virulence factor

S. aureus is known to have various virulence factors in its genome. According to VFDB, virulence factors of *S. aureus* FORC_062 can be categorized as those related to adherence, exoenzyme, host immune evasion, secretion system and toxin.

FORC_062 had major virulence related genes such as α-, β-, δ-, γ-hemolysin encoding genes (FORC62_1190, 1191, 2026, 2428, 2429, 2430). And, this microorganism had genes related to intoxication diseases, such as food poisoning (FORC62_1912~ FORC62_1917), staphylococcal scalded skin syndrome (FORC62_1200) and toxic shock syndrome (FORC62_2034). Also, it had type VII secretion system (T7SS) (FORC62_0274 ~ FORC62_0281). Additionally, the genome of this strain FORC_062 included various adherence related genes; fibrinogen-binding protein encoding genes (FORC62_1184, 2508, 2509), Clumping factors encoding genes (FORC62_0778, 2638).

Furthermore, FORC_062 had a lot of virulence genes which did not describe here. These genes which localized in FORC_062 genome are summarized in table 5.

Table 5. Virulence factors of *S. aureus* FORC_062

Virulence factor	Annotation	Location (Locus_tag)	Function
Adherence			
atl	Autolysin	1084991-1088737 (FORC62_1014)	Autolysin
ebh	Extracellular matrix-binding protein	1561143-1572815 (FORC62_1468) 1540941-1561082 (FORC62_1467)	fibronectin binding
clfA, clfB	Clumping factors	869632-872079 (FORC62_0778), 2808317-2810950 (FORC62_2638)	Fibrinogen binding
ebp	Cell surface elastin binding protein	1621805-1623265 (FORC62_1512)	Elastin binding protein
efb	Fibrinogen-binding protein	1238713-1239210 (FORC62_1184)	Fibrinogen binding proteins
fnbA, fnbB	Fibronectin-binding protein	2665057-2671739 (FORC62_2508 ~ FORC62_2509)	Host cell attachment
icaA, icaB, icaC, icaD, icaR	Intracellular adhesin	2859795-2863934 (FORC62_2673 ~ FORC62_2677)	Intracellular adhesin
sdrC, sdrD, sdrE	Ser-Asp repeat protein	626721-637657 (FORC62_0554 ~ FORC62_0556)	Attachment
Spa	Staphylococcal protein A	117420-118772 (FORC62_0104)	Staphylococcal protein A
Exoenzyme			
Geh	Glycerol ester hydrolase	359702-361777 (FORC62_0310)	Lipase
hysA	Hyaluronate lyase	2370123-2372552 (FORC62_2218)	Hyaluronate lyase

splA, splB, splC, splD, SplF	Serine protease	2008777-2012825 (FORC62_1896 ~ FORC62_1900)	Autolysis
sspA	Serine protease	1080297-1081325 (FORC62_1010)	Autolytic
sspB	SspB	1079034-1080215 (FORC62_1009)	Pathogenesis
sspC	Staphostatin B	1078667-1078996 (FORC62_1008)	Cysteine protease
coa	Staphylocoagulase	259445-261421 (FORC62_0221)	Promotes pathogenesis
nuc	Thermonuclease	1424373-1424906 (FORC62_1356)	Thermonuclease
lip	Triacylglycerol lipase	2864269-2866314 (FORC62_2678)	Lipase
Host Immune evasion			
-	Capsular polysaccharide synthesis	162180 - 178748 (FORC62_0141 ~ FORC62_0156)	Antiphagocytosis:
Secretion system			
esxA, esaA, essA, esaB, essB, essC, esaC, esxB	Type VII secretion system	321827-332464 (FORC62_0274 ~ FORC62_0281)	Secretion
Toxin			
hla	Alpha hemolysin	1243513-1244471 (FORC62_1190, FORC62_1191)	cellular damage
hlb	Beta hemolysin	2153026-2153849 (FORC62_2026)	Beta hemolysin
hld	Delta hemolysin	2182522 - 2182656	lysing erythrocytes

sec, seg, sei, set7, set7, set6, set7, set8, set9, set10, set11, set12, set13, set14, set15	Exotoxin	461137-474861 (FORC62_0415~FORC_0426), 2161498-2162298 (FORC62_2032), 2024018-2024794 (FORC62_1911), 2026796-2027524 (FORC62_1915)	Food-poisoning
yent1, yent2, sell, selm, seln, selo	Enterotoxin	2025077-2029341 (FORC62_1912 ~ FORC62_1917), 2160607-2161329 (FORC62_2031)	Food poisoning
eta	Exfoliative toxin type A	1253292-1254239 (FORC62_1200)	Exfoliative toxin
hlgA, hlgB, hlgC	Gamma hemolysin	2574646-2430 (FORC62_2428~FORC62_2430)	Pore formation
lukD, lukE	Leukotoxin	2016955-2018875 (FORC62_1905, FORC62_1906)	Pore formation
tsst	Toxic shock syndrome toxin	2164524-2165228 (FORC62_2034)	Toxic shock syndrome

Mobile genetic elements of *S. aureus* FORC_062

S. aureus is known to have various mobile genetic elements (MGEs) in its genome. SCC*mec* (staphylococcal cassette chromosome *mec*), transposons, prophage, pathogenicity island and insertion regions are included to MGEs. MGE is closely related to bacterial evolution, as its genetic plasticity facilitates the evolution and acquisition of virulence and drug-resistant factors.

FORC_062 had lots of major MGE. For instance, type II SCC*mec* (FORC62_0030~FORC62_0054) containing *MecA* which causes resistance to methicillin. Also, this strain possesses 3 types of pathogenicity islands and each of them has various kinds of virulence factors.

The MGEs which localized in FORC_062 genome are summarized in table 6.

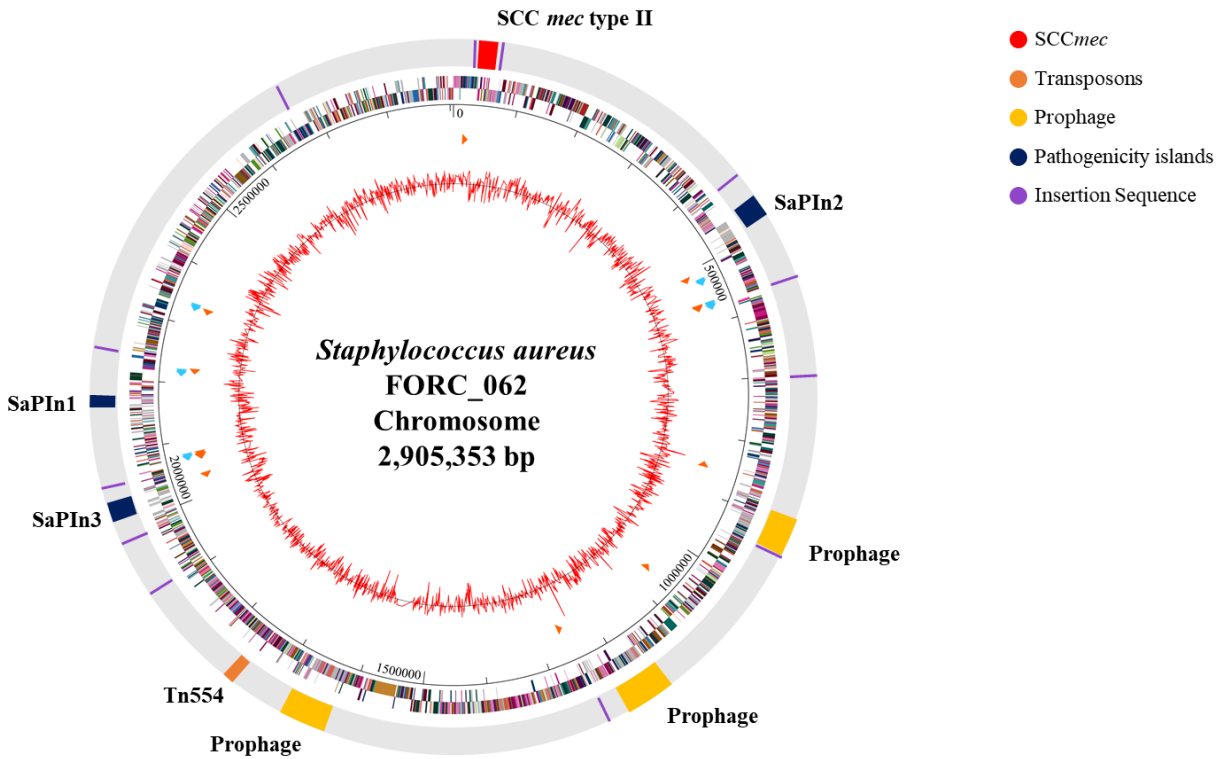


Fig 5. Mobile genetic elements (MGE) of *S. aureus* FORC_062. The mobile genetic elements were indicated on the base of genome map of FORC_062. The annotated MGEs are colored differently according to the categories as indicated above.

Table 6. Mobile genetic elements of *S. aureus* FORC_062

Mobile genetic elements	Region		Feature
SCC*mec*			
Type II SCC*mec*	36958~61167	(FORC62_0030~FORC62_0054)	Methicillin resistance
Transposons			
Tn554	1753784~1759007	(FORC62_1746~FORC62_1751)	Erythromycin, spectinomycin resistance
Prophage			
region I	893071~944346	(FORC62_0807~FORC62_0883)	
region II	1154701~1221018	(FORC62_1079~FORC62_1167)	
region III	1623345~1685236	(FORC62_1531~FORC62_1593)	Type II toxin-antitoxin system MazEF
Pathogenicity islands			
SaPIn1	2160607~2175720	(FORC62_2031~FORC62_2051)	Toxic shock syndrome toxin
SaPIn2	456194~485740	(FORC62_0409~FORC62_0439)	Exotoxin
SaPIn3	2003086~2029323	(FORC62_1891~FORC62_1917)	Enterotoxin, luekocidine
Insertion region			
IS1181	420793~422304, 575583~577094, 699661~701172, 952438~953949, 1241916~1243427, 1909304~1910815, 2062394~2063905, 2237788~2239299, 2663456~2664967		
IS431mec	35855~36644,43552~44341		
ISSau3	1987722~1988803		
ISSau4	64941~66201		

Phylogenetic analysis

To analysis the phylogenetic position of *S. aureus* FORC_062, the 16s rRNA sequence of FORC_062 was compared with other *Staphylococcus* species (*S. aureus, S. epidermidis, S. haemolyticus, S. saprophyticsu*). The result show that FORC_062 belongs to *S. aureus* (Fig. 5).

Furthermore, ANI analysis was conducted to figure out the evolutionary relationship within same species (*S. aureus*). The completed genome sequences of 20 strains (*S. aureus* COL, FORC_001, FORC_012, FORC_026, FORC_027, FORC_039, JH1, JH9, MRSA252, MRSA476, Mu3, Mu50, MW2, N315, NCTC8325, Newman, FR122, USA300_FPR3757, USA300_TCH1516 and FORC_062) were used for the analysis. The result demonstrated that the strain N315 is the mostly close strain with FORC_062, of which the ANI value is the highest (99.94). The strain N315 is a clinical isolate from Japan (Kuroda M *et al*., 2001) (Fig. 6). Consequently, the strain N315 was selected as the subject of comparative analysis.

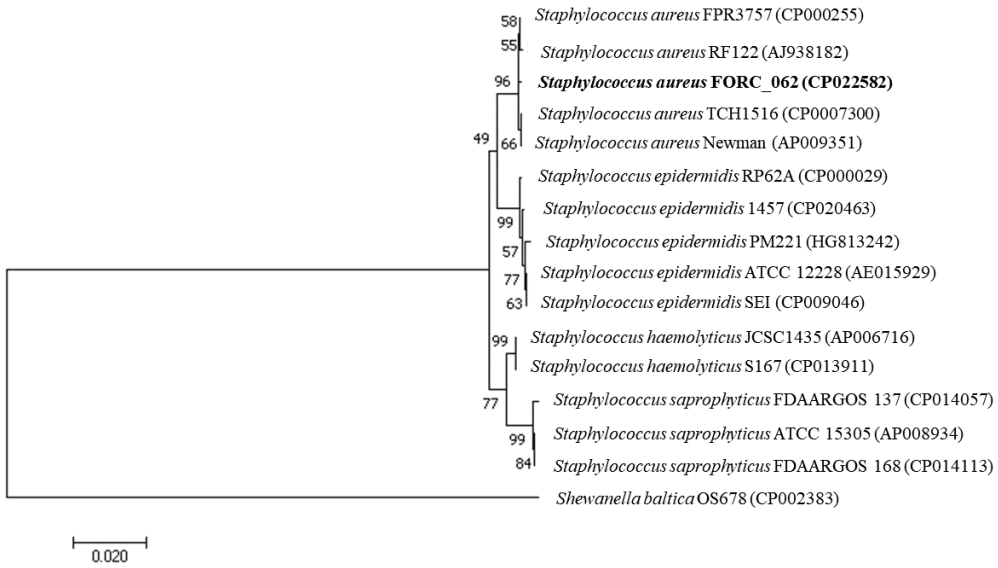


Fig 6. Phylogenetic analysis of *S. aureus* strains. The 16S rRNA sequences from several *Staphylococcus* species (*S. aureus*, *S. epidermidis*, *S. haemolyticus* and *S. saprophyticus*) were aligned by using ClustalW. The phylogenetic tree was constructed by using neighbor joining method with 1000 bootstrap replicates. For the out group, *Shewanella baltica* OS678 was used. The scale bar is below the tree and the bootstrap values are on the branch.

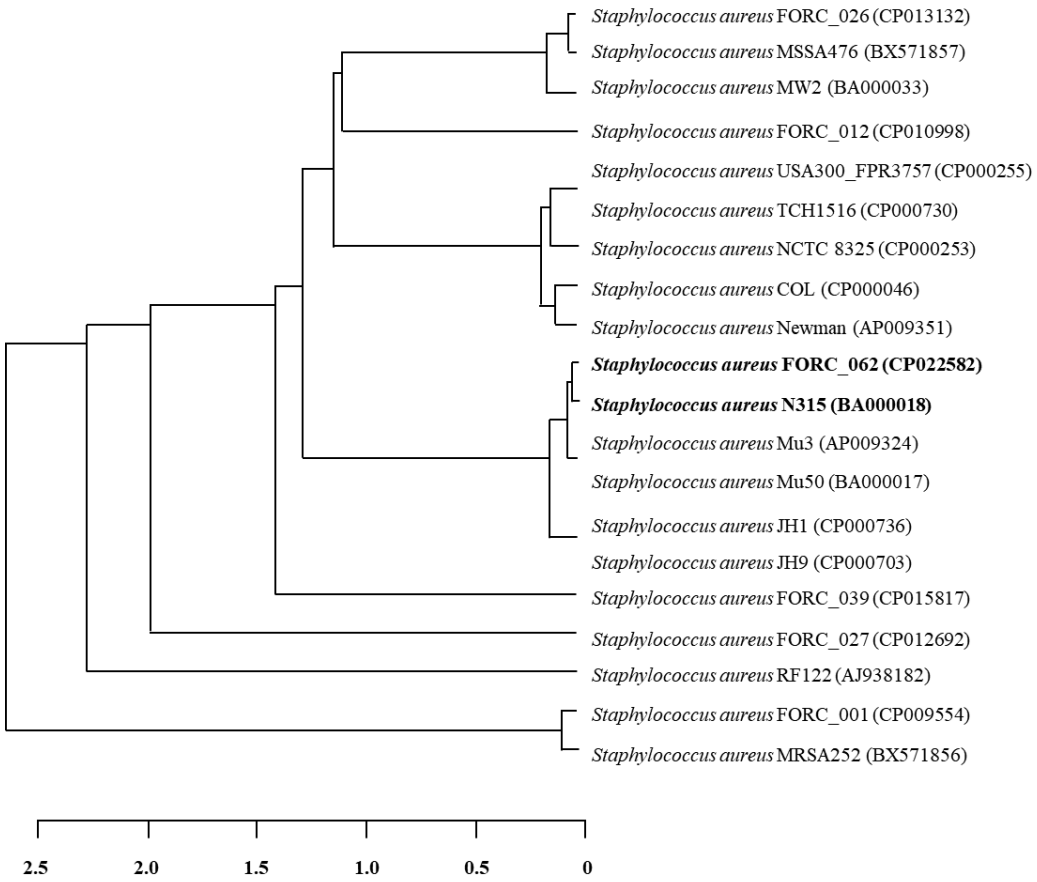
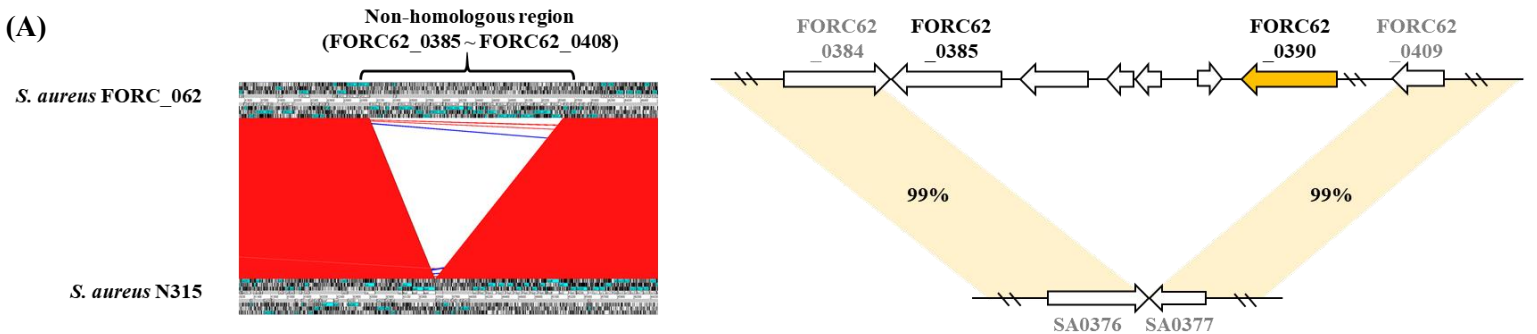


Fig 7. ANI analysis of *S. aureus* strains. The ANI values were computed based on chromosomal sequences of the selected strains. R was used to construct the tree.

Comparative genome analysis between FORC_062 and *S. aureus* N315.

Comparative analysis of whole genome sequence between FORC_062 and *S. aureus* N315 was conducted. It is identified that FORC_062 has additional genes, which may be related to antibiotic resistances (Fig. 7A, B). FORC62_0390 and FORC62_1582 are the regions encoding tetracycline resistance protein TetM and type II toxin-antitoxin system MazEF, respectively.


(A)
Non-homologous region
(FORC62_0385 ~ FORC62_0408)
S. aureus FORC_062
S. aureus N315
FORC62 _0384
FORC62 _0385
FORC62 _0390
FORC62 _0409
99%
99%
SA0376
SA0377

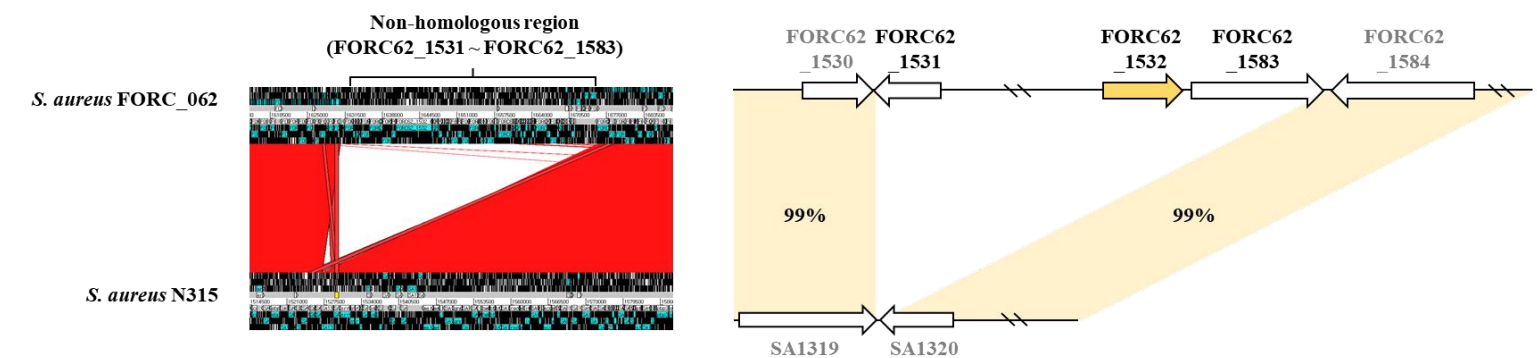

(B)
Non-homologous region
(FORC62_1531 ~ FORC62_1583)
S. aureus FORC_062
S. aureus N315
FORC62 _1530
FORC62 _1531
FORC62 _1532
FORC62 _1583
FORC62 _1584
99%
99%
SA1319
SA1320

Fig 8. Comparative genome analysis between FORC_062 and N315.

The regions (A) and (B) included the tetracycline resistance protein tetM (FORC62_0390) and type II toxin-antitoxin system MazEF (FORC62_1532), respectively. On the left figure, the homologue regions between FORC_062 and N315 are indicated in red color, and the non-homologous regions are indicated in white color. On the right figure, each arrow represents each corresponding gene. The orange arrow represents the functionally important gene in FORC_062. The percentage in the light yellow regions indicates the sequence similarity of the homologous region.

Identification of antimicrobial resistances of FORC_062 and N315.

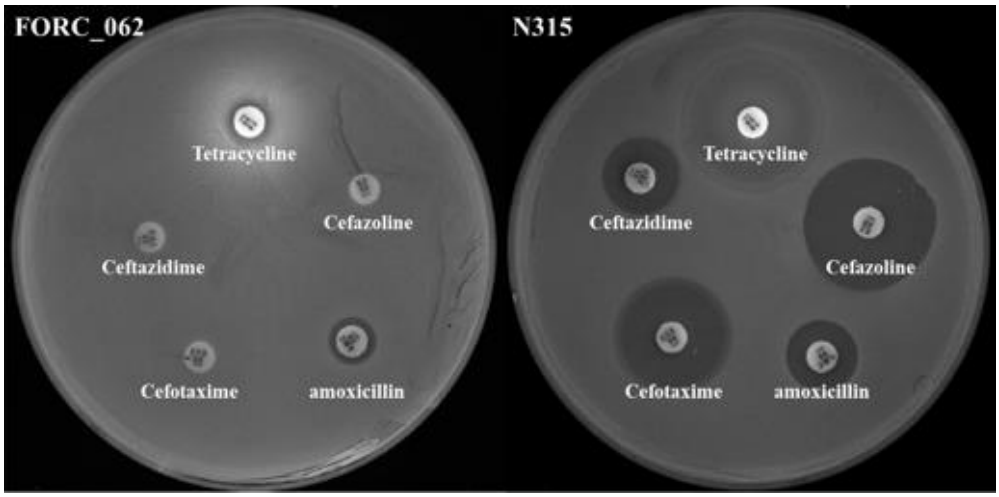
According to the results of comparative analysis, FORC_062 has additional tetracycline resistance gene (FORC62_0390) and type II toxin-antitoxin system MazEF (FORC62_1532). Previous study indicated that the MazEF toxin-antitoxin system alters the β-lactam susceptibility of *S. aureus* (Christopher F. Schuster *et al.,* 2015).

To confirm the prediction of genome sequence phenotypically, the antibiotics susceptibilities of the two strains, FORC_062 and N315, were tested by Kirby-Bauer Disk Susceptibility Test. According to Clinical and Laboratory Standards Institute (CLSI) standards, the result demonstrated that FORC_062 is more resistant to tetracycline and β-lactams (amoxicillin, cefazoline, cefotaxime, ceftazidime) than N315 (Fig. 8A) (Table 6).

Additional antibiotics (ampicillin, ciprofloxacin, erythromycin, linezolid, sulfamethoxazole) susceptibility tests were conducted, as there are various genes related to multidrug resistances. The results of two strains were similar (Fig. 8B).

S. aureus ATCC 29213 served as a quality control strain in the Kirby-Bauer disk susceptibility test

(A)


FORC_062
Tetracycline
Cefazoline
Ceftazidime
Cefotaxime
amoxicillin
N315
Tetracycline
Ceftazidime
Cefazoline
Cefotaxime
amoxicillin

(B)

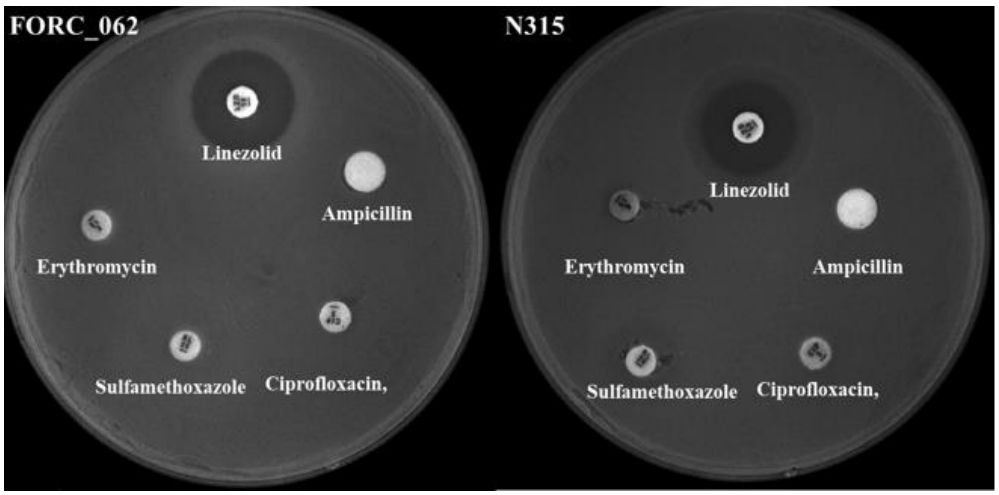

FORC_062
Linezolid
Ampicillin
Erythromycin
Sulfamethoxazole
Ciprofloxacin,
N315
Linezolid
Erythromycin
Ampicillin
Sulfamethoxazole
Ciprofloxacin,

Fig 9. Kirby-Bauer disk susceptibility test to identify antibiotic resistances of FORC_062 and N315. (A) The susceptibilities of FORC_062 and N315 to tetracycline and β-lactams (amoxicillin, cefazoline, cefotaxime, ceftazidime) were identified using Kirby-Bauer disk susceptibility test. **(B)** The susceptibilities to 5 other antibiotics (ampicillin, ciprofloxacin, erythromycin, linezolid, sulfamethoxazole) were identified using Kirby-Bauer disk susceptibility test. The diameters of clear zone formed by each antibiotic were categorized as susceptible (S), intermediate (I), or resistant (R) according to CLSIs. The experiment repeated in biological duplication and technical triplication. The information of 5 antimicrobial discs (Oxoid, UK) were listed in table 6.

Table 7. Antibiotic resistances of *S. aureus* FORC_062 and N315

Antimicrobial agent	[a] Disk content (µg)	[b] Interpretive criteria			[c] Zone diameter	
		S	I	R	FORC_062	N315
Amoxicillin	30	≥18	14-17	≤13	10 (R)	14 (I)
Cephazolin	30	≥15	-	≤14	- (R)	24 (S)
Cefotaxime	30	≥26	23-25	≤22	- (R)	23 (I)
Ceftazidime	30	≥21	18-20	≤17	- (R)	15 (R)
Tetracycline	30	≥15	12-14	≤11	9 (R)	24 (S)
Ampicillin	10	≥13	14-16	≤17	- (R)	- (R)
Ciprofloxacin	5	≥31	21-30	≤20	- (R)	- (R)
Erythromycin	15	≥23	14-22	≤13	- (R)	- (R)
linezolid	10	≥23	20-23	≤20	19 (R)	20 (I)
Sulfamethoxazole	25	≥16	11-15	≤10	- (R)	- (R)

[a] Antibiotic-impregnated discs (6 mm) with amount, µg shown in bracket
[b] Interpretive criteria according to the recommendations of the Clinical and Laboratory Standards Institute
[c] Diameter of inhibition from three individual experiments. **S, susceptible; I, intermediate; R, resistant.**

Identification of DEGs (Differentially Expressed Genes) of FORC_062 under exposure to raw chicken breast

To understand the transcriptional change of *S. aureus* FORC_062 when it is exposed to raw chicken breast, transcriptome analysis was conducted. Experimental groups (with raw chicken breast) and control groups (without raw chicken breast) were incubated in M9 minimal media for 2 h and 4 h. After extraction of total RNA sample, RNA-sequencing was performed by using the samples. Samples of each time point were conducted in biological triplication. The average RPKM values of samples were used to represent the expression level of genes. Volcano plots of time points of 2 h and 4 h (Fig. 9A, B) was constructed to represent the overall profile of RNA-sequencing results. The genes which were differently expressed significantly were identified in the statistical criteria of fold change $\geq \pm 2$ and P-value < 0.05. The results of time points 2 h and 4 h were similar. At time point of 2 h incubation, a total of 340 genes were differentially expressed, where 155 genes were up-regulated and 185 genes were down-regulated. The number of genes expressed differentially was 453 when the infection time was 4 h, among them 227 genes were up-regulated and 226 genes were down-regulated. These genes were categorized by their function according to the WebMGA server (Fig. 10A, B).

Generally, genes related with amino acid synthesis were down-regulated, while other genes related with amino acid such as permease and transporter were up-regulated. For the carbohydrate metabolism, genes related with glucose metabolism were up-regulated, on the contrast, genes related with other carbohydrate source were

down-regulated. At the same time, genes encoding protein related to deamination and long-chain lipid degradation were up-regulated. In addition, many virulence factor associated genes like enterotoxin, adhesion and type VII secretion system were up-regulated significantly. (Table. 7)

Furthermore, the results of RNA-sequencing were depicted using heat map (Fig. 11). To examine the reliability of the RNA-sequencing results, qRT-PCR was conducted (Fig. 12).

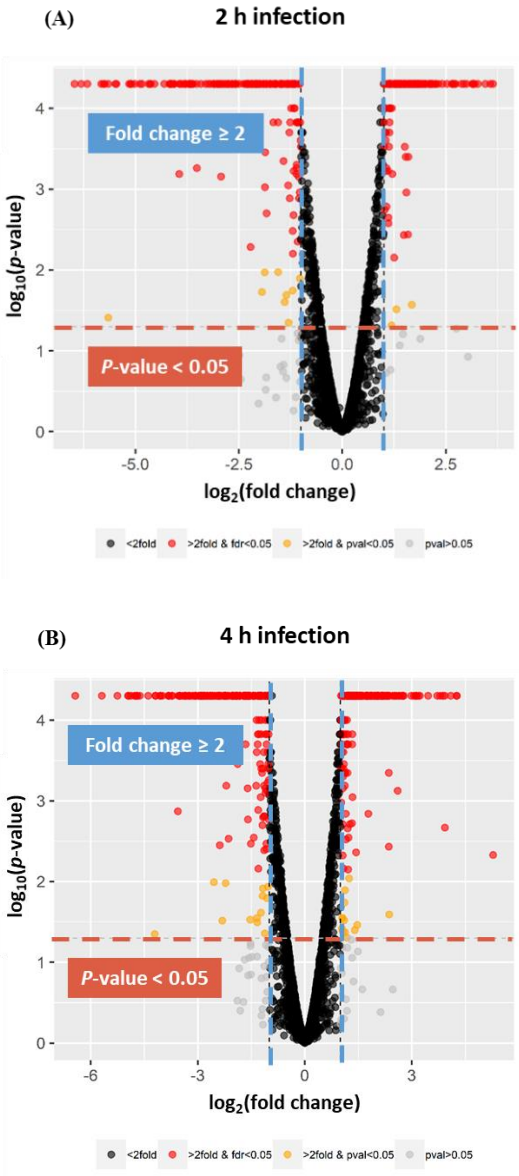


Fig 10. Transcriptome comparison of the RNA sequencing samples. The volcano plot was expressed to visualize differentially total expressed genes when *S. aureus* FORC_062 was exposed to raw chicken breast for 2 h (A) and 4 h (B).

Table 8. List of genes differentially expressed when FORC_062 exposed to raw chicken breast.

Locus tag	Annotation	2 h infection		4 h infection	
		Fold change [a]	*P*-value	Fold change [a]	*P*-value
Amino acid transporter					
FORC62_1470	Amino acid permease	2.94	0.00005	3.92	0.00005
FORC62_1773	Lysine-specific permease	1.17	0.00005	1.53	0.00005
FORC62_0717	Di/tripeptide permease YjdL			1.13	0.00045
FORC62_1630	Glycine dehydrogenase			1.06	0.00475
FORC62_1631	Glycine dehydrogenase			1.21	0.0071
FORC62_1632	Aminomethyltransferase			1.35	0.00005
FORC62_0009	Histidine ammonia-lyase	1.42	0.00005	1.76	0.00005
FORC62_0228	Oligopeptide ABC transporter,OppA	1.80	0.00005	1.76	0.00005
FORC62_2446	D-serine/D-alanine/glycine transporter	1.35	0.00005		
FORC62_2458	Amino acid transporter	1.15	0.00005	1.42	0.00005
FORC62_2641	Arginine/ornithine antiporter ArcD	-1.60	0.00005		
FORC62_2421	Molybdenum transport ATP-binding protein ModC	-1.86	0.00035	-2.03	0.00005
FORC62_2422	L-Cystine ABC transporter, permease protein TcyB	-1.87	0.00095	-1.96	0.00025
FORC62_1945	Glutamate transport ATP-binding protein	-3.89	0.00005	-2.97	0.00005
FORC62_1946	Glutamate ABC transporter	-4.17	0.00005	-3.33	0.00005
FORC62_0566	L-Proline/Glycine betaine transporter ProP			1.07	0.0005
FORC62_2298	Eukaryotic-type low-affinity urea transporter			1.47	0.00005
FORC62_1703	Allophanate hydrolase 2 subunit 2	1.70	0.00005		
FORC62_1704	Allophanate hydrolase 2 subunit 1	1.60	0.0004		
FORC62_2299	Urease gamma subunit			5.28	0.0047
FORC62_2300	Urease beta subunit			3.93	0.00215

FORC62_2301	Urease alpha subunit	1.16	0.0001	2.77	0.00005
FORC62_2302	Urease accessory protein UreE			1.78	0.00005
FORC62_2303	Urease accessory protein UreF			1.46	0.00005
FORC62_2304	Urease accessory protein UreG			1.08	0.0069
FORC62_2305	Urease accessory protein UreD			1.34	0.00005
Amino aicd synthesis					
FORC62_0200	Gamma-glutamyltranspeptidase	-2.66	0.00005	-2.23	0.00005
FORC62_0926	Argininosuccinate lyase	-3.69	0.00005	-2.23	0.00005
FORC62_0927	Argininosuccinate synthase	-4.68	0.00005	-3.50	0.00005
FORC62_1197	Amino acid kinase family			-1.07	0.0002
FORC62_2642	Ornithine carbamoyltransferase	-2.00	0.00005		
FORC62_1402	Anthranilate phosphoribosyltransferase	-5.15	0.00005	-4.19	0.00005
FORC62_1403	Indole-3-glycerol phosphate synthase	-5.82	0.00005	-4.37	0.00015
FORC62_1404	Phosphoribosylanthranilate isomerase	-5.74	0.00005	-4.21	0.0448
FORC62_1405	Tryptophan synthase beta chain	-5.09	0.00005	-3.68	0.0002
FORC62_1406	Tryptophan synthase alpha chain	-3.51	0.00055	-2.22	0.0105
FORC62_0109	siderophore biosynthesis protein SbnA		0.00045	-2.22	0.00005
FORC62_0452	Cystathionine beta-synthase	-1.39	0.02485		
FORC62_0453	Cystathionine gamma-lyase	-1.30	0.00005		
FORC62_0505	Cysteine synthase	-1.91	0.00005	-1.57	0.00005
FORC62_0464	Glutamate synthase large chain	-1.73	0.00005	-1.93	0.00005
FORC62_1813	Phosphoserine aminotransferase, putative			-1.51	0.0034
FORC62_1362	Homoserine kinase	-1.08	0.00445	-1.20	0.00045
FORC62_2687	ATP phosphoribosyltransferase	-1.55	0.01065	-2.14	0.00295
FORC62_2688	ATP phosphoribosyltransferase regulatory subunit	-1.51	0.00005	-1.99	0.00005
FORC62_2686	Histidinol dehydrogenase	-1.29	0.00005	-1.73	0.00005
FORC62_2074	Dihydroxy-acid dehydratase			-1.07	0.00015

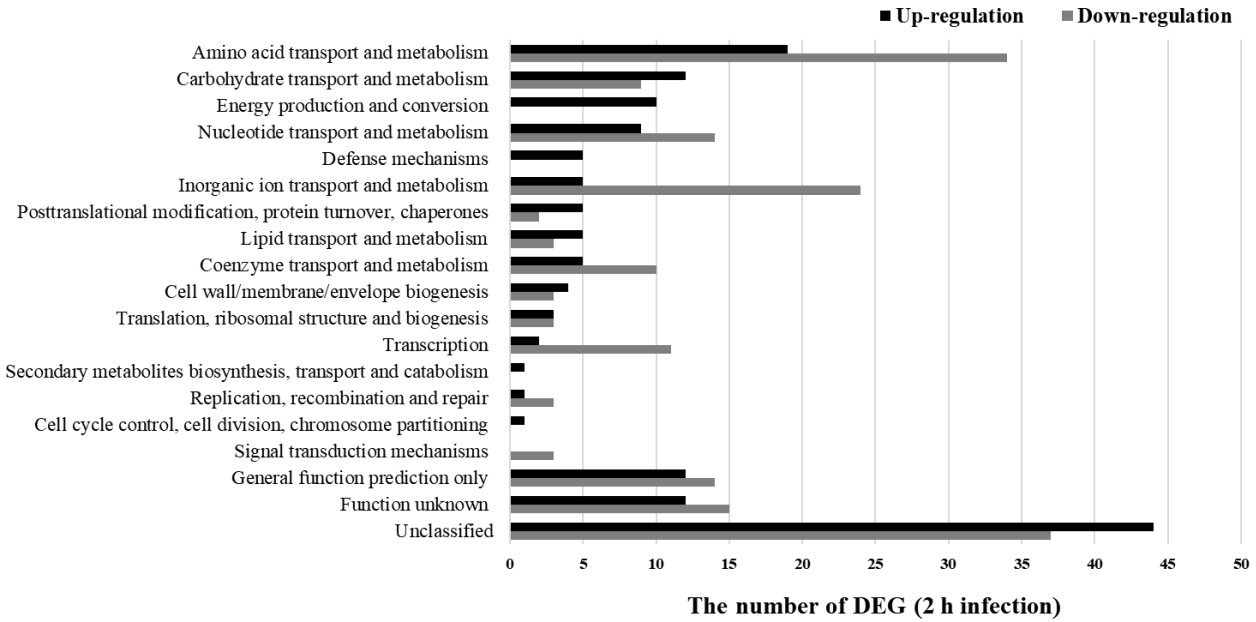
FORC62_2077	Ketol-acid reductoisomerase			-1.55	0.00005
FORC62_2078	2-isopropylmalate synthase			-1.70	0.00005
FORC62_2080	3-isopropylmalate dehydratase large subunit			-1.15	0.00025
FORC62_2081	3-isopropylmalate dehydratase small subunit			-1.32	0.0002
FORC62_2477	Diaminopimelate epimerase		0.00005	-2.65	0.00005
FORC62_0110	Ornithine cyclodeaminase			-2.24	0.00005
FORC62_1063	Spermidine Putrescine ABC transporter permease component PotB	-1.16	0.00005		
FORC62_1064	Spermidine Putrescine ABC transporter permease component potC	-1.20	0.01795		
FORC62_2538	L-serine dehydratase, beta subunit			1.10	0.00005
Carbohydrate metabolism					
FORC62_1778	NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase	1.07	0.00165		
FORC62_2595	Ribulosamine/erythrulosamine 3-kinase potentially	1.16	0.00005		
FORC62_2696	Lactonase Drp35	1.18	0.00005		
FORC62_1469	putative transporter	2.74	0.00005	4.25	0.00005
FORC62_0180	PTS glucose EIICBA component	1.75	0.00005	3.11	0.00005
FORC62_0764	Phosphoglycerate kinase	1.92	0.00005	1.87	0.00005
FORC62_0763	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	1.82	0.00005	1.84	0.00005
FORC62_0766	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	1.75	0.00005	1.84	0.00005
FORC62_0765	Triosephosphate isomerase	1.68	0.00005	1.74	0.00005
FORC62_0131	Phosphopentomutase	1.58	0.00005	1.71	0.00005
FORC62_0233	PTS systerm, maltose and glucose-specific IIB component			1.42	0.00005
FORC62_1816	PTS glucose transporter subunit IIBC			1.25	0.00005
FORC62_2539	Transcriptional regulator pfoR			1.23	0.00005
FORC62_2545	PTS system, glucose-specific IIBC component			1.20	0.00005
FORC62_0210	Inosose isomerase			1.12	0.00065
FORC62_2425	Phosphoglycerate mutase			1.08	0.0004
FORC62_0238	PTS system, galactitol-specific IIC component	-1.24	0.00005	-1.14	0.00005

FORC62_2211	Galactose-6-phosphate isomerase, LacA subunit			-1.19	0.002
FORC62_0237	PTS system, galactitol-specific IIB component	-1.51	0.00005	-1.24	0.0009
FORC62_0213	Glycerol-3-phosphate transporter			-1.25	0.00035
FORC62_0791	Phosphoglycerate mutase family	-1.47	0.00005	-1.26	0.00005
FORC62_2207	PTS system, lactose-specific IIA component			-1.30	0.00695
FORC62_0689	1-phosphofructokinase	-1.67	0.00015	-1.32	0.00015
FORC62_0690	PTS system, fructose-specific IIA component	-1.17	0.00005	-1.44	0.00005
FORC62_2173	PTS system, mannitol-specific IIBC component	-1.28	0.0002	-1.61	0.00005
FORC62_0320	PTS system, Lactose/Cellobiose specific IIB subunit	-1.05	0.0013		
Deamination					
FORC62_1471	Threonine dehydratase, catabolic	3.46	0.00005	4.09	0.00005
FORC62_1472	Alanine dehydrogenase	3.20	0.00005	3.87	0.00005
FORC62_2538	L-serine dehydratase, beta subunit			1.10	0.00005
Lipid metabolism					
FORC62_0568	3-ketoacyl-CoA thiolase	-1.15	0.00005		
FORC62_1701	Biotin carboxylase	1.12	0.00265		
FORC62_1702	Biotin carboxyl carrier protein	1.11	0.0006		
FORC62_0582	Mevalonate kinase			1.72	0.00005
FORC62_0584	Phosphomevalonate kinase			1.72	0.00005
FORC62_0583	Diphosphomevalonate decarboxylase			1.52	0.00005
FORC62_0948	3-oxoacyl-(acyl-carrier-protein) synthase, KASIII			1.41	0.00005
FORC62_0223	Enoyl-CoA hydratase	2.18	0.00005	1.33	0.00015
FORC62_0224	Long-chain-acyl-CoA dehydrogenase	2.25	0.00005	1.33	0.0002
FORC62_0222	3-ketoacyl-CoA thiolase	2.12	0.00005	1.18	0.00005
FORC62_2553	Hydroxymethylglutaryl-CoA synthase			1.10	0.00015
FORC62_1817	1-acyl-sn-glycerol-3-phosphate acyltransferase			1.05	0.0001
FORC62_1257	Malonyl CoA-acyl carrier protein transacylase			1.04	0.038

FORC62_0166	butyryl-CoA dehydrogenase	-2.81	0.00005	-2.43	0.00005
Virulence factor					
FORC62_1696	Enterotoxin, phage associated	1.59	0.00365		0.0302
FORC62_1912	Enterotoxin, phage associated	1.12	0.00005		
FORC62_1913	Enterotoxin, phage associated	1.25	0.00705		
FORC62_1914	Enterotoxin, phage associated	1.49	0.0037	1.23	0.00285
FORC62_1915	Superantigen enterotoxin SEK	1.39	0.00005	1.03	0.0001
FORC62_1916	Superantigen enterotoxin SEK	1.49	0.00005		
FORC62_1917	Enterotoxin, phage associated	1.76	0.00005	1.71	0.00005
FORC62_2031	Superantigen enterotoxin SEL	1.13	0.00005	1.2	0.00005
FORC62_0554	MSCRAMM family adhesin SdrC	2.1	0.00005	1.28	0.00005
FORC62_0555	MSCRAMM family adhesin SdrD	1.88	0.00005	1.02	0.00105
FORC62_2654	Predicted cell-wall-anchored protein SasF (LPXAG motif)	2.66	0.00005	2.34	0.00005
FORC62_0274	ESAT-6/Esx family secreted protein EsxA/YukE	1.54	0.00005	1.96	0.00005
FORC62_0275	Putative secretion accessory protein EsaA/YueB	1.27	0.00005	1.83	0.00005
FORC62_0276	Putative secretion system component EssA			2.35	0.0037
FORC62_0278	Putative secretion system component EssB/YukC	1.44	0.00005	2.13	0.00005
FORC62_0279	putative secretion system component EssC/YukA	1.3	0.00005	2.16	0.00005
FORC62_0280	protein EsaC			2.2	0.00005
FORC62_0281	ESAT-6/Esx family secreted protein EsxB			2.35	0.00045
FORC62_0273	Secretory antigen precursor SsaA	1.49	0.00005	1.84	0.00005

[a] The $\log_2$ mRNA expression level of the *S. aureus* FORC_062 experimental groups relative to the control groups

(A)


Up-regulation
Down-regulation
Amino acid transport and metabolism
Carbohydrate transport and metabolism
Energy production and conversion
Nucleotide transport and metabolism
Defense mechanisms
Inorganic ion transport and metabolism
Posttranslational modification, protein turnover, chaperones
Lipid transport and metabolism
Coenzyme transport and metabolism
Cell wall/membrane/envelope biogenesis
Translation, ribosomal structure and biogenesis
Transcription
Secondary metabolites biosynthesis, transport and catabolism
Replication, recombination and repair
Cell cycle control, cell division, chromosome partitioning
Signal transduction mechanisms
General function prediction only
Function unknown
Unclassified
0
5
10
15
20
25
30
35
40
45
50
The number of DEG (2 h infection)

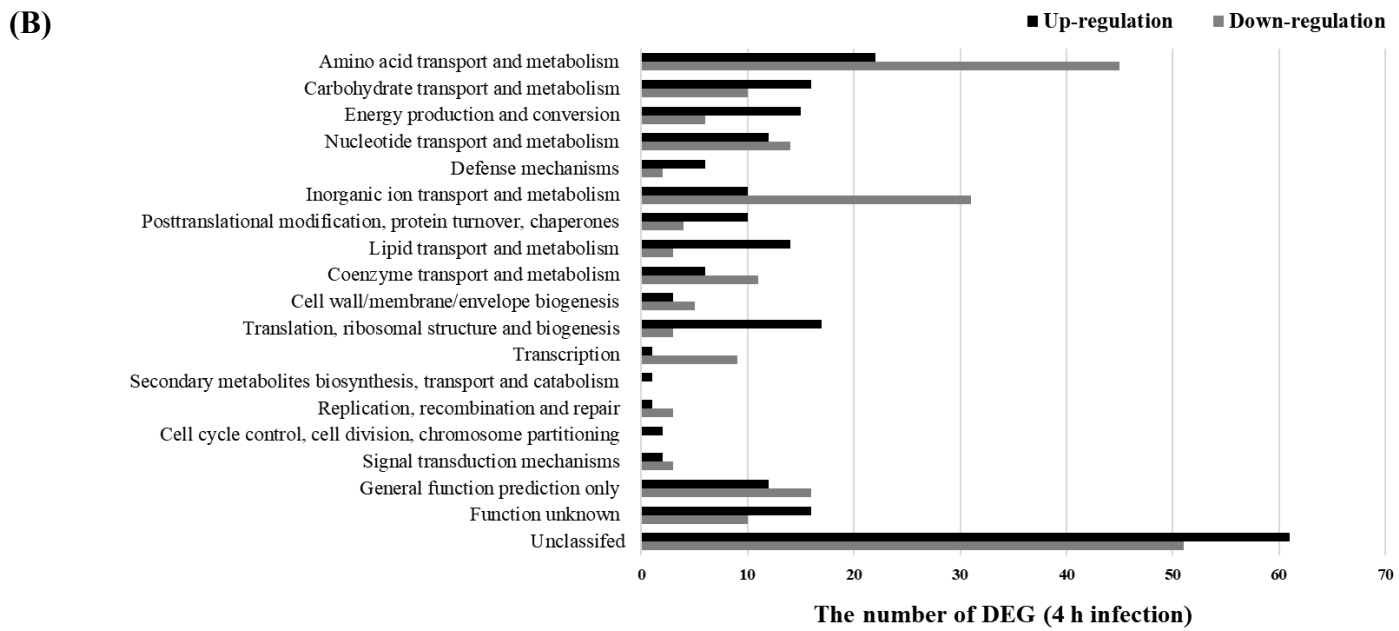
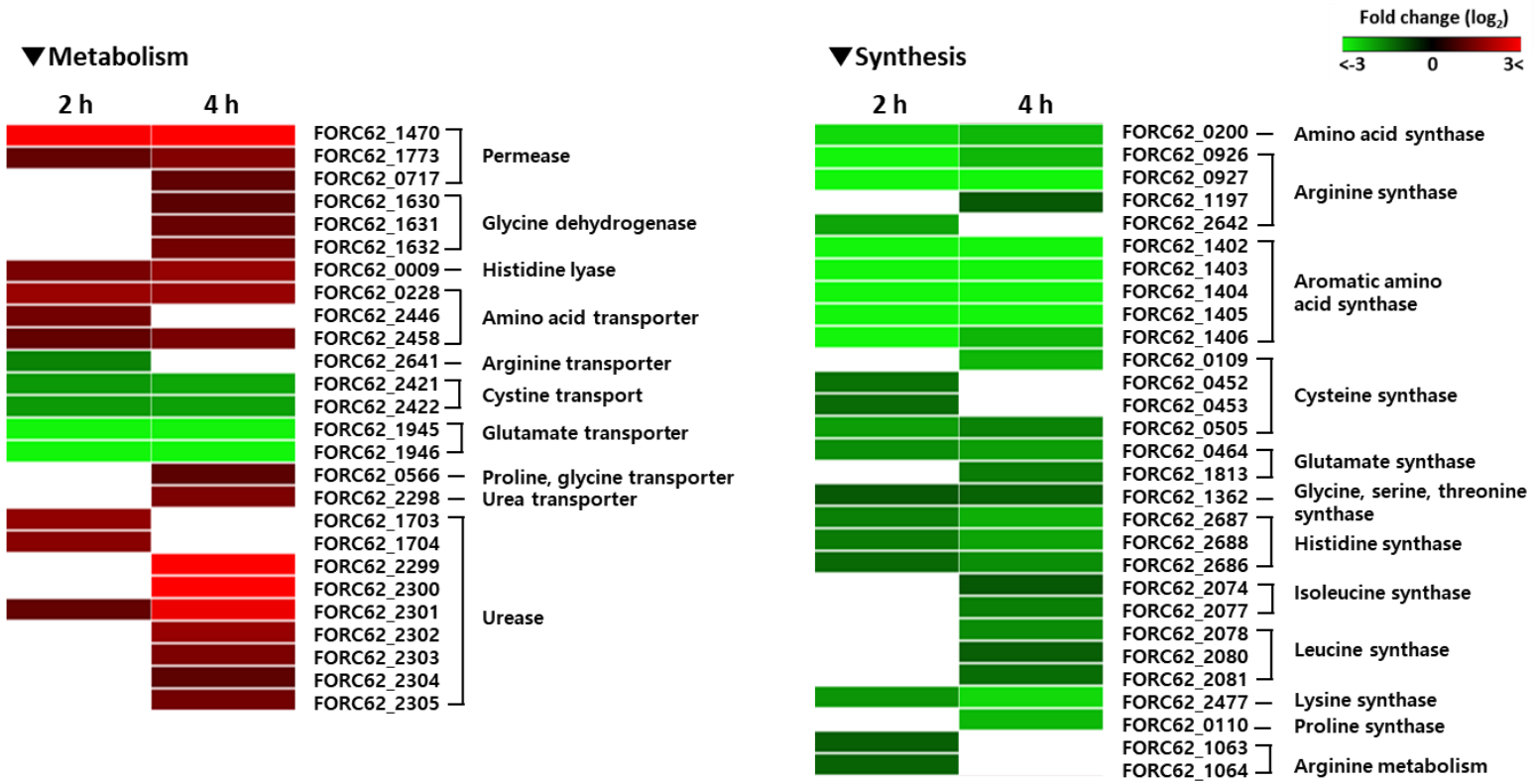


Fig 11. Functional categorization of genes differentially expressed under exposure to raw chicken breast. Gene expressed differentially was classified by their function. The black bars indicate up–regulated genes, while the gray bars indicate down-regulated genes. DEG; differentially expressed gene. (A) 2 h infection (B) 4 h infection


▼Metabolism
2 h
4 h
FORC62_1470
FORC62_1773
FORC62_0717
Permease
FORC62_1630
FORC62_1631
FORC62_1632
Glycine dehydrogenase
FORC62_0009
Histidine lyase
FORC62_0228
FORC62_2446
FORC62_2458
Amino acid transporter
FORC62_2641
Arginine transporter
FORC62_2421
FORC62_2422
Cystine transport
FORC62_1945
FORC62_1946
Glutamate transporter
FORC62_0566
Proline, glycine transporter
FORC62_2298
Urea transporter
FORC62_1703
FORC62_1704
FORC62_2299
FORC62_2300
FORC62_2301
FORC62_2302
FORC62_2303
FORC62_2304
FORC62_2305
Urease
▼Synthesis
Fold change (log2)
<-3
0
3<
2 h
4 h
FORC62_0200
Amino acid synthase
FORC62_0926
FORC62_0927
FORC62_1197
FORC62_2642
Arginine synthase
FORC62_1402
FORC62_1403
FORC62_1404
FORC62_1405
FORC62_1406
Aromatic amino acid synthase
FORC62_0109
FORC62_0452
FORC62_0453
FORC62_0505
Cysteine synthase
FORC62_0464
FORC62_1813
Glutamate synthase
FORC62_1362
Glycine, serine, threonine synthase
FORC62_2687
FORC62_2688
FORC62_2686
Histidine synthase
FORC62_2074
FORC62_2077
Isoleucine synthase
FORC62_2078
FORC62_2080
FORC62_2081
Leucine synthase
FORC62_2477
Lysine synthase
FORC62_0110
Proline synthase
FORC62_1063
FORC62_1064
Arginine metabolism

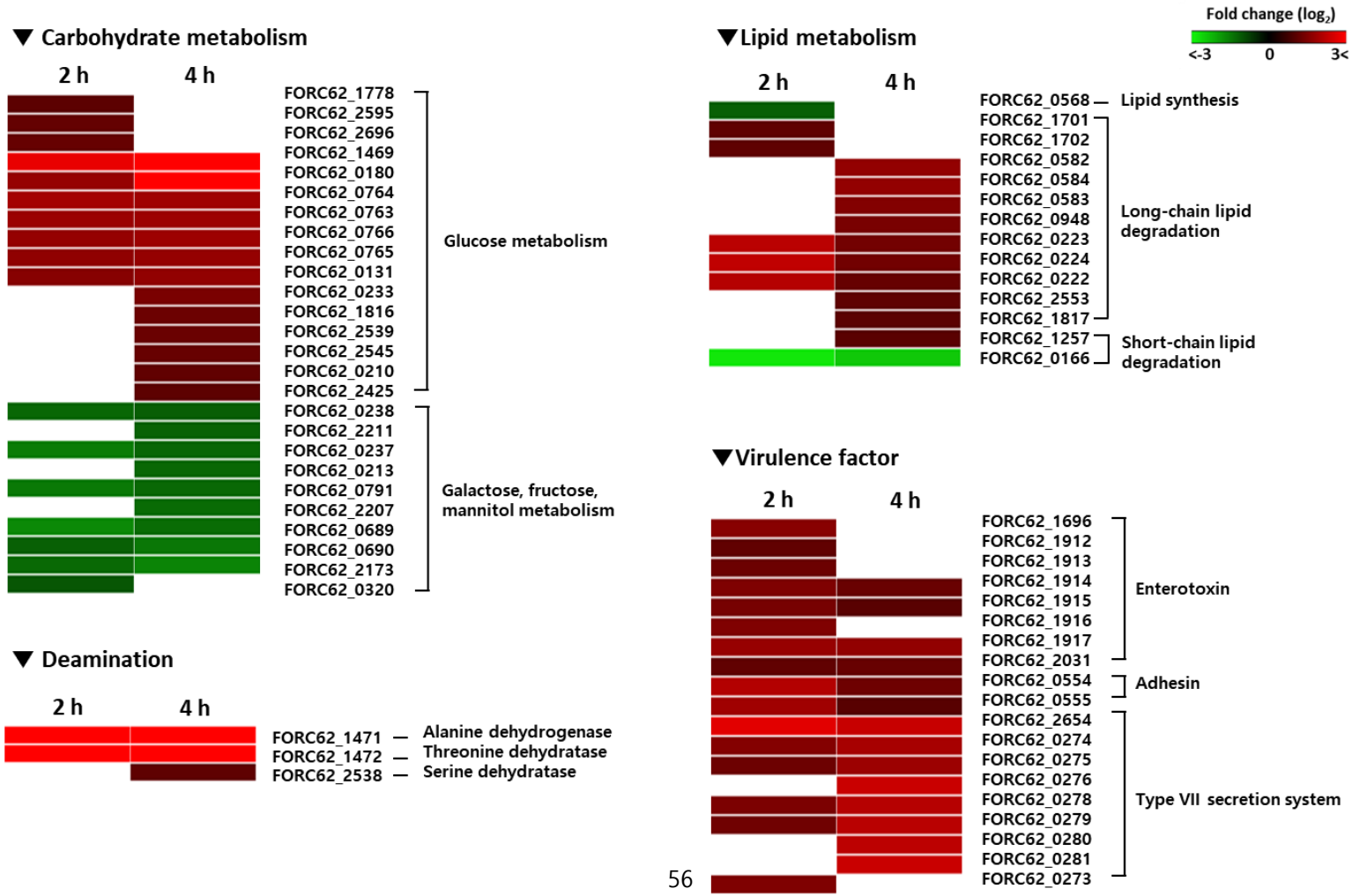
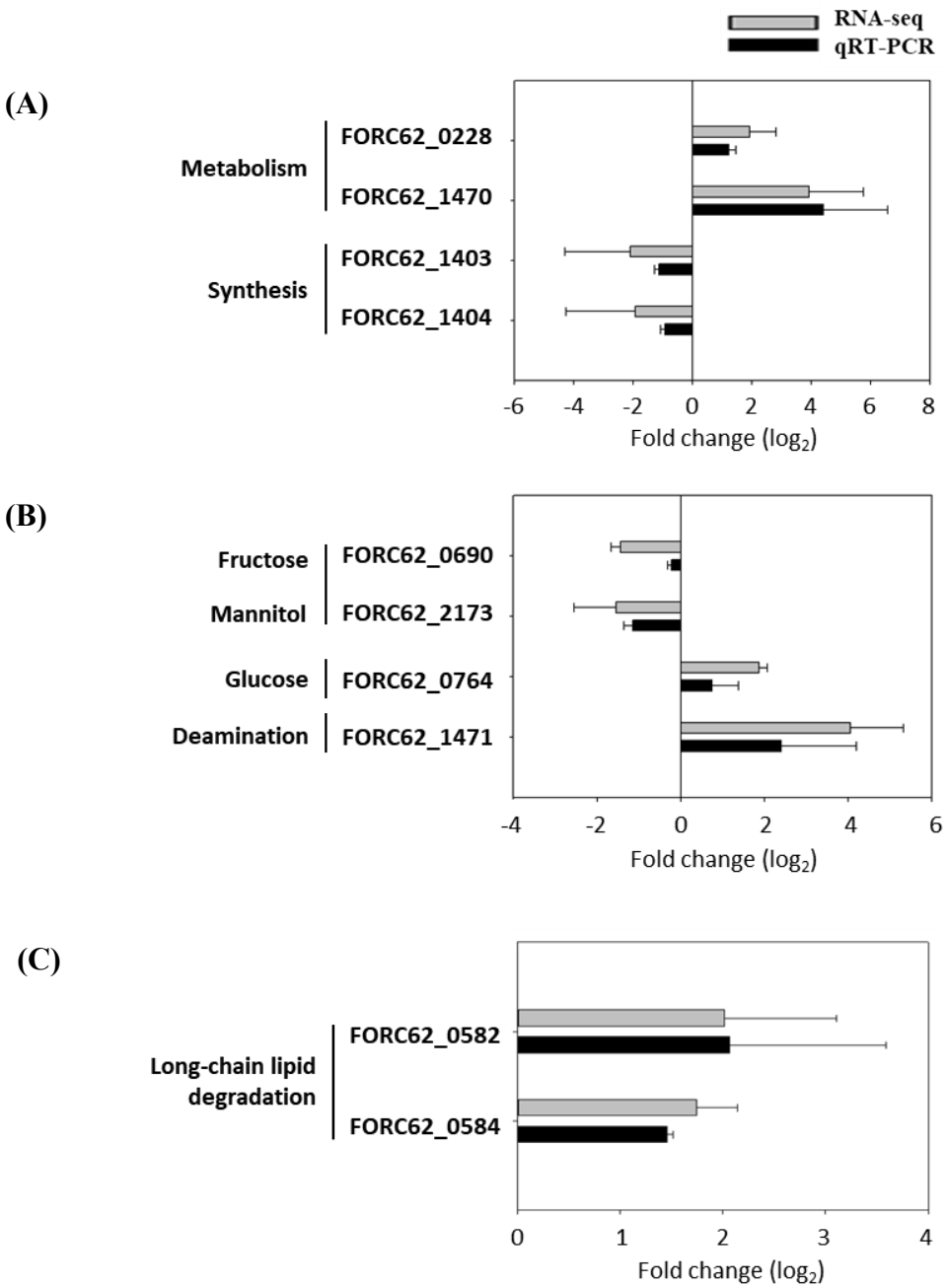

▼ Carbohydrate metabolism
2 h
4 h
FORC62_1778
FORC62_2595
FORC62_2696
FORC62_1469
FORC62_0180
FORC62_0764
FORC62_0763
FORC62_0766
FORC62_0765
FORC62_0131
FORC62_0233
FORC62_1816
FORC62_2539
FORC62_2545
FORC62_0210
FORC62_2425
Glucose metabolism
FORC62_0238
FORC62_2211
FORC62_0237
FORC62_0213
FORC62_0791
FORC62_2207
FORC62_0689
FORC62_0690
FORC62_2173
FORC62_0320
Galactose, fructose, mannitol metabolism
▼ Deamination
2 h
4 h
FORC62_1471
FORC62_1472
FORC62_2538
Alanine dehydrogenase
Threonine dehydratase
Serine dehydratase
Fold change (log2)
<-3
0
3<
▼Lipid metabolism
2 h
4 h
FORC62_0568
Lipid synthesis
FORC62_1701
FORC62_1702
FORC62_0582
FORC62_0584
FORC62_0583
FORC62_0948
FORC62_0223
FORC62_0224
FORC62_0222
FORC62_2553
FORC62_1817
Long-chain lipid degradation
FORC62_1257
FORC62_0166
Short-chain lipid degradation
▼Virulence factor
2 h
4 h
FORC62_1696
FORC62_1912
FORC62_1913
FORC62_1914
FORC62_1915
FORC62_1916
FORC62_1917
FORC62_2031
Enterotoxin
FORC62_0554
FORC62_0555
Adhesin
FORC62_2654
FORC62_0274
FORC62_0275
FORC62_0276
FORC62_0278
FORC62_0279
FORC62_0280
FORC62_0281
FORC62_0273
Type VII secretion system

Fig 12. Heat map of DEGs under exposure to raw chicken breast. The results of RNA-sequencing were depicted using heat map. The red bars indicated up-regulated genes, and the green bars indicated the down-regulated genes. The genes were categorized according to their functions. The results of both 2 h infection and 4 h infection were showed. The scale bar is in the upper right of the page. Heat map was constructed using the Gitools.


(A)
RNA-seq
qRT-PCR
Metabolism
FORC62_0228
FORC62_1470
Synthesis
FORC62_1403
FORC62_1404
-6 -4 -2 0 2 4 6 8
Fold change (log2)
(B)
Fructose
FORC62_0690
Mannitol
FORC62_2173
Glucose
FORC62_0764
Deamination
FORC62_1471
-4 -2 0 2 4 6
Fold change (log2)
(C)
Long-chain lipid degradation
FORC62_0582
FORC62_0584
0 1 2 3 4
Fold change (log2)

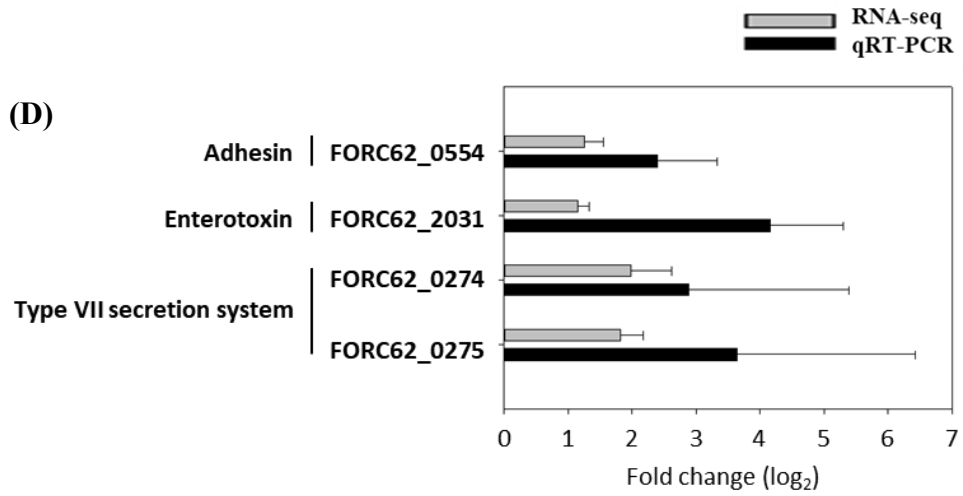


Fig 13. Comparison between RNA sequencing and qRT-PCR. The results of RNA sequencing were confirmed using qRT-PCR by targeting the represented genes. (A) Amino acid metabolism, (B) carbohydrate metabolism and deamination, (C) lipid metabolism, (D) virulence factors were identified, respectively. The gray bars indicated RNA-sequencing result and the black bars indicated qRT-PCR results. The products and more information of each genes were listed in Table 7. The error bars represent the standard deviations.

Growth kinetics of FORC_062 exposed to raw chicken breast

As protein and lipid are abundant components of raw chicken meat (Koh *et al*., 2015) (M. De Marchi *et al.*, 2012), it is possible for FORC_062 to utilize protein and lipids for growth and survival when the strain exposed to raw chicken breast. To understand the kinetic growth of FOCR_062 when it was exposed to raw chicken breast, it was incubated in M9 minimal media with or without chicken breast.

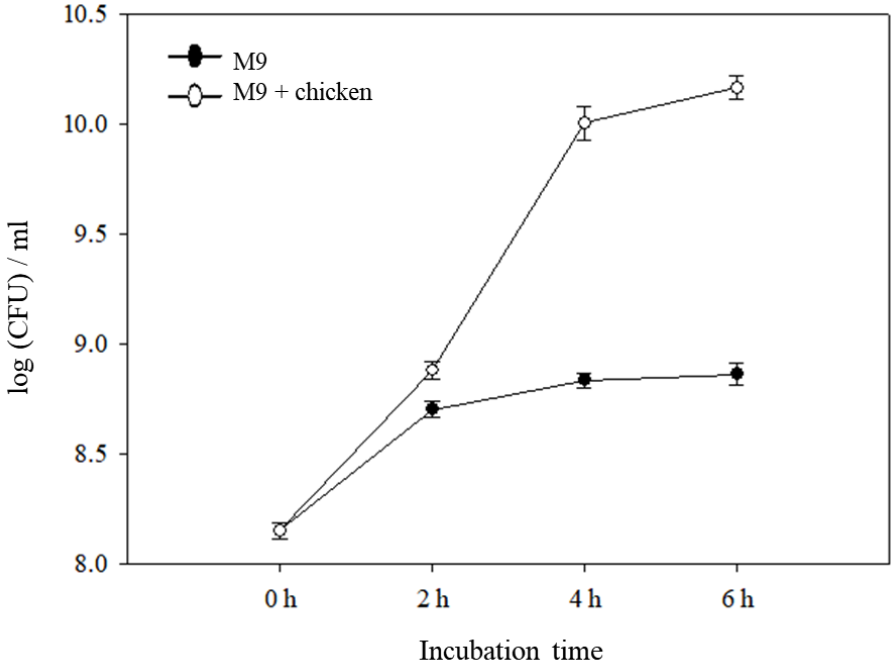


Fig 14. Growth of *S. aureus* FORC_062 under exposure to raw chicken breast. FORC_062 grown to A_{600} of 0.7 was harvested and washed, then inoculated in M9 media or M9 media containing raw chicken breast. Growth of cell was measured by CFU counting at specific time points (0 h, 2 h, 4 h, 6 h). The experiments were performed by biological and technical triplication. Open circle (○), cells grown in M9; closed circle (●), cells grown in M9 containing chicken.

IV. DISCUSSION

Staphylococcus aureus is an opportunistic microorganism and causes skin ailments to life-threating disease such as sepsis. The importance of studying *S. aureus* comes from yearly increasing infections and antibiotics resistance. Notably, in South Korea, MRSA ratio is more than 67%, which is frightening statistics. However, the types of antibiotics that *S. aureus* has resistance is increasing rapidly. To prevent future outbreaks, there is the necessity of studying the genome and transcriptome of the species. In this study, a clinical *S. aureus* isolate from blood of patient in South Korea was analyzed.

To estimate the virulence of the isolate F2742, PCR screening targeting major virulence genes and cytotoxicity test were conducted. This strain possesses various major virulence genes, including *mecA* (Fig. 1). Cytotoxicity test showed than it has higher cytotoxicity than other clinical isolates (Fig. 2). The results suggested that this strain may be more virulent than other strains, so *S. aureus* F-2742 was designates as 'FORC_062'.

S. aureus FORC_062's genomic DNA was extracted. The whole genome sequenced by Pacbio was used for analysis. FORC_062 had many virulence genes and antibiotic resistance genes (Fig. 4). Also, there were many MGEs on genomes (table 6), which is closely related to bacterial evolution, as its genetic plasticity facilitates the evolution and acquisition of virulence and drug-resistant factors.

The ANI analysis suggested that *S. aureus* N315 (ANI value 99.94) isolated from Japanese patient was the most closely related strain to FORC_062 in their genome sequences. Furthermore, to understand the genome properties that FORC_062 has uniquely, comparative genome analysis was conducted between FORC_062 and N315. FORC_062 has several non-homologous regions, which of them there are two non-homologous region related to contributing its antibiotics resistance. One region has *TetM* (FORC62_0930) encoding tetracycline resistance protein. The other region has type II toxin –antitoxin system MazEF. According to the previous study, MazEF system alters the β-lactam susceptibility of *S. aureus* (Christopher F. Schuster *et al.*, 2015). To confirm the *in silico* prediction, Kirby-Bauer Disk Susceptibility Test was conducted (Fig. 9). As our prediction, FORC_062 showed much higher resistance to tetracycline than N315. To compare the resistance to β-lactam antibiotics between FORC_062 and N315, amoxicillin, cephazolin, cefotaxime, ceftazidime, and ampicillin discs were used for the test. Except for ampicillin, FORC_062 showed more resistance to other β-lactam antibiotics (Table 7). In addition, other type of antibiotics were tested. To antibiotics of ciprofloxacin, erythromycin, sulfamethoxazole, two strains showed similar high resistance. Against linezolid, FORC_062 (zone of diameter 19 mm) was slightly more resistant than N315 (zone of diameter 20 mm). These data suggested that FORC_062 may have an advantage from antibiotics to survive and the treatment for the patient infected to this strain might be tough.

To study the behavior of *S. aureus* FORC_062 when this strain is exposed to raw chicken breast, RNA sequencing was conducted. FORC_062 was incubated in M9 minimal media with or without containing raw chicken breast. As figure 12 showed, various kinds of genes were differently expressed. The genes related to metabolism such as transporters and permeases were up-regulated while the genes related to amino acid synthesis were down–regulated. This may resulted from the protein which is main components of chicken. For the carbohydrate metabolism, the genes related to glucose metabolism were up-regulated, on the contrary, the genes related other carbohydrate source such as galactose and fructose were down-regulated. At the same time, the expression of deamination associated genes like alanine dehydrogenase and threonine dehydrogenase were increased. So it is supposed that FORC_062 might use the glucose by deamination of amino acid. Lipids are abundant in chicken besides water and proteins, and long-chain fatty acids take up most of it (Marchi *et al*., 2012). The expression of genes encoding protein related to long-chain lipid degradation were up regulated. In addition to the nutrient metabolism, there are plenty of DEGs encoding virulence factors, such as enterotoxin, adhesin and type VII secretion system. Enterotoxin is closely related to food poisoning, which is heat-stable and causes nausea, diarrhea and stomachache. And the adhesin might help the strain to facilitate attachment to the raw chicken breast. The increased expression of type VII secretion system is involved in lipid metabolism. According to the previous study, host-derived fatty acids activate type VII secretion in *S. aureus* (Michael S. Lopez, 2017). The results described above suggested the behavior of FORC_062 when this

strain was exposed to raw chicken breast; (1) increased attachment, (2) utilization of amino acid and long-chain lipid from raw chicken meat, (3) increased enterotoxin, type VII secretion system. This result suggests that the strain FORC_062 recognizes the raw chicken breast as a reservoir, then the bacteria utilize the component and convert it into what itself needs. This study contributes the accumulation of database for the *Staphylococcus aureus* and helps to cope the outbreak from poultry meat in South Korea.

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VI. 국문초록

황색포도상구균은 그람양성 통성혐기성 세균으로, 가벼운 피부질환부터 생명을 위협하는 패혈증 등 여러 질병을 야기한다. 황색포도상구균이 내성을 갖는 항생제의 종류가 빠르게 증가하면서, 감염 시 치료의 어려움 역시 날로 증가하고 있다. 특히 국내의 경우 황색 포도상구균의 메티실린 저항성 균주가 전체의 67% 이상으로, 이는 해외 다른 국가보다 매우 높은 수치이다. 본 연구는 국내 환자의 혈액에서 분리한 황색포도상구균 FORC_062 균주에 대한 특성을 알아보기 위해 유전체 분석과 전사체 분석을 진행하였다.

FORC_062는 한 개의 염색체로 구성되어 있으며, 2,721개의 개방형 해독틀, 60개의 운반 RNA와 16개의 리보좀 RNA를 지니고 있다. FORC_062는 병원성에 기여하는 다양한 독성유전자들과 항생제 저항성 유전자를 가지고 있는 것을 확인하였으며, 세포독성 실험을 통해 다른 임상분리 균주보다 높은 독성을 확인하였다. 또한, NCBI상 등재된 20개의 다른 황색포도상구균의 종 간 유사도를 확인한 결과, FORC_062는 일본 환자로부터 분리한 N315와 계통학적으로 가장 유사하였다. FORC_062와 N315의 유전체 비교 분석을 통해 FORC_062가 tetracycline 저항 유전자 tetM과 type II toxin-antitoxin system MazEF를 암호화 하는 유전자를

추가적으로 가지고 있음을 확인하였다. MazEF system이 황색포도상구균의 베타-락탐 계열 항생제 저항성을 증가시킨다는 선행 연구 결과가 있음으로, 유전체 상의 분석결과를 표현형으로 확인하기 위해 항생제 저항성을 실험을 진행하였다. 실험결과 FORC_062가 N315보다 tetracycline과 베타-락탐 계열 항생제에 더욱 저항성을 가지고 있는 것을 확인하였다.

닭 가슴살은 전세계적으로 소비량이 많은 식품이다. 또한, 국내 유통되는 생닭의 다수가 황색포도상구균에 감염이 되어있다. 따라서 닭 가슴살을 모델식품으로 선정하여 전사체 분석을 진행하였다. FORC_062를 닭 가슴살에 노출되었을 때의 유전자 발현 양상을 RNA-sequencing을 통해 확인하였다. 아미노산의 수송, 탈아미노화, 포도당 대사, 지방산 대사와 관련된 유전자들의 발현은 증가한 반면, 아미노산의 생합성, 포도당을 제외한 다른 탄수화물의 대사를 관여하는 유전자들의 발현은 감소하였다. 또한, type VII secretion system, adhesin과 enterotoxin 등 여러 독성 유전자의 발현이 증가하는 것을 확인하였다.

결론적으로, 국내 환자 혈액에서 분리된 황색포도상구균 FORC_062는 여러 독성 인자를 가지고 있으며, 다른 임상 균주보다 높은 독성과 항생제 저항성을 가지고 있다. 또한, 모델 식품인 닭 가슴살에 노출되었을 때, 닭 가슴살을 하나의 저장소로 인지하여 생존과 성장을

유리하게 할 것으로 예측된다. 또한 독성유전자의 발현량의 증가에 따라, 인체 감염 시, 질병 발생의 가능성을 높일 것으로 예측된다.

본 연구를 통해 국내 환자에게서 분리한 식중독균의 데이터베이스를 축적함으로써, 국내 황색포도상구균의 특징에 대한 이해를 넓히고 질병 발생 시 효율적으로 대처할 수 있을 것으로 기대된다.

주요어: 황색포도상구균, 닭 가슴살, 유전체 분석, 전사체 분석

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