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The transcriptomic response of *Staphylococcus equorum* KS1030 to Lincomycin stress reveals transporters associated with horizontal gene transfer

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The spread of antibiotic resistance through horizontal gene transfer (HGT) in food-associated bacteria represents an emerging public health concern. *Staphylococcus equorum* strain KS1030, isolated from a high-salt fermented food, carries plasmids encoding the lincomycin resistance gene *lnuA* and the relaxase gene *rlx*, both of which contribute to resistance dissemination. Previous studies have shown that strain KS1030 can transfer the *lnuA* gene both within and across subspecies when exposed to lincomycin. To investigate the transcriptional basis of this phenomenon, we performed RNA sequencing (RNA-Seq) to analyze the global gene expression profile of KS1030 under lincomycin stress (30 mg/L). Transcriptome analysis revealed more differentially expressed genes (DEGs) at 2 h than at 4 h, with enriched categories including amino acid transport and metabolism (22.9%), transcription (19.3%), and inorganic ion transport and metabolism (14.7%). Genes involved in ornithine, Fe^{3+}, siderophore, and tryptophan metabolism, as well as stress regulators such as *sigB*, *dcuSR*, and helix-turn-helix transcriptional regulators, were strongly induced. Genome analysis further identified the competence (Com) operon and DNA translocase (*ftsK*) as potential transport systems, with *comGC* classified as a DEG. To capture short-term dynamics not resolved by RNA-Seq, quantitative real-time PCR was performed at 30-min intervals. Several genes, including *comC*, *comEC*, *comFA*, and *ftsK*, peaked at 1.5 h, while *lnuA* and *rlx* peaked at 1 h. Although the roles of the Com and FtsK systems in HGT remain unresolved, their induction under lincomycin stress suggests a potential contribution to plasmid transfer, offering new insight into the adaptive and gene transfer responses of *S. equorum*. However, as this study relies solely on transcriptional data from a single strain and antibiotic condition, functional validation—such as targeted gene disruption—will be required to confirm the involvement of these candidate HGT-related genes.

Keywords Staphylococcus equorum, Secretion system, Horizontal gene transfer, Com operon, DNA translocase, RNA sequencing

The extensive use of antibiotics for infection control and disease treatment has accelerated the emergence of antibiotic-resistant microorganisms[1]. Resistance is further exacerbated when antibiotic resistance genes are horizontally transferred within microbial communities, thereby increasing the prevalence of resistant strains[2]. This phenomenon has resulted in clinical challenges where conventional antibiotics become ineffective during patient treatment or surgical procedures[3]. Recognizing this threat, the U.S. Centers for Disease Control and Prevention (CDC) issued a landmark report in 2013 highlighting the risks posed by the horizontal transfer of antibiotic resistance genes[4]. The report emphasized that resistance determinants could potentially be transmitted not only between humans but also across food-to-human and animal-to-human interfaces. Subsequently, the World Health Organization published a global priority list of antibiotic-resistant bacteria in 2017[5], and the CDC

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identified 18 antibiotic-resistant bacterial and fungal pathogens[4], both underscoring the urgent need for global intervention strategies.

Given this context, increasing attention has been directed toward the safety of microorganisms employed in food and feed applications. The European Union has established guidelines prohibiting the use of strains harboring acquired antibiotic resistance genes[6]. Similarly, microorganisms registered under the Generally Recognized As Safe system in the U.S. must demonstrate antibiotic susceptibility[7]. In Korea, candidate strains for provisional approval as food sources are also required to be confirmed as non-resistant[7]. Despite these regulatory measures, reports of horizontal transfer of antibiotic resistance genes by food-associated microorganisms in fermented foods remain scarce. Moreover, experimental evidence confirming horizontal transfer from foodborne microorganisms to the human gut microbiota is limited.

In previous work, we analyzed microbial communities in high-salt fermented foods and identified coagulase-negative staphylococci as dominant members[8]. To investigate their functional potential, candidate starter strains were screened for antibiotic susceptibility and enzymatic activities that could enhance the quality of fermented foods[9,10]. Several candidates were shown to contribute to flavor compound development during fermentation[11–13]. Although most food-derived strains were antibiotic-sensitive, four isolates displaying acquired resistance to lincomycin were identified[14]. Resistance was attributed to the lincosamide O-nucleotidyltransferase gene (*lnuA*), located on plasmids capable of intra- and interspecific transfer[14,15]. Strain KS1030, unlike strain KM1031, exhibited horizontal transfer activity, and genomic analysis revealed that only strain KS1030 carries the plasmid pKS1030-3, which contains the relaxase gene[14,16]. This finding suggests that the horizontal transfer of antibiotic resistance requires not only resistance genes themselves but also mobile genetic elements such as relaxase[16]. Typically, relaxase-mediated relaxasome complexes are transferred though a Type IV secretion system (T4SS); however, interestingly, no genes associated with a T4SS were identified in the genome of *S. equorum* including strain KS1030[16,17]. Therefore, the objective of this study was to identify candidate genetic systems that may contribute to antibiotic resistance gene transfer in strain KS1030 and to establish a basis for further functional studies. By analyzing gene expression profiles in response to lincomycin as an inducer, we sought to generate hypothesis-driven insights into genes associated with secretion and to characterize transcriptional responses potentially linked to horizontal transfer under antibiotic exposure.

Materials and methods

Bacterial strains and culture conditions

Staphylococcus equorum strain KS1030, which isolated from a high-salt fermented seafood and possessed lincomycin resistance gene, *lnuA*, and mobile element relaxase gene, *rlx*, in plasmids[8,14,16,18], was subjected to genomic and transcriptomic analyses. *S. equorum* strains were cultured in tryptic soy broth (TSB; Becton, Dickinson and Co., Franklin Lakes, NJ, USA) supplemented with 30 mg/L lincomycin—a sub-inhibitory concentration previously shown to maintain normal cell growth while inducing antibiotic-responsive gene expression[19]—at 30 °C for 24 h.

Extraction and purification of RNA

S. equorum strain KS1030 was initially cultured overnight in TSB supplemented with 30 mg/L lincomycin. The overnight culture was then inoculated into fresh TSB medium at 1% (v/v) and incubated at 30 °C with shaking. When the culture reached an optical density of 0.5 at 600 nm, lincomycin was added again at a final concentration of 30 mg/L. The cultures were subsequently incubated for an additional 2 and 4 h at 30 °C. A control group was prepared under identical conditions without the addition of the antibiotic. Cells were harvested by centrifugation, treated with lysostaphin (1 mg/mL) at 37 °C for 20 min, and total RNA was extracted using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA). Total RNA from each sample was subjected to rRNA removal using the NEBNext rRNA Depletion Kit (New England BioLabs, Wrexham, UK) according to manufacturer instructions.

RNA sequencing analysis

The purified RNA was then used for mRNA-Seq library construction with the Illumina TruSeq RNA Sample Preparation Kit v2 (Illumina, San Diego, CA, USA). RNA sequencing was performed on the Illumina NovaSeq 6000 platform using paired-end 150 bp reads. All RNA-Seq datasets generated and analyzed in this study, including whole transcriptome profiles (Supplementary Table 1), have been deposited in the Sequence Read Archive (accession numbers SRR28760434-SRR28760435 for 2 h-exposure and SRR32872724-SRR32872725 for 4 h-exposure). The reference-genome sequence was obtained from the NCBI database (http://ncbi.nlm.nih.gov/genomes), and quality-filtered reads were aligned to this reference using Bowtie2[20]. Sequencing reads were mapped to the *S. equorum* KS1030 genome and normalized using the CLRNASeq program (version 1.00.06; CJ Bioscience, Inc., Seoul, Republic of Korea) based statistical packages like edgeR and DEseq2. Gene expression levels were quantified using the RPKM (Reads Per Kilobase of transcript, per Million mapped reads) method. Statistical significance (p-values) was calculated using edgeR, and subsequently, the FDR correction was applied to these p-values. Fold changes were determined as the ratio of RPKM values under antibiotic treatment to those under control conditions (RPKM_antibiotic/RPKM_control). Differentially expressed genes (DEGs) were defined as those with FDR-corrected $p < 0.05$ and an absolute fold change greater than 2. These DEGs were visualized using the CLRNASeq program. Functional classification of all strain KS1030 genes was performed using the evolutionary genealogy of genes: Non-supervised Orthologous Groups (eggNOG) database (version 5.0)[21], and the proportion of DEGs in each functional category was subsequently calculated. Functional enrichment analysis including Kyonto Encyclopedia of Genes and Genomes was performed[22].

Genomic analyses

The complete genome sequence of *S. equorum* strain KS1030 was published previously (GenBank accession: CP068576–CP068580). CLgenomics™ software (version 1.55; CJ Bioscience, Inc) and the web-hosted BLAST programs of NCBI were used to find genes and gene products with sequence identity. Gene functions were analyzed by a search against the COG database.

Quantitative real-time PCR (qRT-PCR)

The expression levels of selected genes predicted to be involved in DNA uptake or export were validated using qRT-PCR. Reactions were performed with gene-specific primer sets (Supplementary Table 2) designed based on the strain KS1030 genome sequence, using the iQ™ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) on a C1000 Thermal Cycler (Bio-Rad). Thermal cycling conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. The 16 S rRNA gene served as the reference gene for normalization[23]. RNA samples used for qRT-PCR were extracted and reverse-transcribed to cDNA using the same procedures as those employed for transcriptome analysis. To precisely evaluate time-dependent gene expression under short-term antibiotic exposure, samples were collected not only at 2 h and 4 h but also at 0.5 h, 1 h, and 1.5 h following lincomycin treatment. Untreated cultures grown under identical conditions served as controls. Gene expression levels were quantified from three independent biological replicates, and normalized fold changes were calculated using the 2^(-ΔΔCt) method[24].

Statistical analysis

Duncan's multiple range test following one-way analysis of variance was applied to evaluate significant differences between the average values obtained in qRT-PCR. All statistical analysis was performed using the SPSS software package (version 29.0; IBM SPSS Statistics, Armonk, NY, USA).

Results

Comprehensive transcriptome analysis under Lincomycin stress

S. equorum strain KS1030 harbors the *lnuA* on plasmid pSELNU1 and the relaxase gene on plasmid pKS1030-3, both involved in the horizontal transfer of resistance determinants[14,16]. Previous studies demonstrated that strain KS1030 is capable of transferring the lincomycin resistance gene between strains and even across subspecies boundaries[14]. Based on these findings, we hypothesized that exposure to lincomycin would induce the expression of resistance- and transfer-associated genes. To evaluate this, transcriptome profiles were generated from RNA samples collected after 2 h and 4 h of lincomycin (30 μg/mL) exposure, time points selected based on a previous study to capture both early (short-term) and sustained (long-term) transcriptional responses[19]. RNA-Seq data were processed as described in the methods (Supplementary Table S1).

The strain KS1030 genome comprises 2,948 predicted genes, of which 2,788 and 2,781 were expressed following 2 h and 4 h lincomycin exposure, respectively (Supplementary Table S3). Compared with the control, 352 genes were DEGs after 2 h, whereas only 119 DEGs were detected after 4 h ($\log_2$ fold-change value; Fig. 1A and Supplementary Table S3).

At 2 h, excluding genes of unknown function, 101 were upregulated and 8 downregulated. The most affected functional category was amino acid transport and metabolism (22.9%; 25/109), followed by transcription (19.3%; 21/109) and inorganic ion transport and metabolism (14.7%; 16/109) (Fig. 1B). At 4 h, DEG numbers decreased markedly to 16 upregulated and 6 downregulated genes, with the largest decreases observed in transcription (31.8%; 7/22) and inorganic ion transport and metabolism (27.3%; 6/22). Overall, lincomycin exposure significantly altered genes related to amino acid metabolism, transcriptional regulation, and ion transport.

Lincomycin and differentially expressed genes (DEGs)

Lincomycin exposure differentially modulated gene expression over time. Notably, the number of upregulated DEGs declined from 315 at 2 h to 37 at 4 h, while downregulated DEGs slightly increased from 37 to 39 (Fig. 1B). We hypothesized that resistance- and transfer-related genes would be selectively upregulated under lincomycin stress. Therefore, subsequent analyses focused on the upregulated DEGs (Table 1).

Effects of Lincomycin on amino acid transport and metabolism

Among the 315 DEGs upregulated after 2 h of lincomycin exposure, excluding those of unknown function, the most enriched COG category was "amino acid transport and metabolism" (Table 1). Genes involved in ornithine biosynthesis from glutamate were enriched (Fig. 2A and Supplementary Fig. S1). Ornithine has been reported to confer resistance or tolerance to antibiotic stress[25]. Consistent with this, our results suggest that lincomycin exposure induces the expression of genes responsible for ornithine biosynthesis from glutamate, thereby contributing to resistance or tolerance against the antibiotic.

In addition, genes responsible for tryptophan biosynthesis from chorismate were detected in an operon structure and identified as DEGs after 2 h of lincomycin exposure (Fig. 2B and Supplementary Fig. 1). As tryptophan is a precursor of indole, which promotes horizontal gene transfer (HGT) and antibiotic resistance[26,27]. This finding suggests an adaptive role for tryptophan metabolism. Interestingly, genes encoding biosynthesis of the siderophore staphyloferrin B were also upregulated at 2 h (Fig. 2C and Supplementary Fig. S1). Siderophores play a key role in iron acquisition under iron-limiting conditions[28], and reduced intracellular Fe^{3+} availability during antibiotic stress may drive siderophore-mediated uptake[29].

Effects of Lincomycin on transcription

Transcription-related genes represented the second largest category of DEGs at 2 h (Table 1). Approximately 10% (20/196) of transcription-associated genes in the genome were differentially expressed, including six

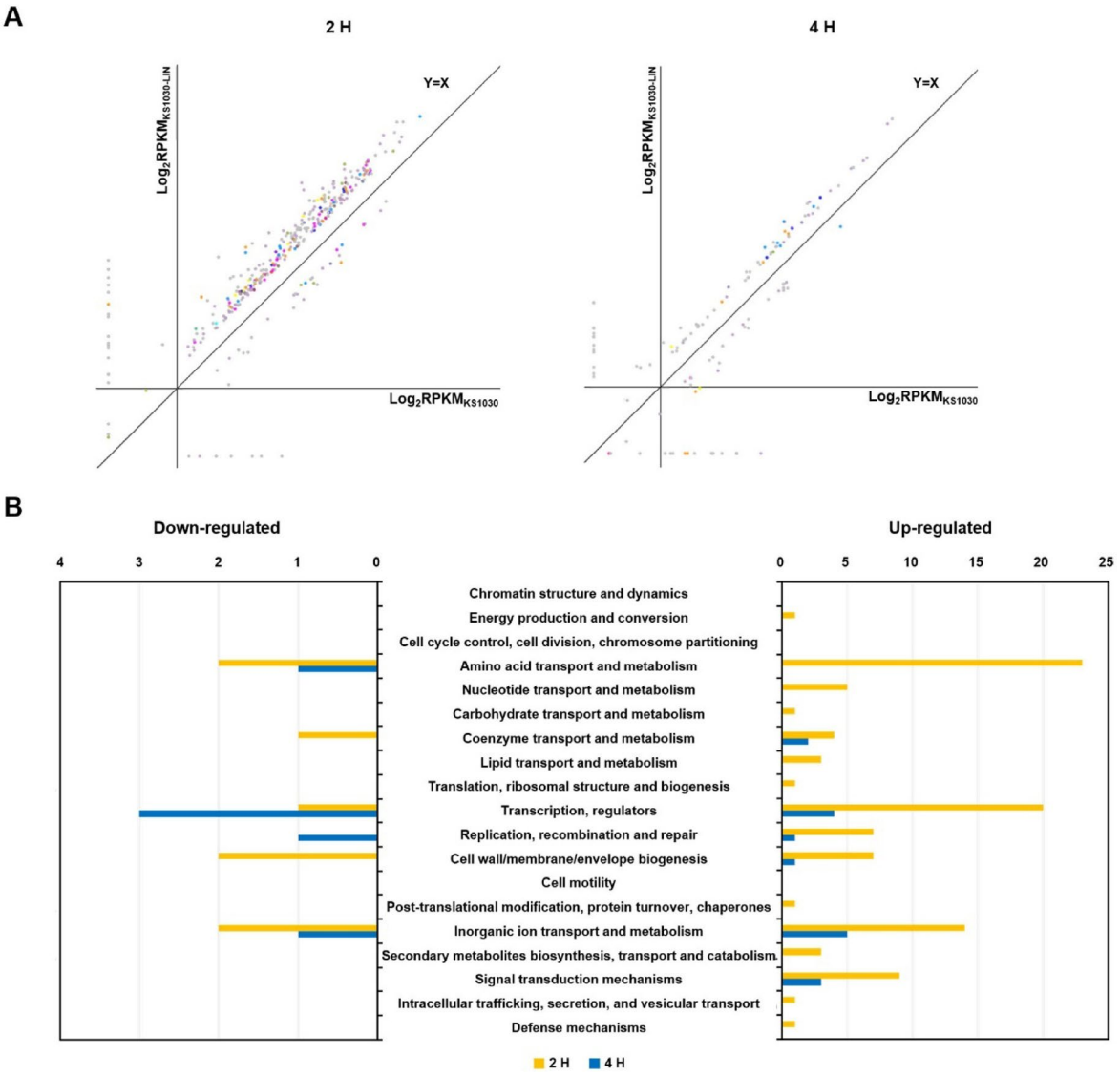

Fig. 1. Classification of differentially expressed genes (DEGs) from RNA-Seq analysis. (**A**) DEGs in *S. equorum* strain KS1030 after 2 h and 4 h of lincomycin treatment compared with untreated controls. The x-axis represents log-scaled Reads Per Kilobase of transcript per Million mapped reads (RPKM) values under untreated conditions, while the y-axis represents log-scaled RPKM values following lincomycin exposure. (**B**) Functional categorization of DEGs based on the Clusters of Orthologous Groups (COG) database. Genes were considered significantly differentially expressed with a P-value ≤ 0.05 and a fold-change ≥ 2.

helix-turn-helix transcriptional regulators, which are known to contribute to antibiotic resistance[30]. These findings suggest that transcriptional regulators involved in antibiotic resistance were upregulated in response to lincomycin stress. Interestingly, the expression of a metalloregulator belonging to the ArsR/SmtB family, which was low at 2 h, was significantly increased at 4 h of exposure. Members of this family are well known as stress-inducible regulators[31]. Thus, it can be inferred that strain KS1030 recognized lincomycin as an external stressor, resulting in delayed induction of this transcription factor relative to other regulators. It is expected that exposure to lincomycin would require transcriptional factors to activate genes involved in environmental adaptation, including antibiotic resistance genes. Consistent with this notion, *S. equorum* KS1030 showed a high number of differentially expressed genes related to transcription, including transcriptional regulators—the second most enriched functional category among the DEGs. However, the specific relationship between these transcriptional regulators and HGT remains to be elucidated in future studies.

Effects of Lincomycin on inorganic ion transport and metabolism

The third most enriched COG category among the DEGs upregulated after 2 h of lincomycin exposure was "inorganic ion transport and metabolism" (Table 1). At 4 h, this category contained the largest number of DEGs overall. As shown in Table 1, no clear trend was observed across all transport genes; however, specific patterns were evident. For example, genes encoding transporters of betaine and choline (JL104_RS01920 and JL104_

Gene locus	Product	Gene	KEGG	EC No.	Log_2 (Fold change)	
					2h	4h
Amino acid transport and metabolism						
JL104_RS00550	N-(2-amino-2-carboxyethyl)-L-glutamate synthase	*sbnA*	K21949	2.5.1.140	1.55	0.40
JL104_RS00555	N-((2S)-2-amino-2-carboxyethyl)-L-glutamate dehydrogenase	*sbnB*	K21721	1.5.1.51	1.62	0.25
JL104_RS00585	Diaminopimelate decarboxylase	*sbnH*	K01586	4.1.1.20	1.36	0.21
JL104_RS00765	Succinyl-diaminopimelate desuccinylase	*dapE*	K01439	3.5.1.18	1.43	-1.14
JL104_RS01435	Polyamine-transporting ATPase	*potA*	K11072	3.6.3.31	1.41	0.91
JL104_RS01445	Ornithine aminotransferase	*rocD|argD*	K00819	2.6.1.13, 2.6.1.11	1.42	0.82
JL104_RS01450	N-acetyl-gamma-glutamyl-phosphate reductase	*argC*	K00145	1.2.1.38	1.47	0.52
JL104_RS01455	Glutamate N-acetyltransferase	*argJ*	K00620	2.3.1.35, 2.3.1.1	1.27	0.19
JL104_RS01460	Acetylglutamate kinase	*argB*	K00930	2.7.2.8	1.02	0.64
JL104_RS02245	ring-cleaving dioxygenase	*gloA*	K15975	-	1.61	0.03
JL104_RS03885	Arginase	*rocF*	K01476	3.5.3.1	1.34	-0.15
JL104_RS06590	Cysteine desulfurase	*iscS|NFS1*	K04487	2.8.1.7	1.67	0.63
JL104_RS06655	5-oxoprolinase subunit PxpB	*pxpB*	K06351	3.5.2.9	1.03	-0.06
JL104_RS07750	Tryptophan synthase	*trpA*	K01695	4.2.1.20	1.25	0.09
JL104_RS07755	Tryptophan synthase	*trpB*	K01696	4.2.1.20	1.07	-0.08
JL104_RS07760	Phosphoribosylanthranilate isomerase	*trpF*	K01817	5.3.1.24	1.24	0.11
JL104_RS07770	Anthranilate phosphoribosyltransferase	*trpD*	K00766	2.4.2.18	1.33	-0.28
JL104_RS07775	Anthranilate synthase	*trpG*	K01658	4.1.3.27	1.02	-0.29
JL104_RS12125	APC family permease	*potE*	K16261	-	1.06	0.26
JL104_RS12185	sodium:alanine symporter family protein	*alsT*	K03310	-	1.50	0.84
JL104_RS12380	glutathione-dependent formaldehyde dehydrogenase	*adh2*	K18369	1.1.1.-	1.77	0.84
JL104_RS12830	Protein arginine kinase	*mcsB*	K19405	2.7.14.1	1.19	-0.08
JL104_RS14035	alcohol dehydrogenase catalytic domain-containing protein	*adh2*	K18369	1.1.1.-	1.19	0.56
Transcription						
JL104_RS00590	bifunctional transcriptional regulator/O-phospho-L-serine synthase SbnI	*sbnI*	-	-	1.35	0.17
JL104_RS00675	PadR family transcriptional regulator	*padR*	-	-	1.54	-0.89
JL104_RS01290	helix-turn-helix transcriptional regulator	*xre*	K07729	-	4.17	-0.23
JL104_RS01380	GNAT family N-acetyltransferase	*phnO*	-	-	1.32	1.08
JL104_RS01745	winged helix DNA-binding protein	*marR*	-	-	1.16	0.34
JL104_RS02240	MarR family transcriptional regulator	*mhqR*	K15973	-	1.27	0.34
JL104_RS04355	RNA polymerase sigma factor SigB	*sigB*	K03090	-	1.32	0.67
JL104_RS08155	helix-turn-helix domain-containing protein	-	-	-	1.36	-0.19
JL104_RS08240	ORF6C domain-containing protein	*antB*	K07741	-	2.22	0.14
JL104_RS08305	helix-turn-helix domain-containing protein	*rghR*	K22299	-	1.05	-0.10
JL104_RS09795	metalloregulator ArsR/SmtB family transcription factor	*arsR*	K03892	-	-0.25	1.24
JL104_RS10765	helix-turn-helix domain-containing protein	*xre*	K07729	-	3.53	0.07
JL104_RS11105	helix-turn-helix domain-containing protein	*hipB*	K07726	-	3.30	1.18
JL104_RS11170	Rha family transcriptional regulator	*yoqD|pRha*	K07741	-	1.29	0.42
JL104_RS12840	CtsR family transcriptional regulator	*ctsR*	K03708	-	1.52	0.41
JL104_RS13325	LysR family transcriptional regulator	*ilvY*	K02521	-	1.32	0.29
JL104_RS13530	Rrf2 family transcriptional regulator	*rrf2*	-	-	1.44	0.36
JL104_RS13685	TM2 domain-containing protein	-	-	-	1.40	-1.69
JL104_RS14085	PRD domain-containing protein	*licR*	K03491	-	1.36	-0.76
JL104_RS14305	metalloregulator ArsR/SmtB family transcription factor	*arsR*	K03892	-	-0.22	1.53
JL104_RS14555	helix-turn-helix transcriptional regulator	-	-	-	1.62	0.09
JL104_RS14735	transcriptional regulator AryK	*araC|xynB*	-	-	1.04	-0.68
Inorganic ion transport and metabolism						
JL104_RS00545	iron-siderophore ABC transporter substrate-binding protein	*sirA*	K02016	-	1.52	0.19
JL104_RS00900	DsrE/DsrF/DrsH-like family protein	*tusA|pspE|yrkE*	K04085	2.8.1.-	1.28	-0.25
JL104_RS01245	2-keto-3-deoxygluconate permease	*kdgT*	K02526	-	-1.13	1.04
JL104_RS01350	ABC transporter substrate-binding protein	*fepB*	K02016	-	1.45	-0.14
JL104_RS01920	BCCT family transporter	*opuD*	K05020	-	1.92	0.70

Table 1. List of differentially expressed genes (DEGs) in *S. equorum* KS1030 after 2 h and 4 h of Lincomycin exposure. DEGs are categorized according to the clusters of orthologous groups (COG) classification. RNA expression levels are indicated both numerically and by color shading within the boxes. Gene expression is represented using the log_2 fold change values having adjusted p-value of less than 0.05. Red and blue colors correspond to downregulated or upregulated gene expression level, respectively, with a range from –4 to 4.

JL104_RS01975	MFS transporter	*lmrB*	K18926	-	1.05	0.18
JL104_RS02385	ABC transporter substrate-binding protein	*fepB*	K02016	-	1.69	0.12
JL104_RS02445	Polyphosphate kinase	*ppk*	K00937	2.7.4.1	1.19	0.64
JL104_RS03745	BCCT family transporter	*opuD*	K05020	-	3.27	0.60
JL104_RS03780	ABC transporter substrate-binding protein	*fepB*	K02016	-	1.01	-0.13
JL104_RS07695	PstS family phosphate ABC transporter substrate-binding protein	*pstS*	K02040	-	1.91	-0.36
JL104_RS09790	arsenite efflux transporter membrane subunit ArsB	*arsB*	K03893	-	-0.30	1.32
JL104_RS10330	Nitronate monooxygenase	*ncd2|npd*	K00459	1.13.12.16	1.56	0.70
JL104_RS11380	siderophore ABC transporter substrate-binding protein	*yclQ*	K02016	-	1.43	0.80
JL104_RS13985	ABC transporter substrate-binding protein	*fepB*	K02016	-	1.79	0.35
JL104_RS14300	arsenite efflux transporter membrane subunit ArsB	*arsB*	K03893	-	-0.21	1.34
JL104_RS14315	arsenical pump-driving ATPase	*arsA*	K01551	3.6.3.16, 3.6.3.-	0.36	2.07
JL104_RS14320	arsenite efflux transporter metallochaperone ArsD	*arsD*	K01551	3.6.3.16, 3.6.3.-	0.05	1.74
JL104_RS14455	Hydroxyacylglutathione hydrolase	*cstA*	K01069	3.1.2.6	1.42	-0.01
Signal transduction mechanisms						
JL104_RS00330	Histidine kinase	*dcuS*	K11614	2.7.13.3	1.59	0.16
JL104_RS00335	response regulator	*dcuR*	K07702	-	1.39	0.29
JL104_RS01760	universal stress protein	*uspA*	-	-	1.10	0.89
JL104_RS04340	Phosphoserine phosphatase	*rsbU*	K07315	3.1.3.3	1.02	0.20
JL104_RS04345	anti-sigma factor antagonist	*rsbV*	K04749	-	1.60	0.47
JL104_RS04350	Non-specific serine/threonine protein kinase	*rsbW*	K04757	2.7.11.1	1.47	0.77
JL104_RS08550	helix-turn-helix domain-containing protein	*yesN*	-	-	1.38	-0.49
JL104_RS09785	Arsenate reductase (glutaredoxin)	*arsC*	K03741	1.20.4.1	-0.18	1.32
JL104_RS10870	type II toxin-antitoxin system PemK/MazF family toxin	*mazF|ndoA|chpA*	K07171	3.1.-.-	1.15	-0.43
JL104_RS13980	universal stress protein	*uspA*	-	-	1.82	1.46
JL104_RS14295	Arsenate reductase (glutaredoxin)	*arsC*	K03741	1.20.4.1	-0.12	1.14
Replication, recombination and repair						
JL104_RS01805	DNA-3-methyladenine glycosylase II	*alkA*	K01247	3.2.2.21	2.26	0.53
JL104_RS05390	sporulation protein	*spo0M*	K06377	-	1.61	0.15
JL104_RS08230	single-stranded DNA-binding protein	*ssb*	K03111	-	2.28	0.10
JL104_RS08250	ATP-binding protein	*dnaC*	K02315	-	2.35	0.47
JL104_RS10380	IS3 family transposase		K07497	-	0.58	1.50
JL104_RS11080	Protein-N(pi)-phosphohistidine--lactose phosphotransferase	*lacE*	K02787, K02788	-	1.77	0.76
JL104_RS11095	ATP-binding protein	*dnaC*	K02315	-	1.70	0.27
JL104_RS11115	single-stranded DNA-binding protein	*ssb*	K03111	-	1.51	0.47
Cell wall/membrane/envelope biogenesis						
JL104_RS00790	transglycosylase family protein	*rpfB*	K21688	-	2.77	0.14
JL104_RS00800	transglycosylase family protein	*ncoa2*	K11255	-	2.18	0.15
JL104_RS04230	transglycosylase family protein	*rpfB*	K21688	-	1.95	0.56
JL104_RS05760	CDP-ribitol ribitolphosphotransferase	*tarL*	K18704	2.7.8.14, 2.7.8.47	1.70	-0.25
JL104_RS07920	large conductance mechanosensitive channel protein MscL	*mscL*	K03282	-	1.79	0.08
JL104_RS12190	6-phospho-3-hexuloisomerase	*hxlB*	K08094	5.3.1.27	1.91	0.29
JL104_RS13040	septation regulator SpoVG	*spoVG*	K06412	-	1.04	1.09
Nucleotide transport and metabolism						
JL104_RS02450	Exopolyphosphatase	*ppx*	K01524	3.6.1.11, 3.6.1.40	1.59	0.86
JL104_RS05250	class Ib ribonucleoside-diphosphate reductase assembly flavoprotein NrdI	*nrdI*	K03647	-	1.66	-0.14
JL104_RS08185	dUTP diphosphatase	*dut*	K01520	3.6.1.23	1.81	-0.12
JL104_RS09630	Phosphoribosylformylglycinamidine synthase	*purS*	K01952	6.3.5.3	1.05	-0.31
JL104_RS10600	DNA topology modulation protein	*adk*	-	-	1.06	0.21
Coenzyme transport and metabolism						
JL104_RS00975	sulfur carrier protein ThiS	*thiS*	K03154	-	1.40	-0.86
JL104_RS01610	2-dehydropantoate 2-reductase	*panE*	K00077	1.1.1.169	1.49	1.14
JL104_RS01815	6-pyruvoyltetrahydropterin synthase	*queD|ptpS*	K01737	4.2.3.12, 4.1.2.50	1.88	1.57
JL104_RS09820	Lipoate--protein ligase	*lplA|lplJ*	K03800	6.3.1.20	1.05	0.52
Lipid transport and metabolism						
JL104_RS06665	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	*accB*	K02160	-	1.53	0.46
JL104_RS10530	Lysophospholipase	*pldB*	K01048	3.1.1.5	1.16	0.67
JL104_RS13975	Propionate CoA-transferase	*pct*	K01026	2.8.3.1	1.03	0.50

Table 1. (continued)

RS03745), which function as osmoprotectants and contribute to salt tolerance, were highly expressed at 2 h. In contrast, genes associated with arsenite efflux showed elevated expression at 4 h. Betaine and choline uptake promotes the intracellular accumulation of osmoprotectants, thereby protecting cells from environmental stress[19], whereas arsenite efflux systems remove toxic compounds such as arsenite from the cell[32]. Accordingly, the observed transcriptional changes suggest that strain KS1030 responds to lincomycin-induced stress by enhancing osmoprotectant accumulation at early time points and activating efflux systems to eliminate harmful compounds during prolonged exposure.

Secondary metabolites biosynthesis, transport and catabolism						
JL104_RS00560	Aerobactin synthase	*sbnC*	K03895	6.3.2.39	1.48	0.34
JL104_RS00570	N(2)-citryl-N(6)-acetyl-N(6)-hydroxylysine synthase	*sbnE/iucA*	K03894	6.3.2.38	1.38	0.19
JL104_RS00575	Aerobactin synthase	*sbnF*	K03895	6.3.2.39	1.36	0.20
Carbohydrate transport and metabolism						
JL104_RS03970	Cytosine deaminase	*codA*	K01485	3.5.4.1, 3.5.4.21	1.22	0.38
Defense mechanisms						
JL104_RS02215	ATP-binding cassette domain-containing protein	*mdlB*	K18889	-	1.07	-0.46
Energy production and conversion						
JL104_RS03305	Opine dehydrogenase	*odh*	K04940	1.5.1.28	1.83	0.30
Intracellular trafficking, secretion, and vesicular transport						
JL104_RS06960	prepilin-type N-terminal cleavage/methylation domain-containing protein	*comGC*	K02245	-	2.03	0.21
Post-translational modification, protein turnover, chaperones						
JL104_RS06140	signal peptide peptidase SppA	*sppA*	K04773	3.4.21.-	1.27	0.04
Translation, ribosomal structure and biogenesis						
JL104_RS05560	tRNA (cytidine(34)-2'-O)-methyltransferase	*trmL*	K03216	2.1.1.207	1.09	-0.07

Gene expression is represented using the $\log_2$ fold change values having adjusted p-value of less than 0.05. Red and blue colors correspond to downregulated or upregulated gene expression level, respectively, with a range from –4 to 4.

Table 1. (continued)

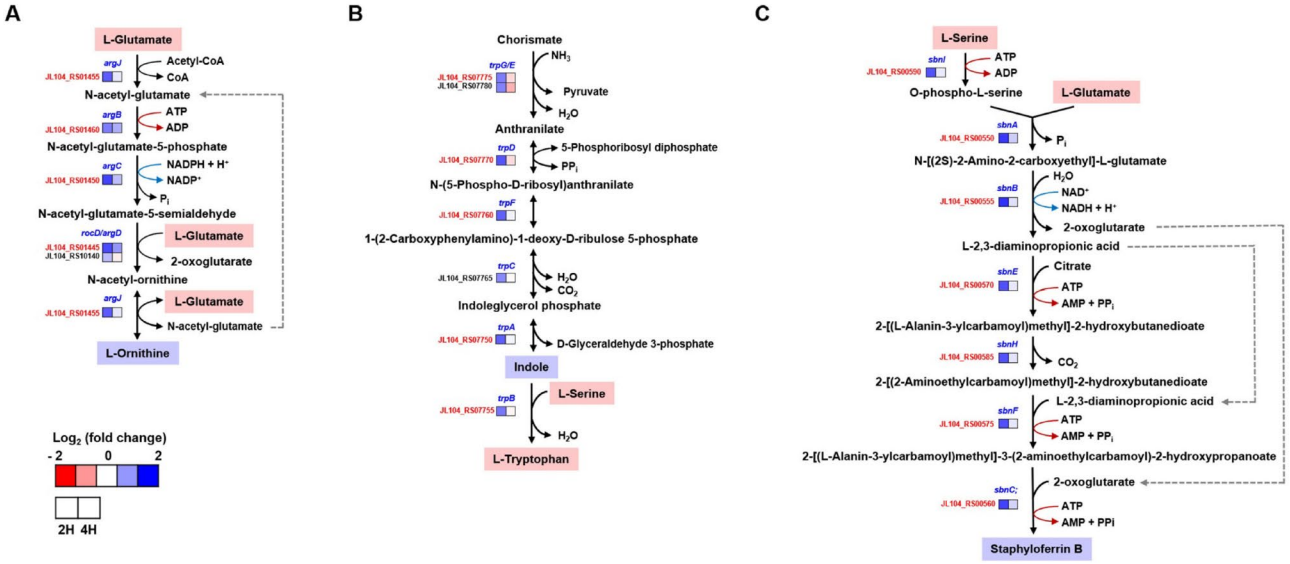

Fig. 2. Metabolic pathways associated with DEGs of *S. equorum* KS1030 under lincomycin exposure. (**A**) Ornithine biosynthesis, (**B**) tryptophan biosynthesis, and (**C**) staphyloferrin B biosynthesis pathways are shown. Gene names are labeled in blue, and DEG locus tags are highlighted in red. Biosynthetic compounds are represented in light purple boxes, and related amino acids are shown in light pink boxes. Expression levels at 2 h and 4 h after lincomycin exposure are indicated adjacent to each locus tag on $\log_2$ fold-change values.

Notably, five of the 14 DEGs identified in the COG category "inorganic ion transport and metabolism" after 2 h of lincomycin exposure were associated with Fe^{3+} uptake (Table 1). Previous studies have reported that the intracellular accumulation of exogenous Fe^{3+} enhances bacterial resistance to antibiotics[33]. These findings therefore suggest that, in addition to activating lincomycin resistance genes, strain KS1030 may increase Fe^{3+} uptake as a complementary mechanism to strengthen its tolerance against lincomycin stress.

Effects of Lincomycin on the regulator genes

In addition to the genes described above, increased expression was also observed for several genes involved in signal transduction. In particular, the DcuSR two-component system (TCS), known to regulate genes in response to citrate and to control both virulence factors and antibiotic resistance determinants[34–36], showed elevated expression (Table 1 and Supplementary Fig. 1). Genes encoding universal stress proteins, which play protective roles against antibiotics and other environmental challenges[37], were also upregulated at 2 h, and their expression remained high at 4 h (Table 1). Furthermore, the expression of the stress-responsive sigma factor *sigB*

was significantly increased at 2 h, along with its regulatory partners *rsbV*, *rsbU*, and *rsbW*, which are required to maintain *sigB* activation (Table 1 and Supplementary Fig. 1).

Changes of DEGs during exposure time of Lincomycin

Analysis of the transcriptional profiles of strain KS1030 after 2 h and 4 h of lincomycin exposure revealed increased expression of transport- and biosynthesis-related genes associated with ornithine, Fe^{3+}, siderophores, and tryptophan, all of which are linked to cellular protection against environmental stress (Table 1; Fig. 2)[25–29,33]. In addition, regulatory genes required for adaptation to external stressors, including *sigB*, *dcuSR* TCS, and several helix-turn-helix transcriptional regulators, were also highly expressed (Table 1)[30–32,34–37]. To further assess the temporal differences in gene expression, a Venn diagram analysis was conducted (Fig. 3). While DEGs analysis excluded genes classified as "function unknown", the Venn diagram analysis included these genes to provide a more comprehensive view of transcriptional changes under lincomycin stress (Fig. 3 and Supplementary Table 4).

A total of 37 genes were identified as common DEGs after both 2 h and 4 h of lincomycin exposure (Fig. 3 and Supplementary Table 4). Excluding hypothetical proteins, 22 genes remained, including two helix-turn-helix domain-containing proteins, as well as universal and general stress protein genes. These findings suggest that certain regulatory and protective genes are consistently expressed throughout lincomycin exposure to facilitate bacterial adaptation. By contrast, genes upregulated at 2 h—such as those involved in ornithine, Fe^{3+}, siderophore, and tryptophan transport and biosynthesis, as well as *sigB* and *dcuSR* TCS—were no longer identified as DEGs at 4 h. Instead, the DEGs at 4 h included three arsenite efflux genes (JL104_RS09790, JL104_RS14300, and JL104_RS14320), which are known to mediate the export of intracellular arsenic acid but can also contribute to antibiotic efflux[38,39]. This suggests that, during prolonged exposure, strain KS1030 activates efflux systems to expel accumulated lincomycin.

Interestingly, the sigma-70 family RNA polymerase sigma factor, a general transcriptional regulator under normal conditions, was also detected as a DEG at 4 h. Moreover, the *lnuA* gene (JL104_RS14695), which confers lincomycin resistance, was strongly expressed at 2 h but markedly reduced at 4 h. Although this gene is classified as "function unknown" in COG analysis and thus not highlighted in DEG categorization, its induction at 2 h under lincomycin exposure is consistent with its known role in resistance. Taken together, these results indicate that strain KS1030 mounts a rapid adaptive response within the first 2 h of lincomycin exposure, upregulating metabolic, stress-response, and resistance genes. By 4 h, however, expression largely returns toward baseline, with efflux systems becoming the predominant mechanism of defense.

Putative horizontal gene transfer related genes

We hypothesized that exposure of strain KS1030 to lincomycin for 2 h and 4 h would induce expression of lincomycin resistance genes and genes required for their horizontal transfer across species or genera. Transcriptome analysis at the DEG level confirmed that the lincomycin resistance gene *lnuA* was differentially expressed. However, neither the relaxase gene (JL104_RS14645) nor the mobilization relaxasome protein gene *mobC* (JL104_RS14640)—both previously proposed to contribute to horizontal transfer[16]—showed significant changes in expression (Table 2). Nevertheless, despite the lack of strong induction, we reasoned that genes supporting horizontal transfer of antibiotic resistance must exist in strain KS1030 and therefore extended our analysis to membrane-associated proteins potentially involved in DNA transfer. Notably, genome analysis did not identify a T4SS, the classical mechanism implicated in horizontal transfer of resistance genes, in strain KS1030 or in other *S. equorum* strains examined.

Based on these findings, we next searched the genome of strain KS1030 for genes associated with "secretion," "translocase," and "DNA export/DNA uptake" (Table 3). Genes related to the general secretion system (Sec-SRP) and secretion accessory proteins were identified under the "secretion" category. Although the Sec-SRP system is primarily involved in protein secretion[40], genome analysis confirmed that strain KS1030 possesses the

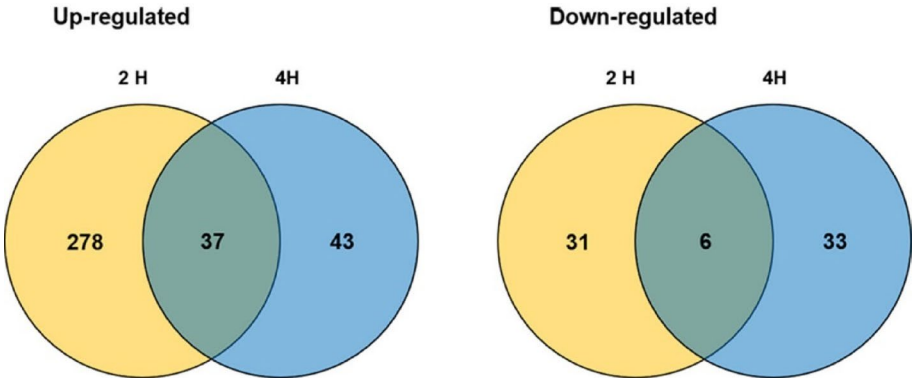

Fig. 3. Venn diagram of DEGs in *S. equorum* strain KS1030 following 2 h and 4 h of lincomycin treatment. The overlapping region represents genes that were differentially expressed at both 2 h and 4 h of lincomycin exposure.

Gene locus	Product	COG	Gene	KEGG	EC No.	Log_2 (Fold change)	
						2h	4h
JL104_RS14640	plasmid mobilization relaxosome protein MobC	S	*mobC*	-	-	0.00	0.00
JL104_RS14645	relaxase/mobilization nuclease domain-containing protein	-	*rlx*	-	-	0.00	0.00
JL104_RS14650	DUF334 domain-containing protein	-	*orf2*	-	-	0.26	-0.11
JL104_RS14695	lincosamide nucleotidyltransferase Lnu(A)'	S	*lnuA*	K19545	-	1.16	0.12

Table 2. Lincomycin resistance genes and horizontal gene transfer–related genes in *S. equorum* KS1030, and their expression levels under Lincomycin exposure. RNA expression levels are indicated both numerically and by color shading within the boxes. Gene expression is represented using the log_2 fold change values having adjusted p-value of less than 0.05. Red and blue colors correspond to downregulated or upregulated gene expression level, respectively, with a range from − 2 to 2.

Keywords	Gene locus	Product	COG	Gene	KEGG	EC No.	Log_2 (Fold change)	
							2h	4h
Secretion	JL104_RS00940	YhgE/Pip family protein	S	*yhgE*	K01421	-	-0.09	-0.47
	JL104_RS03655	preprotein translocase subunit SecY	U	*secY*	K03076	-	-0.04	-0.11
	JL104_RS06510	protein translocase subunit SecDF	U|U	*secDF*	K12257	-	-0.18	-0.07
	JL104_RS06955	type II secretion system F family protein	U	*comGB*	K02244	-	0.06	-0.03
	JL104_RS10800	preprotein translocase subunit SecG	U	*secG*	K03075	-	-0.09	-0.19
	JL104_RS12770	preprotein translocase subunit SecE	U	*secE*	K03073	-	-0.44	1.19
Translocase	JL104_RS03655	preprotein translocase subunit SecY	U	*secY*	K03076	-	-0.04	-0.11
	JL104_RS06005	DNA translocase FtsK	D	*ftsK*	K03466	-	0.33	-0.35
	JL104_RS06505	preprotein translocase subunit YajC	U	*yajC*	K03210	-	0.03	0.04
	JL104_RS06510	protein translocase subunit SecDF	U|U	*secDF*	K12257	-	-0.18	-0.07
	JL104_RS08665	DNA translocase FtsK	D	*ftsK*	K03466	-	0.10	-0.05
	JL104_RS10800	preprotein translocase subunit SecG	U	*secG*	K03075	-	-0.09	-0.19
	JL104_RS11290	preprotein translocase subunit SecA	U	*secA*	K03070	-	0.02	0.03
	JL104_RS12770	preprotein translocase subunit SecE	U	*secE*	K03073	-	-0.44	1.19
DNA uptake	JL104_RS06405	Prepilin peptidase	NOU	*comC*	K02236	3.4.23.43, 2.1.1.-	-0.68	0.56
	JL104_RS06730	helix-hairpin-helix domain-containing protein	L	*comEA*	K02237	-	-0.11	-0.36
	JL104_RS06740	DNA internalization-related competence protein ComEC/Rec2	S|S	*comEC*	K02238	-	-0.03	-0.13
	JL104_RS06950	Flp pilus assembly complex ATPase component TadA	U	*tadA*	K02243	-	-0.23	-0.13
	JL104_RS06955	type II secretion system F family protein	U	*comGB*	K02244	-	0.06	-0.03
	JL104_RS08810	DNA-processing protein DprA	L	*dprA*	K04096	-	0.15	-0.30
	JL104_RS09960	competence protein	S	*coiA*	K06198	-	0.25	-0.25
	JL104_RS11300	ComF family protein	S	*comFC*	K02242	-	-0.43	0.33
	JL104_RS11305	DEAD/DEAH box helicase family protein	L	*comFA*	K02240	-	0.01	0.25
	JL104_RS13165	YbaB/EbfC family nucleoid-associated protein	S	*ybaB*	K09747	-	0.22	0.48

Table 3. Genes related to DNA transfer in the genome of *S. squorum* KS1030. RNA expression levels are indicated both numerically and by color shading within the boxes. Gene expression is represented using the log_2 fold change values having adjusted p-value of less than 0.05. Red and blue colors correspond to downregulated or upregulated gene expression level, respectively, with a range from − 2 to 2.

complete set of Sec-SRP components (Fig. 4). Transcriptome analysis showed that only *secE* (JL104_RS12770) was classified as a DEG at 4 h, while expression changes in other genes were minimal.

When searched under the "translocase" keyword, two DNA translocase *ftsK* genes were detected, in addition to the Sec-SRP components (Table 3; Fig. 4). FtsK is known to function in chromosome segregation and cell division, but has also been implicated in HGT[41]. Although not classified as DEGs, both *ftsK* genes showed increased expression at 2 h.

Finally, no genes associated with "DNA export" were identified, whereas genes related to "DNA uptake" were detected in the competence (Com) operon (Table 3; Fig. 4). The Com operon is reported to mediate HGT through the uptake of extracellular DNA[42], and all required competence-associated genes were present in the strain KS1030 genome. Transcriptome data revealed that *comGC* (JL104_RS06960), encoding the type IV pilus major pilin, was a DEG at 2 h, whereas the other Com operon genes did not show significant differential expression.

Taken together, genome analysis indicated that strain KS1030 harbors several membrane-associated transport systems, including the Sec-SRP system, the Com operon, and DNA translocase FtsK (Fig. 4). Since our aim was to identify potential secretion or transport systems involved in HGT under lincomycin stress, we cautiously propose that the Com operon and FtsK may contribute to gene transfer in this strain. However, as their expression was generally low, further research is required to determine their functional relevance. The

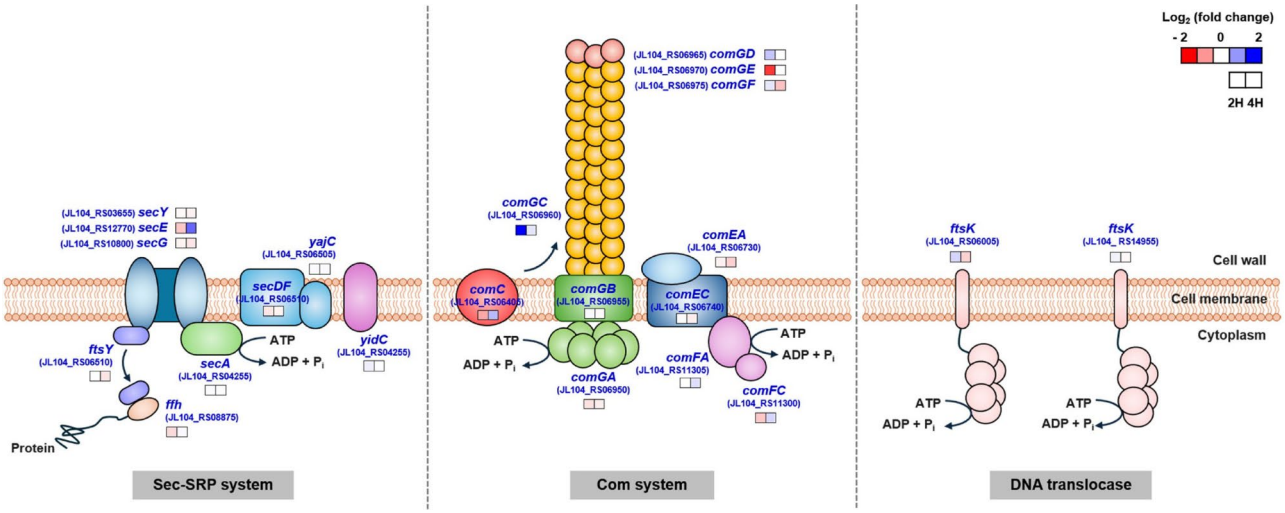

Fig. 4. Genome-based analysis of DNA and protein transport systems located in the cell membrane of *S. equorum* KS1030. Gene names and locus tags are shown in blue. Expression levels after 2 h and 4 h of lincomycin exposure are indicated in boxes adjacent to each locus tag.

transient upregulation of *comGC* at 2 h suggests that competence-related genes may be induced early, followed by rapid decline, highlighting the need for finer time-point analyses. Consequently, we performed qRT-PCR at 30-min intervals to more precisely assess temporal expression dynamics.

Validation of RNA-Seq data by qRT-PCR

To validate the transcriptional profiles of *S. equorum* strain KS1030 obtained by RNA-Seq under lincomycin exposure, qRT-PCR was performed. While RNA-Seq was conducted at 2 h and 4 h post-exposure, qRT-PCR included these time points as well as additional earlier time points (0.5 h, 1 h, and 1.5 h) to capture short-term transcriptional dynamics. Target genes included those predicted to be involved in DNA uptake or export and located at the membrane, specifically the Com operon genes (*comC*, *comEC*, *comFA*, *comGC*) and two DNA translocase genes (*ftsK* JL104_RS06005 and JL104_RS14955). In addition, the lincomycin resistance gene *lnuA* and the transfer-related genes *rlx* and *mobC* were analyzed.

qRT-PCR results at 2 h and 4 h were consistent with RNA-Seq data, showing relatively modest expression changes (Fig. 5). However, as expected, several genes showed earlier peaks in expression. At 1.5 h, five genes (*comC*, *comEC*, *comFA*, *ftsK* JL104_RS06005, and *ftsK* JL104_RS14955) exhibited their highest expression levels. The resistance gene *lnuA* and *rlx* peaked at 1 h before declining, while *comGC* and *mobC* showed maximal expression at 2 h. Although fold change values were not large enough to classify these genes as DEGs, their consistent upregulation compared with untreated controls confirms their induction by lincomycin. Thus, RNA-Seq results were validated by qRT-PCR, and additional short-term analysis revealed transient induction of several transport- and transfer-associated genes.

Discussion

S. equorum strain KS1030 harbors the lincomycin resistance gene *lnuA* on plasmid pSELNU1, as well as a relaxase gene on plasmid pKS1030-3[14,16,18]. Previous studies demonstrated that strain KS1030 is capable of horizontally transferring the resistance plasmid both within and across subspecies boundaries when exposed to lincomycin[14,15]. Furthermore, resistance gene transfer was observed not only under in vitro conditions but also when strain KS1030 was used as a starter culture in fermented foods or administered orally, followed by antibiotic exposure[15]. These findings strongly suggest that strain KS1030 possesses the genetic potential to mediate plasmid transfer. However, despite the presence of a transferable plasmid and relaxase, the specific secretion system required for gene transfer has remained unclear. Therefore, we hypothesized that lincomycin exposure would induce not only lincomycin resistance genes but also genes required for horizontal gene transfer, and we used RNA-Seq to identify candidate secretion systems potentially involved in this process. Transcriptomic analysis confirmed that the lincomycin resistance gene *lnuA* was differentially expressed; however, the relaxase gene and the mobilization relaxosome gene *mobC*, both previously implicated in horizontal transfer, showed no significant transcriptional changes. This outcome was unexpected, given earlier findings that plasmids carrying a relaxase gene mediate the horizontal transfer of *lnuA*. One possibility is that the conditions used in this study were not optimal to induce their expression. Alternatively, these genes may function without requiring substantial transcriptional upregulation, as some resistance-associated genes are known to exert effects even at low expression levels[19].

Contrary to our initial expectation that lincomycin exposure would trigger marked expression of secretion system components facilitating plasmid transfer, the major DEGs were instead associated with stress adaptation. Genes involved in osmoprotection (e.g., *opuD* encoding glycine-betaine uptake), environmental adaptation (e.g., *sigB*), and iron acquisition (e.g., staphyloferrin B biosynthesis) were significantly upregulated (Table

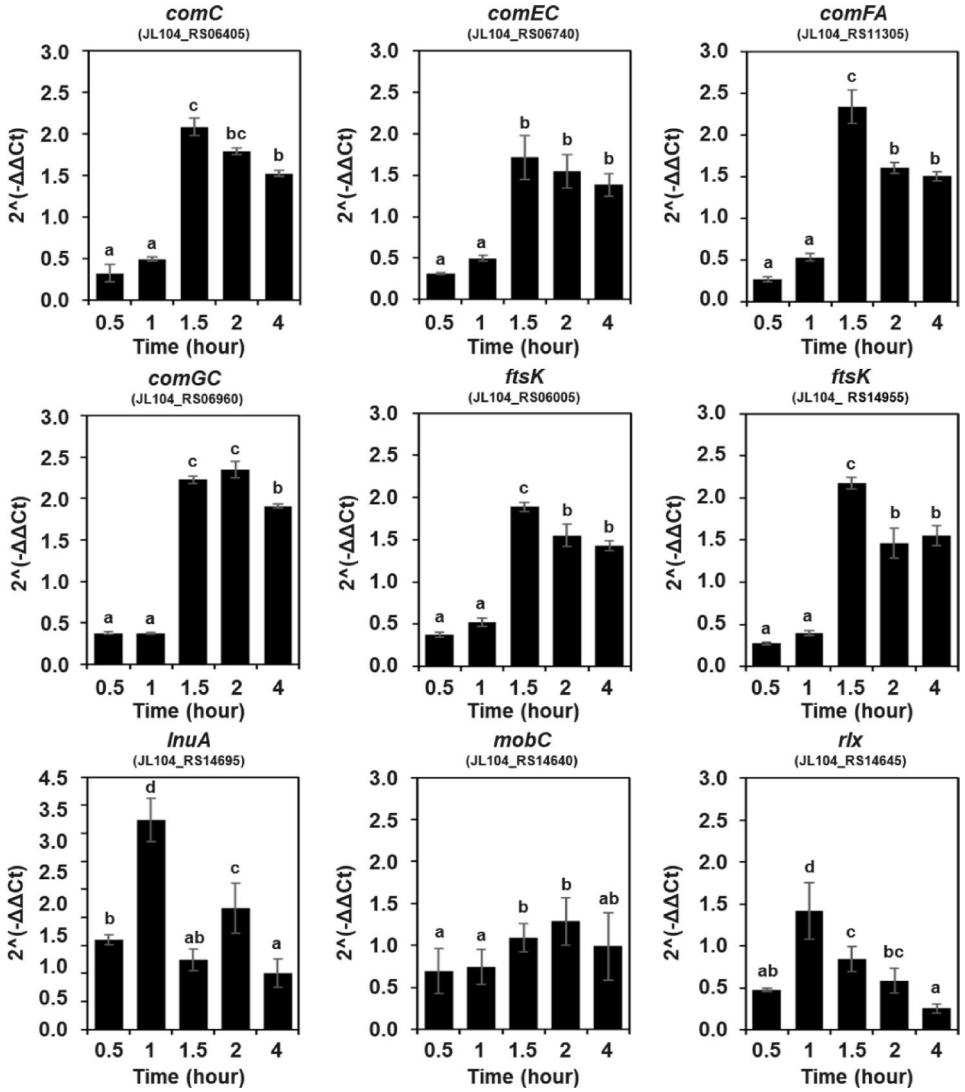

Fig. 5. Relative expression levels of selected genes predicted to be involved in DNA uptake or export in *S. equorum* KS1030 upon lincomycin exposure. Gene expression was measured at 0.5, 1, 1.5, 2, and 4 h using quantitative real-time PCR (qRT-PCR). Expression levels were normalized against the 16 S rRNA gene, and fold changes were calculated using the 2^(-ΔΔCt) method. Data represent the mean ± standard deviation of three independent experiments. Different letters above bars indicate statistically significant differences between time points ($p < 0.05$, Duncan's multiple range test).

1). These results indicate that strain KS1030 primarily mounts a physiological response to survive antibiotic stress rather than directly inducing secretion machinery. Interestingly, among the DEGs, we detected *comGC*, a structural component of the type IV pilus encoded by the Com operon, which is known to mediate DNA uptake. Genomic analysis further confirmed that strain KS1030 contains the full set of Com operon genes (Fig. 4). Although expression changes were modest, qRT-PCR revealed that *comGC* expression peaked at 1.5 h, earlier than the 2 h and 4 h RNA-Seq time points (Fig. 5). This observation is consistent with prior studies reporting that transcriptional responses to antibiotic stress often occur within the first hour of exposure[43–45], suggesting that the sampling times used for RNA-Seq in this study may have been too long to capture peak activity. Taken together, the transcriptomic and qRT-PCR data indicate that early (≤ 1.5 h) time points are optimal for capturing lincomycin-induced expression dynamics.

Our initial hypothesis posited the presence of a T4SS or a related DNA export pathway in strain KS1030. However, transcriptomic data did not reveal clear evidence of such a system. Instead, we hypothesize that weak activation of the Com operon and FtsK may transiently promote DNA mobilization capacity, thereby enhancing plasmid dissemination under antibiotic stress. This hypothesis could explain why plasmid transfer occurs upon lincomycin exposure even when classical secretion systems such as the T4SS are not transcriptionally induced. The Com operon, which encodes pilins structurally analogous to type IV secretion components[46–49], may represent a functional alternative mechanism contributing to horizontal transfer. The DNA translocase FtsK is known to be involved in chromosome segregation and cell division, but it has also been suggested to contribute

to HGT[41]. However, the functional validation of candidate genes presumed to participate in HGT—such as the *com* operon and *ftsK*—has not yet been conducted. Moreover, the RNA-Seq analysis used in this study detects transcriptional changes but cannot directly measure the extent of plasmid transfer. To overcome these limitations, additional experimental validation—including transcriptomic analysis with earlier sampling and functional characterization of the corresponding genes—is required.

This study presents the first integrated genomic and transcriptomic analysis of *S. equorum* KS1030 under lincomycin stress, revealing that the strain primarily activates metabolic and stress-adaptation pathways rather than classical secretion systems. Although *lnuA* was strongly upregulated, the relaxase and *mobC* genes showed no significant induction, while competence-associated components and the DNA translocase *ftsK* exhibited only modest, early transcriptional responses. These findings suggest that KS1030 may rely on alternative or condition-dependent mechanisms for plasmid transfer. By delineating a focused set of candidate genes, this work provides a hypothesis-generating foundation for targeted functional studies—including gene disruption, complementation, protein-level assays, and earlier time-point transcriptomics—to clarify their roles in horizontal gene transfer. Ultimately, the results offer valuable insights into resistance dissemination in food-derived *S. equorum* and support the development of safer fermented-food starter cultures through improved understanding of HGT-associated genetic factors.

Data availability

RNA-Seq data analyzed in this study were deposited in the Sequence Read Archive (SRR28760434-SRR28760435 and SRR32872724-SRR32872725). The data presented in this study are available in the article/Supplementary information file; further inquiries can be directed to the corresponding author.

Received: 18 September 2025; Accepted: 9 December 2025
Published online: 18 December 2025

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Funding

This work was supported by the National Research Foundation of Korea (NRF) [NRF-RS-2024-00334769].

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-32198-1.

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