A Thesis for the Degree of Master of Science

Transcriptomic analysis of *Salmonella*–bacteriophage interaction to improve phage biocontrol in foods

전사체 분석을 통한 살모넬라–박테리오파지 상호작용 연구 및 이를 이용한 식품에서 파지의 병원균 제어능 향상

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Abstract

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Understanding the bacterial response to bacteriophage infection can provide more comprehensive solutions to control pathogens using phages. In this study, we used total RNA sequencing analysis to investigate global gene expression change during phage infection and find new functional genes involved in phage-host interaction. When *Salmonella* Typhimurium LT2 was infected by virulent phage SPC32N, several genes in Fep system, required to ferric iron uptake, were significantly up-regulated. Among these up-regulated genes, we speculated that the gene cluster *fepDGC,* encoding an inner membrane transporter complex, is thought be an important factor for phage infection because experiments using knockout strain Δ*fepDGC* showed that the plaque formed by SPC32N became significantly turbid while at the same time this deletion mutant seemed resistant to SPC32N in challenge assay. In addition, the adsorption rate of SPC32N against the Δ*fepDGC* was significantly reduced compared to the wild-type strain and for mimicking the RNA sequencing data of this system, ferric iron chelators were used and we

can identify depletion of ferric irons induces delay of host cells' resistance acquisition. From this understanding, to overcome the limit of phage as biocontrol agent at foods, deferiprone, one of ferric iron chelating agents which is allowed as edible agent for human by FDA was selected as a synergetic resource. By treating deferiprone to food, it was shown that bacterial resistance to phage was significantly postponed.

Keywords: RNA sequencing, Phage-host interaction, Iron uptake system

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1 INTRODUCTION

1.1 *Salmonella* Typhimurium and its Fe^{3+} uptake systems

Salmonella enterica subsp. *enterica* serotype Typhimurium (*S.* Typhimurium) has been studied as a notorious food-borne pathogen which causes a gastroenteritis, characterized by fever, acute intestinal inflammation, and diarrhea in human (Coburn, Grassl et al. 2006, Thiennimitr, Winter et al. 2012). In addition, *S.* Typhimurium is a model organism for studying genetics and microbial pathogenesis of Gram-negative bacteria. The genus *Salmonella* comprises the Gram-negative, rod-shaped, facultative anaerobes with multiple flagella projecting in all directions and it is a member of the family *Enterobacteriaceae. Salmonella* strains which cause human salmonellosis can be grouped into typhoid *Salmonella* and non-typhoid *Salmonella* (NTS) by the clinical patterns. *S. enterica* serovars Typhi, Paratyphi A, Paratyphi B, and C have been classified as typhoid *Salmonella,* whereas others are grouped as NTS. Typhoid *Salmonella* strains are human host-restricted organisms that cause typhoid fever and paratyphoid fever, together referred to as enteric fever. NTS strains may be host generalists, infecting or colonizing a broad range of vertebrates (Crump, Sjölund-Karlsson et al. 2015). Symptoms of salmonellosis like diarrhea, nausea, fever and abdominal cramps start to develop within 12 to 74 hrs after infection, and usually resolve in 5 to 7 days

in healthy peoples often without treatments other than oral fluids meanwhile infection can result in more serious symptoms and in some cases death, particularly in young children, the elderly, and immunocompromised patients (Fournier, Knox et al. 2015). The transmission of NTS infection to humans can occur through the ingestion of food or water contaminated with infected animals' waste, direct contact with infected animals or consumption of infected food animals. The food products predominantly associated with the outbreaks include animal products such as milk, poultry and eggs as well as vegetables and fruits (KuKanich 2011, Sánchez-Vargas, Abu-El-Haija et al. 2011).

Iron, the fourth most abundant metal in nature, is insoluble in aerobic environments. In aqueous, neutral pH conditions, iron exists as an insoluble polymer, $FeOH_3$ (Biederman and Schindler 1957). At a biological pH, the solubility of free iron is 10^{-18}, which restricts its availability and limits microbial growth. To overcome the low-iron availability, *Salmonella* have evolved multiple mechanisms to acquire iron, including the synthesis and uptake of low-molecular-weight compounds called siderophores. Siderophores bind iron-III (Fe^{3+}) and facilitate its solubilization and transportation into the cell through iron-regulated outer membrane proteins. Uptake of Fe^{3+} is powered from the proton motive force generated by the

periplasmic protein complex, TonB-ExbB-ExbD with the regulation of ferric uptake regulator (Fur) (Tsolis, Bäumler et al. 1995). Siderophores are categorized in catecholate, hydroxamate, α-hydroxycarboxylate, and mixed type structural groups (Leong and Neilands 1976, Sheldon, Laakso et al. 2016). In *S. enterica,* the Ent-TolC system to synthesize and secrete a catecholate type of siderophores called enterobactin that binds Fe^{3+}. This enterobactin-Fe^{3+} goes into outer membrane proteins (FepA or IroN or CirA) and then inner membrane proteins (Fep system) to deliver iron. In addition to several other iron uptake systems, the hydroxamate-Fe^{3+} uptake genes *fhuACDB* and *sitABC* (Fhu system) have been reported in various *Salmonella* serovars (Zhou, Hardt et al. 1999). Figure 1 shows a schematic representation of siderophore export and import mechanisms in *Salmonella.*

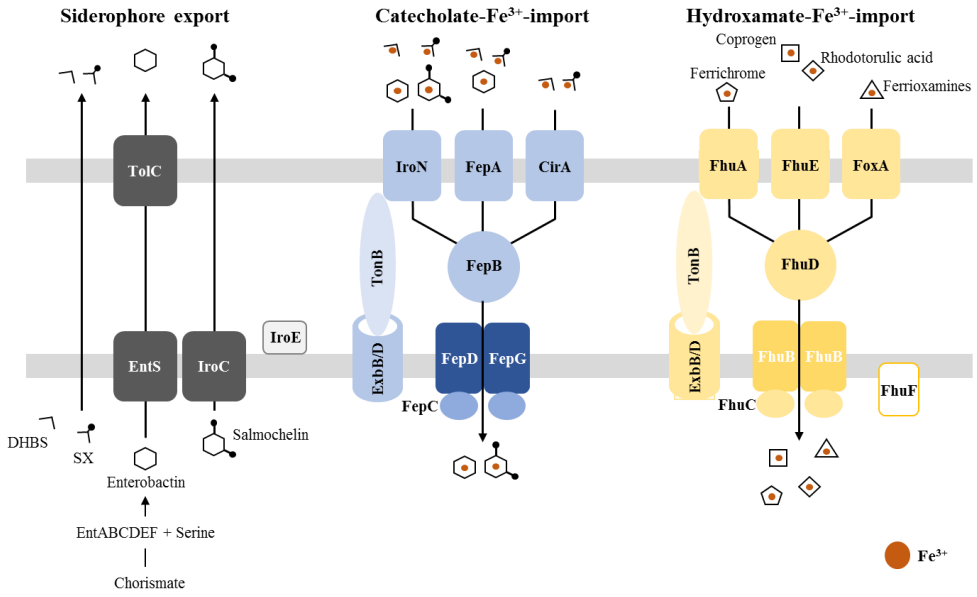

Figure 1. Uptake systems of Fe^{3+} in *Salmonella*

Biosynthesis of enterobactin from chorismate is associated with EntABCDEF and the molecule is exported through EntS and TolC. Salmochelin derives from enterobactin and IroCDE system help its export. When they bind Fe^{3+} outside the cell, they are imported by IroN or FepA or CirA. Hydroxamates are non-*Salmonella* siderophores and mostly recognized by FhuA/E and FoxA.

1.2 Bacteriophages and their biocontrol

Bateriophages (also called as phages) are bactericidal viruses that specifically infect and replicate within the host bacteria. They are known as the most abundant living entities (approximately ~10^{31} phages) on earth, being outnumber their host bacteria by 10-fold (Brüssow and Hendrix 2002). They have been found wherever potential host cells are thrive (Guttman, Raya et al. 2005, Liu and Zhang 2008). According to their morphology and state of nucleic acid, bacteriophages including archaeal viruses as well as bacterial viruses, are classified by the International Committee on Taxonomy of Viruses (ICTV) into one order, 14 families, and 37 genera with at least five other potential families awaiting classification (Ackermann 2009). Phages having tail structure constitute the order *Caudovirales*, and further classified into three families: family *Siphoviridae* have a long, non-contractile tail, wihle family *Myoviridae* possesses a long, contractile tail; phages in family *Podoviridae* are characterized by a short tail.

Phages initially attach to the host bacteria to begin their new life-cycle. This initial attachment step is called as "adsorption." Subsequently, they bind to its specific host receptors using their receptor-binding proteins (RBPs) and intensive studies have been revealed that many cell surface components of bacteria are utilized as phages' receptors (Lindberg 1973). The specific binding between host receptors and phage RBPs triggers the next stage of

phage life cycle, the penetration of phage nucleic acids into the grabbed host cells. *Podoviridae* phages, which includes phage SPC32N used in this study, degrade a portion of host cell membrane by using of their tailspikes containing enzymatic activity, such that inject its genome (Eriksson, Svenson et al. 1979). After the internalization into host cytosol, it is largely divided into temperate phages and virulent phages according to their life-cycle. In virulent phages' life-cycle, they are propagated using the bacterial proteins and their own proteins together and then lyse the cells and release, while temperate phages integrate their genome into the host genome first, which are called as a prophage state (Ravin 2011).

Usually, virulent phages are used for phage biocontrol. Although novel antibiotics have been developed and deployed to control pathogenic bacteria, resistances against virtually every new class of antibiotics were emerged (Clatworthy, Pierson et al. 2007). As a result, so called 'super bacteria' exhibiting resistance against multiple antibiotics was reported in several pathogens, including *Salmonella* (Adzitey, Huda et al. 2012, Eng, Pusparajah et al. 2015). Because of its high specificity to the target bacteria, auto-dosing characteristics and capability of evolution which can overcome the phage-resistant bacterial mutants, phage applications to the medical practice, food and agricultural industries have been reevaluated and received interests in recent years. Especially, in food and agricultural industries, the use

of antibiotics was prohibited in many countries, so several studies focused on a control of livestock-associated pathogens and food stuffs (Desmidt, Ducatelle et al. 1998, Pao, Rolph et al. 2004, Thung, Lee et al. 2019).

Although phages have high potentials as alternatives to antibiotics, still there is hurdle to overcome in immunological perspectives of phage applications (Sulakvelidze, Alavidze et al. 2001, Summers 2001). Huge number of phage particles that entered simultaneously into the body might provoke abnormal immune responses and development of neutralizing antibodies against phages, which would result in a diminished effectiveness of phage treatment. In addition, rapid emergence of phage-resistant bacterial mutants is also another significant problems in application of phage as biocontrol agents (Sanders 1988, Greer 2005). Because phages also threaten the survival of bacteria, the development of resistant mutants against phages is unquestionable. To overcome these problems, many studies have been conducted (Lu and Koeris 2011, Kim, Kim et al. 2014) and various and advanced approaches are required.

1.3 Purpose of this study

Understanding the bacterial response to bacteriophage infection can provide more comprehensive solutions to control pathogens using phages. In this study, we used RNA sequencing analysis to investigate global gene

expression change during phage infection and find new functional genes involved in phage-host interaction. RNA sequencing is a technique that can examine the quantity and sequences of RNA in a sample using next generation sequencing (NGS). It analyzes the transcriptome of gene expression patterns encoded within targets' RNA. In this study, gene expression pattern of SPC32N-untreated groups and SPC32N-treated groups were compared, new functional genes associated with phage-host interaction were explored and more useful and effective solution for phage biocontrol would be suggested from understanding this new possibility.

2 MATERIALS AND METHODS

2.1 Bacterial strains, bacteriophage and growth conditions

All bacterial strains which were derived from *S.* Typhimurium LT2 as the parent are listed in Table 1. Luria-Bertani (LB) medium containing 1% Bacto-tryptone, 0.5% yeast extract, and 1% NaCl (pH 7.5) was used as the complex culture medium for the routine growth of bacteria. All *S.* Typhimurium strains were grown aerobically at 37°C with antibiotics or chemicals supplementation at the following concentration, if required: amplicillin (Ap), 50 μg/mL; kanamycin (Km), 50 μg/mL; chloramphenicol (Cm), 25 μg/mL and L-arabinose, 0.002%.

The bacteriophage used in this study is listed in Table 1. The phage were purified, propagated and concentrated according to methods of Sambrook (Sambrook, Russell et al. 2001) with some modifications. First, the lysate of a single plaque of phage SPC32N was added to LT2 culture (OD_{600} = 0.5), which was then incubated at 37°C for 3 hr 30 min. This culture with cell debris was centrifuged (15,000 x g, 10 min, 4°C). The supernatant of the sample was filtered (0.22-μm pore filter) and precipitated with 10% (w/v) polyethylene glycol (PEG) 6000 in 1 M NaCl at 4°C overnight. After centrifugation (10,000 x g, 20 min, 4°C), precipitated phages were resuspended in SM buffer and separated by CsCl density gradient

ultracentrifugation (78,500 x g, 2 hr, 4). The phage fraction was separated and dialyzed against 1 L of dialysis buffer [50 mM Tris-HCl (pH 8.0), 10 mM NaCl, 10 mM $MgSO_4$] in a Spectra/Por® dialysis membrane (MWCO: 12,000-14,000, Spectrum Laboratories, Inc., US). This step was repeated twice and finally transferred into a sterilized glass ampoule. The titers of the concentrated phage stocks were determined by the overlay assay and the stocks were stored at 4°C until further use.

2.2 Construction of *Salmonella* mutant strains

Lambda Red recombination technique (Datsenko and Wanner 2000) was used to introduce specific gene deletions into the *Salmonella* mutant strains. PCR products including kanamycin resistance gene were constructed from pKD13 using primers carrying homologous regions flanking start and stop codons of the genes to be deleted. The oligonucleotide sequences of primers used are listed in Table 2.

One-percent of overnight culture of the LT2 strain harboring the plasmid pKD46 was inoculated and incubated in 10 mL of LB broth with ampicillin (50 μg/mL) for 30 min to adapt incubation condition and then 100 mM of L-(+)-arabinose was treated. The culture was incubated at 30°C with shaking (220 rpm) until it reached a density of OD_{600} = 0.6. The cells were pelleted by centrifugation (10000 ×g, 5 min, 4°C), resuspended with 1mL of

pre-chilled 10% glycerol twice and then finally resuspended with 50 μL of 10% glycerol. PCR products was electroporated into the electrocompetent LT2 harboring pKD46. Electroporation was performed using Biorad MicroPulser Electroporation System (Cat. #165-2100, CA, USA) following manufacturer's instructions. Then, cells were recovered by resuspending with 1 mL of super optimal catabolite (SOC) media and incubating at 37°C for 1 hour in 220 rpm then plated on a kanamycin plate for the selection of transformants.

Homologous recombination site was confirmed by PCR. The Km^R cassette was removed from the transformants by introducing plasmid pCP20 (Datsenko and Wanner 2000).

2.3 Plasmid construction

Plasmid for complementation of the *fepDGC* mutant was constructed by cloning pBAD18 containing this gene cassette. The insert made by PCR amplification contained the gene *fepDGC,* HindIII and EcoRI sites flanking it. At PCR amplification, the primers fepDGC_pBAD_F and fepDGC_pBAD_R were used. The sequences of them are listed in Table 2.

Table 1. Bacterial strains, bacteriophages and plasmids used in this study

Strain or plasmid	Genotype or relevant characteristics[a]	Reference or source
Bacterial strains (*Salmonella enterica* serovar Typhimurium)		
LT2	Wild-type; host for phage SPC32N	(Erickson, Newman et al. 2009)
	Δ*fepDGC*	This study
	Δ*entB*	This study
	Δ*fepE*	This study
	Δ*ybdZ*	This study
	Δ*fhuB*	This study
	Δ*fepDGC* with pBAD18	This study
	Δ*fepDGC* with p*fepDGC*	This study
	Δ*rfbP*	Laboratory collection
Bacteriophage		
SPC32N	Infect *S.* Typhimurium; O-antigen-specific	(Kim and Ryu 2013)
Plasmids		
Lambda red recombination		
pKD46	P_{BAD}-*gam-beta-exo oriR101 repA101*^{ts}; Ap^R	(Datsenko and Wanner 2000)

pKD13	FRT Km^R FRT PS1 PS4 *oriR6Kγ*; Ap^R	(Datsenko and Wanner 2000)
pCP20	*Ci857* Λp_R*flp oripSC101*^{ts}; Ap^R Cm^R	(Datsenko and Wanner 2000)
Gene complementation		
pBAD18	pBR322 *araC*; arabinose-inducible promoter (P_{BAD}), Ap^R	(Guzman, Belin et al. 1995)
p*fepDGC*	pBAD18-*fepDGC*; Ap^R	This study

[a] Ap^R, ampicillin resistant; Km^R, kanamycin resistant, Cm^R, chloramphenicol resistant.

Table 2. The oligonucleotides for construction of strains used in this study

Oligonucleotides	Sequence (5' → 3')[a]	Purpose
fepDGC_lambda_F	AAG CCG GAT AGC GAT ATC CGG CTT TGT CAT CAG TGT GGG CGA CTA TCG CCG CCC CAG CGG TGT AGG CTG GAG CTG CTT C	Gene (*fepDGC*) deletion
fepDGC_lambda_R	TGC TAA CAT AAC CAA ATA AGA TAG ATA ACG ATA ATT ACT ATC ATT ATC AGG GAA GTT ACT CTG TCA AAC ATG AGA ATT AA	Gene (*fepDGC*) deletion
entB_lambda_F	CGG TTG GCG TCA CGT TCA CCG CTC TGA AGG AGA AAG AGA GTG TAG GCT GGA GCT GCT TC	Gene (*entB*) deletion
entB_lambda_R	TCA CCC ATA CCG TTT TGT CTG AAA AAT CAA AGC CGG TCA TCT GTC AAA CAT GAG AAT TAA	Gene (*entB*) deletion
fepE_lambda_F	ATG TGT TCT TTC ATT GGA TAA AGT TTT CAG GTC ATA CGG CTG TAG GCT GGA GCT GCT TC	Gene (*fepE*) deletion
fepE_lambda_R	ATG ACA AAG CCG GAT ATC GCT ATC CGG CTT TTC GGG TAA ACT GTC AAA CAT GAG AAT TAA	Gene (*fepE*) deletion
fhuB_lambda_F	CGA TGC ACT TTG TCC GCA TCC TGA ATA ACG TGT TGG GAG GCA AAG CGT GAT GTA GGC TGG AGC TGC TTC	Gene (*fhuB*) deletion
fhuB_lambda_R	GTC GGG TTG AGC ATG GCT GCC ACC CGA CAT AAA TGT GGC ACA GCT TTT TT CTG TCA AAC ATG AGA ATT AA	Gene (*fhuB*) deletion

ybdZ_lambda_F	ACA TGT CCA TGA CAG GAG TTG AGT ATG GAA TTC AGT AAT CCC TTC GAT AAT CCG CAG GGA TGT AGG CTG GAG CTG CTT C	Gene (*ybdZ*) deletion
ybdZ_lambda_R	CGC CTC CTG CTT ATC GGC ATA ATG CGC CGG ATT CAG TGT TGT CCA GCG CGT ATT AAG CCA CTG TCA AAC ATG AGA ATT AA	Gene (*ybdZ*) deletion
fepDGC_pBAD_F	AAA GAA TTC ACC AAA TAA GAT AGA TAA CGA TAA TT	Δ*fepDGC* complementation
fepDGC_pBAD_R	AAA AAG CTT CTA TCG CCG CCC CAG CGG TAC CAC CA	Δ*fepDGC* complementation

[a] Restriction enzyme sites are underlined.

2.4 RNA extraction and total RNA sequencing

Overnight culture of bacterial cells cultivated in LB medium were diluted into fresh LB medium at a 1:100 ratio and grown at 37°C until the culture reached a density of OD_{600} = 1.5. After centrifugation (10,000 x g, 5 min, 4°C) and washing by fresh LB medium, the SPC32N stock diluted to a multiplicity of infection (MOI) of 100 was inoculated for 5 min to induce phage attachment. After centrifugation (15,000 x g, 2 min, 4°C) and washing by fresh LB medium to remove planktonic phage virions, the culture was incubated at 37°C with agitation (220 rpm). At 16 min post infection, one volume (200 μL) of bacterial culture were treated with 2 volume of RNAprotect bacterial reagent (Qiagen) for 5 min to quench the transcription of genes and further degradation of RNA molecules. Meanwhile, at the chelated condition, overnight bacterial culture incubated in LB medium were diluted into fresh LB medium at a 1:100 ratio and 200 μM 2, 2'-dipyridyl was treated. Cells were grown at 37°C until the culture reached a density of OD_{600} = 1.5. This culture was sampled as one volume (200 μL) of bacterial culture which treated with 2 volume of RNAprotect bacterial reagent (Qiagen). Total RNA was isolated using a RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and residual DNA was removed with Ambion Turbo DNA-*free*™ (Cat. #AM1907, Texas, USA).

Five micrograms of total RNA from each sample was used as a starting material and subjected to an rRNA-removal process based on the subtractive hybridization/bead capture system of the Ribo-Zero kit (Epicentre Biotechnomogies, Wisconsin, USA). Purified RNA samples were used for mRNA sequencing library construction using the Illumina TruSeq RNA Sample Preparation kit v.2 (Illumina, CA, USA). RNA sequencing was performed by two runs of Illumina HiSeq 2500 to generate single-end-reads around 100 bp in length. Using the CLC Main Workbench 8.0.1 tool (Qiagen), sequencing reads were mapped to the *S.* Typhimurium LT2 genome sequence. Normalization methods employed in RNA sequencing analysis included TPM (transcripts per million) (Robinson and Oshlack 2010, Dillies, Rau et al. 2013, Risso, Ngai et al. 2014). Differentially expressed genes (DEGs) with an absolute [fold change] larger than 2 were filtered and visualized using the CLC Main Workbench 8.0.1 tool, where [fold change] was defined as [$TPM_{SPC32N\text{-}incfected}/TPM_{Control}$]. Clusters of orthologous groups (COG) analysis (Tatusov, Koonin et al. 1997) was used for functional grouping of all genes of *S.* Typhimurium LT2. The proportion of DEGs in each functional group was calculated.

2.5 Heat map generation

Heat map was drawn to analyze the global response patterns categorize them by genes' function. It was visualized that the fold change values of relative gene expression at 16 min post phage infection. Heat map and hierarchical clusters were then generated by using Gitools v.2.3.1.

2.6 Quantitative real-time PCR (qRT-PCR) analysis

From isolated RNA, cDNA was synthesized by using RNA to cDNA EcoDry™ Premix (Clontech, Cat. #639546, CA, USA) following the manufacturer's instruction. Using 2X iQ SYBR Green Supermix (Biorad, Cat. #170-8880, CA, USA) and the CFX Connect™ Real-time PCR Detection System (Biorad, CA, USA). The calculated threshold cycles (Ct) corresponding to target genes were normalized by the Ct of the reference *rpoD* gene (Kjeldgaard, Henriksen et al. 2011). The *rpoD* gene was chosen as the reference gene because it showed no significant variation of *rpoD* expression regardless of experimental conditions. The relative expression of target genes was calculated by using Bio-Rad CFX Manager™ 3.1. Software (Biorad, CA, USA). Experiments were conducted in triplicate. The sequences of primers used in the qRT-PCR analysis are listed in Table 3 (Quellhorst and Rulli 2008).

Table 3. Primers used for qRT-PCR in this study

Oligonucleotides	Sequence (5' → 3')
rpoD_F	CTG AAA ATA CCA CCA GCA CC
rpoD_R	CGG GTC AAC AGT TCA ACA GTG
fepG_F	CTA GGC AGC CCT GAT GTG AT
fepG_R	TCA GCC GGA AAG TTT CAA TCC
fepD_F	TCG GTT TAC TGG CGA TTA CC
fepD_R	ATA TCG GCA AAA AGC AGC AGA G
fepC_F	TAC GCT AAG CCG TCT GAT GA
fepC_R	TAC GCC AGC GGG TAA ATA AC
entB_F	TCT GAT GGC GCT GAA CTA TG
entB_R	CCA GGC CAT AAT CAA TCA GG
ybdZ_F	CCG CAG GGA CAG TTT TAC AT
ybdZ_R	CAT TTC GCC TCC TGC TTA TC
fepE_F	TTG CGT AAC CGT CAA TAC CA
fepE_R	TTT CCA TTT TAC GCG AGA CC
fhuC_F	TGC ATC GTT TAA GCC AAC AG
fhuC_R	TAC CCA TCG GGA TAC CGT AA
fhuD_F	ATG ACG ACG CTT ATC GAT CC
fhuD_R	AGC AGA TCA CAT CCG CTT CT
fhuB_F	CGA TCG TGA TGG TGA TTT TG
fhuB_R	GCC ATC AGT CCA ACG AAA CT
fhuA_F	CAT GCT TGA GCG TGT TGA GT
fhuA_R	TTG CTG CTC ATT TGT CGA AC
fhuE_F	AGT CCT TAT GGC GCA AGC TA
fhuE_R	GTT AAA GGC GCT GAA GTT GC
foxA_F	CCA CTG GAT GAC AAT GAA CG
foxA_R	GCG GAT AGG CCA TAA GAT CA
fur_F	TAA AGA AGG CTG GCC TGA AAG
fur_R	GAT ACC GGC ATC GTC AAA CT

2.7 Bacterial challenge assay

Bacterial overnight culture was inoculated to a LB broth with 100-fold dilution and incubated at 37°C with shaking (220 rpm), until the culture reached a density of OD_{600} = 1.0. SPC32N stock diluted to a MOI of 1 was inoculated and if required, ferric iron chelating chemicals (2, 2'-dipyridyl (Sigma-Aldrich, Cat. #366-18-7, USA), 200 μM; 3-Hydroxy-1, 2-dimethyl-4(1H)-pyridone (deferiporone) (Sigma-Aldrich, Cat. #30652-11-0, USA), 1.2 mM) were treated. Bacterial growth was monitored by measuring OD_{600} every 15 min by SpectraMax® i3x microplate reader (Molecular Devices). As a negative control, one bacterial culture was inoculated with same volume of SM buffer instead of SPC32N.

2.8 Phage adsorption assay

One milliliter of cells in early exponential phase (OD_{600}=1.3-1.5) were harvested and diluted in 8mL of fresh LB medium and SPC32N was added (1 mL, MOI=0.01). This mixture was aliquoted into 5 microcentrifuge tubes (1 mL each) and incubated statically in heat block at 37°C. At each time point, one tube was immediately removed from the heat block, centrifuged (15,000 x g, 1 min, 4°C) and filtered with syringe filter with 0.22-μm of pore size (Polyethersulfone filter). The number of unbound planktonic phage

particles in each tube was determined by overlay assay. The baseline number was determined by adding the same concentration of SPC32N to the bacteria-free LB medium and incubating under the same condition. P_t is the titer of free phage t min after phage attachment.

2.9 One-step growth curve analysis

One-step growth curve analysis was performed as previously described (Lu, Breidt Jr et al. 2003). Bacterial overnight culture was inoculated to a LB broth with 100-fold dilution and incubated at 37°C with shaking (220 rpm), until the culture reached a density of OD_{600} = ~1.5. After centrifugation (6,000 x g, 10 min, 4°C), supernatant was discarded and the pellet was resuspended in fresh LB medium. SPC32N stock which was diluted to a MOI of 0.001 was inoculated for 5 min for phage attachment. This medium was centrifuged (6,000 x g, 10 min, 4°C) and resuspended in fresh LB medium. The culture was incubated at 37°C with shaking (220 rpm). Two sets of samples were collected every 8 min for up to 1 hr. To release the intracellular phages, chloroform was added to one of the tubes, and the eclipse period was determined. Subsequently, titer of each sample was immediately assessed by the overlay assay. With the curved described, latent period, eclipse period and burst size were determined. All the experiments were performed in triplicate.

2.10 LPS extraction of bacterial strains

LPS was extracted from overnight bacterial cultures using hot phenol-water micro-extraction methods (Wang, Zhang et al. 2010). Three milliliters of bacterial cultures was centrifuged (16,000 x g, 1 min, 4°C) and the pellet was washed with 1 mL of DPBS buffer (Dulbecco's phosphate buffered saline, pH 7.2; PBS containing 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$). Then the cells were resuspended in 300 µL ddH_2O and equal volume of pre-heated (68°C) phenol solution was added prior to incubation at 68°C for 15 min with vigorous agitation every 5 min. The samples were chilled on ice for 5 min and the aqueous phase was separated by centrifugation (10,000 x g, 4°C, 5 min). The LPS was extracted again from the phenol phase by adding another 300 µL ddH_2O. After this step, sodium acetate (final concentration of 0.5 M) and 10 volumes of 95% ethanol was added into the collected samples and incubated overnight at -80°C. The crude LPS were sedimented by centrifugation (16,000 x g, 5 min, 4°C) and resuspended in 100 µL of ddH_2O, and precipitated again with 95% ethanol. The precipitated LPS was re-dissolved in 50 µL of ddH_2O and stored at -80°C.

The extracted LPS were analyzed by deoxycholate-polyacrylamide gel electrophoresis (DOC-PAGE) on a 15% acrylamide gels (Reuhs, Geller et al. 1998). The separating gel (15%) containing 5 mL of monomer stock solution [30% (wt/vol) acrylamide, 0.8% (wt/vol) *N, N'*-

methylenebisacrylamide], 2 mL of stacking gel buffer solution (1.875 M Tris base, adjust to pH 8.8), 3 mL of ddH_2O, 17.5 μL of 10% ammonium persulfate and 6.25 μL of TEMED was laid onto the solidified separating gel. The gels were pre-run with a funning buffer (290 mM glycine, 37 mM Tris base and 6 mM sodium deoxycholate) for 1 hr at 15mM/gel using Bio-Rad Mini-PROTEAN® Tetra Cell. Same amount of extracted LPS samples was mixed with equal volumes of sample buffer (containing 2 mL of stacking gel buffer solution, 1 mL of glycerol and 2.5 mg of bromophenol blue, brought up to 10 mL with ddH_2O). Amount of extracted LPS was normalized by CFU/mL of each strain overnight culture. The mixtures were loaded onto the gels for up to 60 min at 15mA/gel. The gels were fluorescently stained using the Pro-Q® Emerald 300 Lipopolysaccharide Gel Stain Kit (Molecular Probes, cat NO. P20495; Eugene, OR, USA) according to the manufacturer's instructions. The samples were visualized under the 300 nm UV by the Red™ Imaging System.

2.11 Antimicrobial activity of SPC32N with deferiprone in food samples

The lytic activity of SPC32N against *S.* Typhimurium LT2 strain was tested in commercial whole-fat pasteurized milk and fresh cucumbers. Samples were obtained from a local supermarket. Inoculation and incubation methods with milk (Tanaka, Yamada et al. 2018) and cucumbers (Vonasek,

Choi et al. 2018, Thung, Lee et al. 2019) were modified from previously described method. In addition, deferiprone, one of ferric iron chelators which is allowed as an edible agent for human by FDA, was treated with phage to get more effective biocontrol in these foods.

A milk sample was inoculated with overnight bacterial cultures diluted to approximately 10^5 CFU/mL and the samples were pre-incubated at 25°C for 1 hr with shaking (100 rpm) to allow bacteria to adapt to food environment. After inoculation of SPC32N solution supplemented with 0.5 mM deferiprone (MOI=100), viable cell count (CFU/mL) were measured at 0, 3, 6, 10, 24 hr post infection time by plating each sample on LB plate. Negative control was treated by same volume of ddH_2O.

Cucumber samples were aseptically cut into 1-cm thick slices with an area of approximately 10 cm^2. Before testing antimicrobial activity of SPC32N with deferiprone, sliced samples were dipped into 70% ethanol for 5 min with slight shaking and placed in a UV chamber for 30 min on each side to remove residual microflora. Overnight culture of *S.* Typhimurium LT2 cells diluted to approximately 10^5 CFU/mL were inoculated onto the surface of each sample and pre-incubated at 25°C until inoculum was dried. Subsequently, each sample was dipped into SPC32N solution (10^9 PFU/mL, 5 mM deferiprone) for 5 min statically and dried. At 0, 3, 8, 20 hr post infection time, bacterial cells were detached from the surface of food samples by

homogenization in 0.1% buffered peptone water (BPW) for 1min by using a stomacher (Medexx) Cell suspensions were serially diluted, plated onto LB plates and incubated at 37°C overnight. Negative control was treated by same method with ddH_2O. All the experiments were performed in triplicate.

2.12 Statistical analysis.

All experiments were replicated three times. GraphPad Prism (version 5.01) was used to conduct statistical analysis. One-way analysis of variance (ANOVA) followed by the one-way Tukey's test for all pairwise comparison (95% confidence interval) were used. The data presented as means with standard deviations. A *P*-value which was lower than 0.05 was considered statistically significant.

3 RESULTS

3.1 Analysis of transcriptome after phage infection and Fe^{3+} uptake system

Based on the one-step growth curve (Figure 5B), 16 min post infection were determined as mid-infection stage and total RNA were extracted at this time point. At the result of RNA sequencing, the [fold change] was defined as [$TPM_{SPC32N\text{-}incfected}/TPM_{Control}$] and the genes whose absolute $\log_2$ [fold change] higher than 2 or lower than -2 were screened as differentially expressed genes (DEGs).

Among DEGs, 6 groups including colonic acid biosynthesis, vitamin B_{12} biosynthesis, flagella biosynthesis, *Salmonella* pathogenicity island 1 (SPI-1), 2 (SPI-2) and iron uptake were functionally classified. From some previously published studies (Boyd 2012, Bohm, Porwollik et al. 2018, Rousset, Cui et al. 2018), it was able to understand the result of RNA sequencing such as DEGs of colonic acid biosynthesis, SPI-1, 2 and flagella biosynthesis. So, DEGs related to iron uptake which did not have much research done were selected as the target group.

DEGs of this group (Figure 1) contain genes encoding enzymes for enterobactin, a catecholate type of siderophores, biosynthesis and genes related to Fep system. Fhu system, one of Fe^{3+} uptake system, was not

screened as DEGs. *fepE* (encoding the enterobactin transport), *ybdB, entD, entB* (encoding enzymes for enterobactin synthesis) were down-relgulated at 16 min time point and all the remaining DEGs were up-regulated at this point. *fepD* showed the greater fold change than other genes, which led to being target genes for the later experiments.

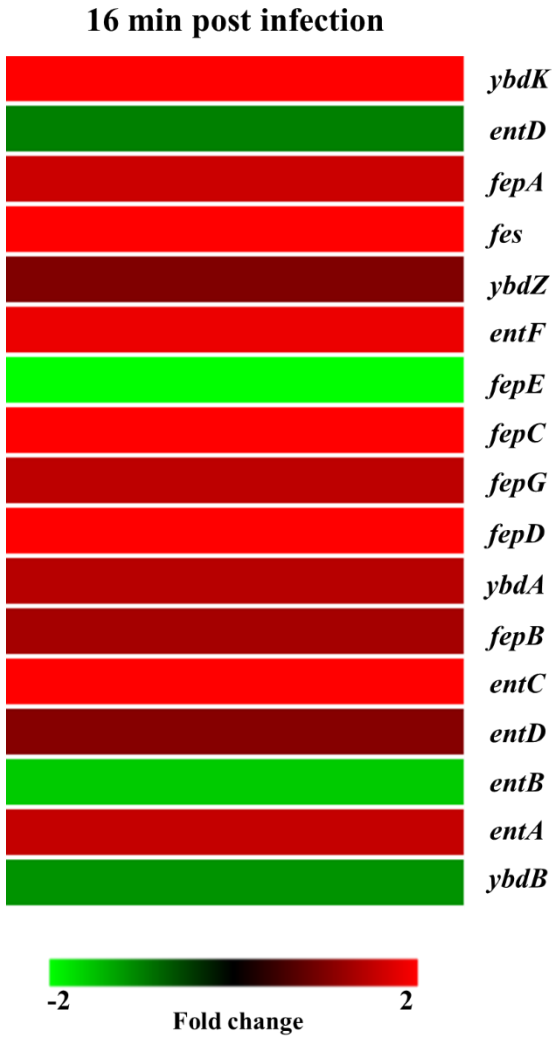

Figure 2. Heat map of differentially expressed genes (DEGs) associated with Fe^{3+} uptake system of *S.* Typhimurium LT2

The fold change values were calculated according to RNA sequencing data. Among several iron uptake systems in *S.* Typhimurium LT2, most of genes on heat map were associated with Fep system and biosynthesis of enterobactin, a catecholate type of siderophores.

3.2 Validation of the expression value of DEGs belonging to Fe^{3+} uptake systems by qRT-PCR

To confirm reliability of transcriptomic data, 6 genes, *fepDGC*, *ybdZ*, *fepE*, and *entB* were subjected to conduct quantitative RT-PCR (qRT-PCR). *fepDGC*, an operon encoding the inner membrane transporter of Fep system, and *ybdZ* showed significant fold change at RNA sequencing (Figure 1). *fepE*, encoding FepE enzyme involving in LPS biosynthesis (Murray, Attridge et al. 2003, Bravo, Silva et al. 2008), was predicted to influence the phage adsorption because the primary receptor of SPC32N was known as LPS of LT2. *entB* is a usual target gene to confirm the function of enterobactin biosynthesis (Crouch, Castor et al. 2008).

The time points of mRNA extraction were same with those of RNA sequencing analysis. Though the expression ratios of transcription levels between phage-free cells and SPC32N-treated cells were different in qRT-PCR compared to RNA sequencing, the pattern of up- or down-regulation was similar with those of RNA sequencing except *entB* (Figure 3). But because these two genes showed relatively small change in transcription level at the RNA sequencing data, difference of tendency between RNA sequencing and qRT-PCR within the error range. Meanwhile, the relative gene expressions of *fur*, the regulator of this system, and all genes related to Fhu system tested for qRT-PCR were not significantly changed as the infection of SPC32N. This

tendency was also shown in RNA sequencing analysis. Consequently, it was confirmed that the RNA sequencing analysis results are biologically significant.

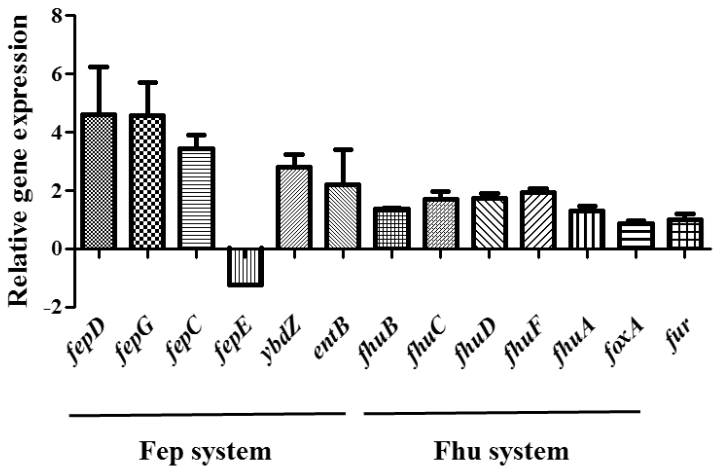

Figure 3. Validation of RNA sequencing data by qRT-PCR

Target genes were selected among the genes related to Fep system and Fhu system. The gene *fur* is encoding the expression regulator of both systems. The reference gene was *rpoD.* The control group was SPC32N-untreated group.

3.3 Effects of various mutants on infectivity

To identify whether 6 genes related to iron uptake can affect phage infectivity, each gene was deleted and phage infectivity against these mutant strains were tested by bacterial challenge assay and spotting assay. Because *fepD, fepG* and *fepC* are located at the same operon on the genome, whole operon was deleted to construct Δ*fepDGC* mutant strain.

As a result of challenge assay, it was confirmed that all deletion mutant strains (Δ*fepE*, Δ*entB* and Δ*ybdZ*) except Δ*fepDGC* showed no differences to the wild-type control in infectivity (Figure 4). This similarity was also observed in spotting assay. However, in a challenge assay with Δ*fepDGC* mutant strains cells were very resistant to the phage infection. The complementation of this gene cassette recovered the phenotype which means the phage resistance in challenge assay was due to the deletion of *fepDGC*. These results indicate that *fepDGC* may play an important role in the phage infection. Therefore, as the screening method for target genes selection, RNA sequencing analysis could be verified again.

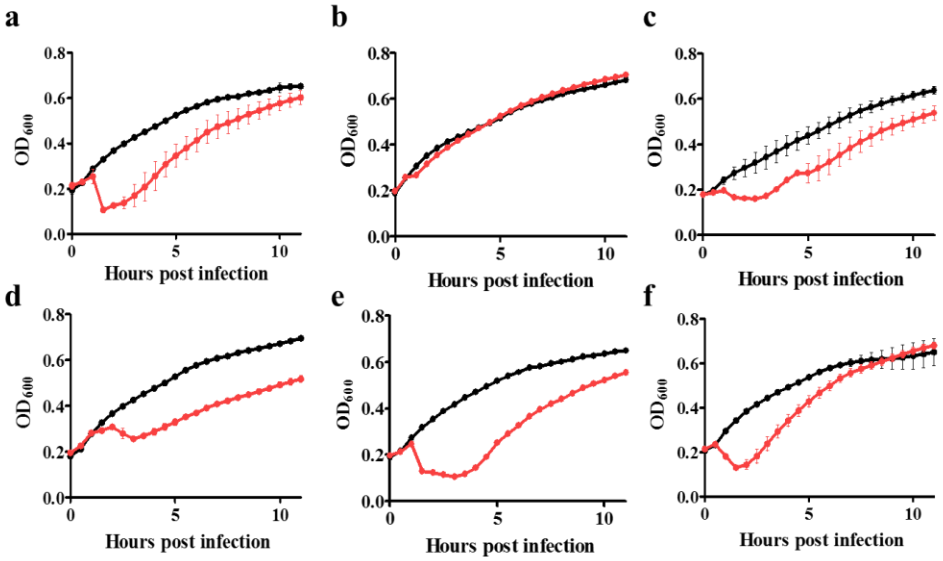

Figure 4. Infectivity of phage SPC32N to *Salmonella* strains

By bacterial challenge assay, phage infection ability to *Salmonella* strains (a, WT (*S.* Typhimurium LT2); b, Δ*fepDGC*; c, Δ*fepDGC*+p*fepDGC*; d, Δ*fepE*; e, Δ*entB*; f, Δ*ybdZ*) were tested. The black lines are SPC32N-untreated groups and the red lines are SPC32N-treated groups. The strain Δ*fepDGC*+p*fepDGC* had been induced by 0.002% L-arabinose during incubation and MOI was 1.

3.4 Adsorption assay and one-step growth curve of Δ*fepDGC*

The data presented earlier (Figure 4) showed that *fepDGC* was likely to play an important role in phage infection. To prove how this gene cassette affect the phage infection, adsorption assay and one-step growth curve analysis were conducted.

By comparing the adsorption ability of phage to WT or mutant strain, it was confirmed that phage has significantly lower adsorption rate against mutant strain. The ln (P_{15}/P_0) values, indicating the adsorption rate (the titer of unbound phage at specific time point / the initial titer of phage), were -4.0642 and -1.8097 against WT and deletion mutant strain, respectively. The adsorption rate was restored by complementation of *fepDGC.* The ratio of adsorbed phages measured after 15 min of attachment in control strain, Δ *fepDGC,* and Δ*fepDGC*+p*fepDGC* were 99.6591%, 82.8333%, 98.181%, respectively. With statistical analysis, it was confirmed that the decreased adsorption rate of Δ*fepDGC* was significant ($P<0.05$) (Figure 5A). However, in one-step growth curve analysis, there was no differences between WT and mutant strains, showing identical eclipse period (24 min), latent period (around 35 min) and burst size (53.11 PFU/infected cell) (Figure 5B).

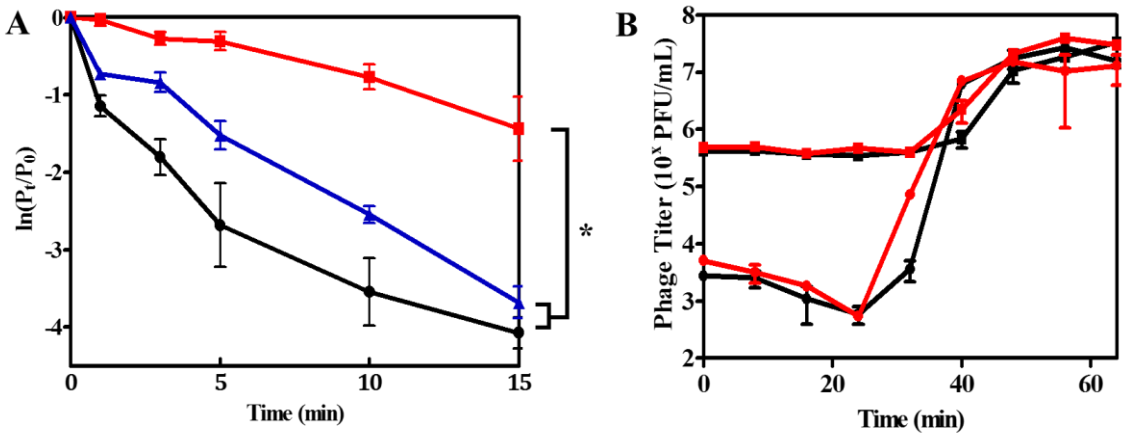

Figure 5. The effect of FepDGC in adsorption assay and one-step growth curve when phage SPC32N infected

At adsorption assay (A), the black line, blue line and red line mean WT (*S.* Typhimurium LT2), Δ*fepDGC* and Δ*fepDGC*+p*fepDGC,* each. The strain Δ*fepDGC*+p*fepDGC* had been induced by 0.002% L-arabinose during incubation. * means $P < 0.05$. At one-step growth curve (B), the black line means WT (*S.* Typhimurium LT2) and the red line means the strain Δ*fepDGC*. The line contained circled points (●) mean the chloroform-treated groups and the lines contained squared points (■) mean chloroform-untreated groups.

3.5 LPS analysis of Δ*fepDGC*

To confirm whether LPS modification is occurred and influence the decreased adsorption rate in Δ*fepDGC*, LPS from WT and mutant strains were isolated and analyzed by DOC-PAGE method (Figure 6). Unfortunately, the result of analysis did not show any band shift, which means that *fepDGC* is not related to LPS modification and thus decreased adsorption rate of phage against mutant strain irrelevant to LPS, major phage receptor.

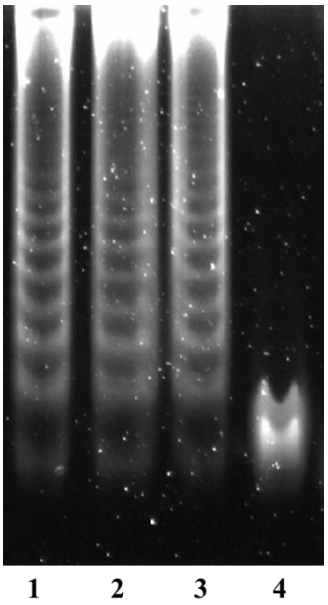

Figure 6. The relationship between FepDGC and LPS modification

By conducting LPS extraction and analysis, the relationship between the function of FepDGC and LPS modification was confirmed (lane 1, WT (*S.* Typhimurium LT2); lane 2, Δ*fepDGC*; lane 3, Δ*fepDGC*+p*fepDGC*; lane 4, Δ*rfbP*). LPS loading concentration was standardized to the amount obtained from 3×10^9 cell forming unit (CFU)/mL. The strain Δ*rfbP* was used as the guide strain for identification of O-antigen region. The Δ*fepDGC*+p*fepDGC* had been induced by 0.002% L-arabinose during incubation.

3.6 Challenge assay under Fe^{3+}-chelated condition

Previously several assays which tested phage infectivity confirmed that Fep system was associated with SPN32N infection. To confirm whether iron depleted condition affect the infectivity, the bacterial cells were incubated under the Fe^{3+}-chelated condition using 2, 2'-dipyridyl, and challenge assay was performed. Prior to challenge assay, qRT-PCR analysis confirmed that 2, 2'-dipyridyl induced up-regulated expression of genes related to Fep system (Figure 7). More specifically, transcription level of *fepD*, *fepG*, *fepC* and *ybdZ* was highly up-regulated while *entB* showed different pattern. The relative expression value of regulator *fur* and *fepE* did not changes at this condition.

As a result, treating 200 μM of 2, 2'-dipyridyl induced continuous growth inhibition with no recovery in OD_{600} value (Figure 8). Because the deletion of *fepDGC* make the cells resistant to SPC32N at the previous data (Figure 4), it could be expected that inducing transcription of genes related to Fep system including *fepDGC* which caused the opposite situation which showed the consistent cell lysis pattern.

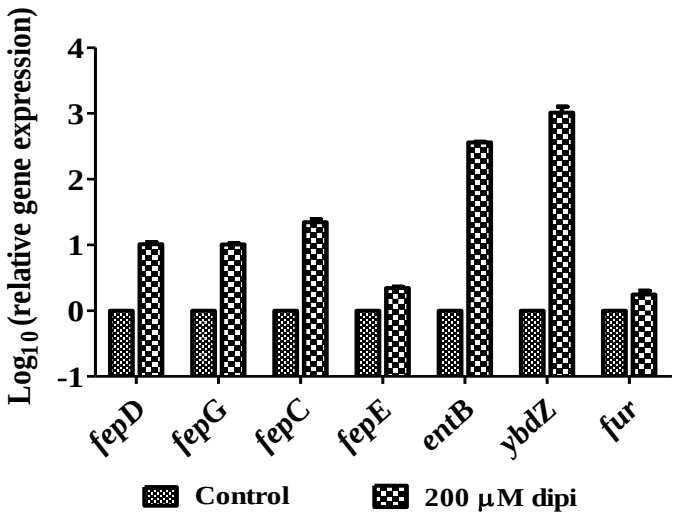

Figure 7. The relative gene expression of genes related to Fep system under the Fe^{3+}-chelated condition

Using 200 μM concentration of 2, 2'-dipyridyl (dipi), *S.* Typhimurium LT2 was incubated until cells reached the exponential phase where phage treated and its mRNAs were extracted. The gene *fur* is encoding the expression regulator of this systems. The control group was incubated at fresh LB medium without 2, 2'-dipyridyl for calculating the relative gene expression of each condition. The reference gene was *rpoD.*

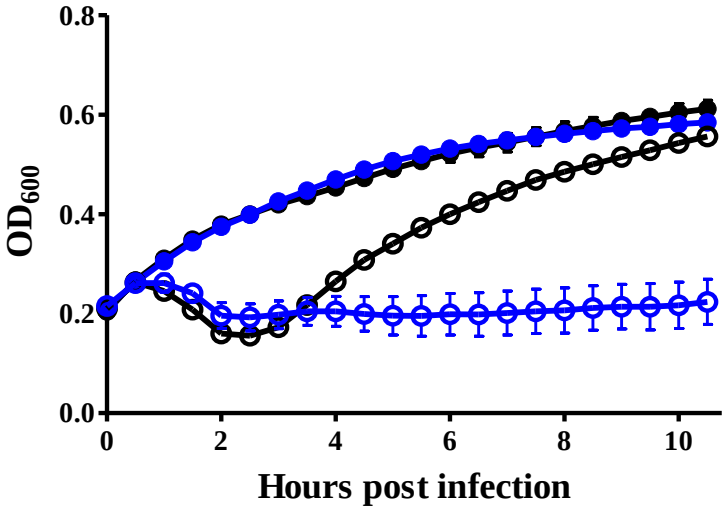

Figure 8. Bacterial challenge assay with the Fe^{3+} chelating agent (2, 2'-dipyridyl)

The optical density OD_{600} of *S.* Typhimurium LT2 was measured every 30 min for 11 hrs. The agent 2, 2'- dipyridyl (black, 0 Mm; blue, 200 μM) was treated in cell cultures with phage SPC32N at the same time. The line contained closed-circle points (●) mean the SPC32N-untreated groups and the lines contained opened-circle points (○) mean SPC32N-treated groups.

3.7 Challenge assay of Δ*fepDGC* and Δ*fhuB* under Fe^{3+}-chelated condition

To confirm the result of bacterial challenge assay with 2, 2'-dipyridyl treatment (Figure 8) is affected by Fep system only, similar experiments with Fhu system, another iron uptake system in *S.* Typhimurium LT2 were performed. Therefore, Δ*fhuB* mutant strain was constructed and similar challenge assay was conducted using deferiprone (Figure 9). *fhuB* encodes FhuB, an inner membrane transporter of Fhu system delivering Fe^{3+} into cytoplasm. Deferiprone, 3-Hydroxy-1, 2-dimethyl-4(1H)-pyridone, is an edible Fe^{3+} chelating agent which was allowed by FDA.

As a result, 1.2 mM of deferiprone significantly suppressed the emergence of phage resistance (Figure 9.A, D). Meanwhile, Δ*fepDGC* showed the same pattern with the deferiprone-untreated control condition, suggesting that phage could not infect the cells of Δ*fepDGC* regardless of deferiprone treatment (Figure 9.B). This result indicates that lack of phage resistance of *S.* Typhimurium LT2 is associated with Fep system rather than Fhu system.

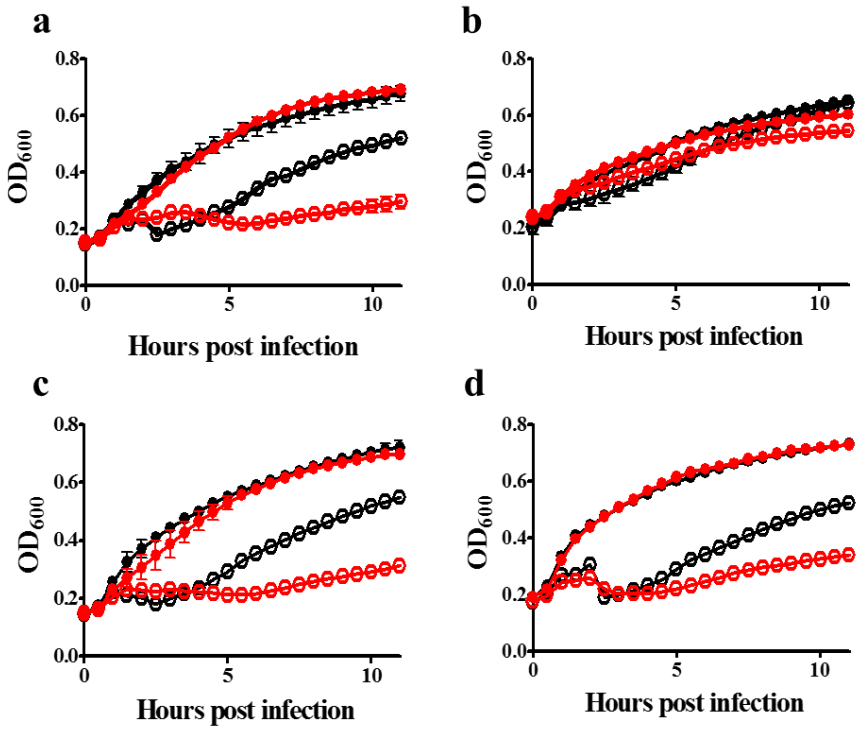

Figure 9. The effect of Fe^{3+} chelating agent, deferiprone to Fe^{3+} uptake systems, Fep and Fhu

1.2 mM deferiprone was treated to each strain culture (a, WT (*S.* Typhimurium LT2); b, Δ*fepDGC*; c, Δ*fepDGC*+p*fepDGC*; d, Δ*fhuB*) with phage SPC32N at the same time. The optical density OD_{600} of *S.* Typhimurium LT2 was measured every 30 min for 11 hrs. The strain Δ*fepDGC*+p*fepDGC* had been induced by 0.002% L-arabinose during incubation. Black, 0 mM deferiprone; Red, 1.2 mM deferiprone; Closed, SPC32N-untreated; Opened, SPC32N-treated.

3.8 The effect of deferiprone to phage biocontrol at foods

To see whether the treatment of Fe^{3+} chelating agents such as deferiprone is applicable in enhancing the phage availability in food industry, food experiments using two types of foods were conducted. The treatment of deferiprone significantly reduced the emergence rate of phage resistance in both food samples, milk and cucumbers (Figure 10) while the control group which treated SPC32N only showed complete recovery of viable cells at 24 hr post infection. Treatment of deferiprone helped the maintenance of 2 log CFU difference at this time point. Statistical analysis confirmed that this difference compared to the control group was significant ($P<0.001$ at milk, $P<0.05$ at cucumbers).

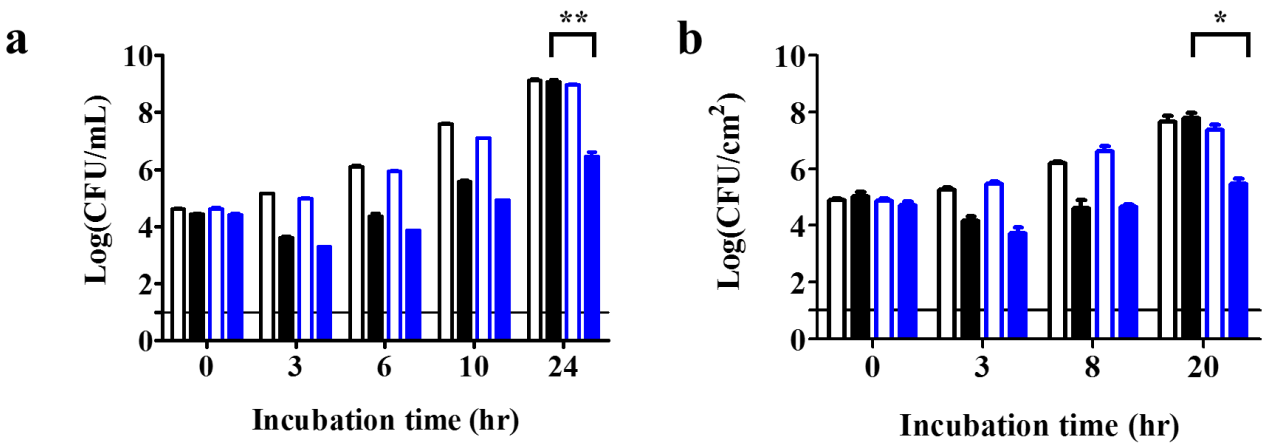

Figure 10. Application of deferiprone and SPC32N to food samples contaminated by *S.* Typhimurium LT2

By treating 0.5 mM (a), 5 mM (b) deferiprone with SPC32N at the same time, the effect of this agent was confirmed. Food samples were milk (a) and cucumbers (b). Milk was incubated with little shaking (100 rpm) and both types were incubated at 25°C. It showed viable bacterial cells at each sampling time point (black, deferiprone-untreated groups; blue, deferiprone-treated groups; empty, SPC32N-untreated groups; filled, SPC32N-treated groups). * means *P*-value<0.05 and ** means *P*-value<0.01.

4 DISCUSSION

To overcome the limitations of phage biocontrol, comprehensive understanding of phages, hosts and their interaction would be needed. Many studies have focused on this theme {Bohm, 2018 #512} {Rousset, 2018 #511} but there are few studies used total RNA sequencing to find important factors in the interaction between *S.* Typhimurium and SPC32N among broad range of genes. Total RNA sequencing in this study could show the responsive pattern of host cells to phage infection at two time points, early- and late-stage of phage infection. As a result, it was speculated that iron uptake system may involve in host-phage interaction because relative expression value of these genes significantly changed during infection. In addition, several DEG groups such as colonic acid biosynthesis, vitamin B_{12} biosynthesis, flagella biosynthesis, *Salmonella* pathogenicity island (SPI)-1 and SPI-2 were also identified, suggesting that studies regarding these DEGs is required to further discover another information about the phage-host interaction.

By qRT-PCR analysis, RNA sequencing results were validated and *fepDGC, fepE, ybdZ* and *entB* was selected as candidate genes for crucial factors in the phage infection. As a result, only Δ*fepDGC* which encodes the inner membrane transporter complex of Fep system showed different infection phenotype compared to other mutant strains in the aspect of infectivity. This

strain became very resistant to SPC32N and this resistance was thought to be the result of decreased adsorption rate. Adsorption rates measured after 15 min of attachment to WT strain, Δ*fepDGC*, and Δ*fepDGC*+p*fepDGC* were 99.6591%, 82.8333%, 98.181%, respectively. Usually, adsorption rate change is known to be related to the modification or mutation of receptors which phage uses at the first stage of infection and the primary receptor of SPC32N is O-antigen of LPS, so adsorption is highly related to this region. Meanwhile, FepDGC is one of inner membrane proteins and FepE, one of Fep system proteins, is already known as the secondary enzyme of LPS modification (especially at variation of LPS length) (Murray, Attridge et al. 2003). However, the O-antigens of LPS, the primary receptor of SPC32N, were not significantly different in WT or mutant strain, suggesting that deletion of *fepDGC* didn't affect the structural variation in LPS. It is possible that Fep system would be a secondary receptor of SPC32N which recognize and access to the surface of host cells. To verify this idea, infectivity of SPC32N against Δ*fepA*, Δ*iroN* and Δ*cirA* mutant strains should be tested because interaction with outer membrane of Fep system was needed in the process. Several tests such as treatment of competitive factors such as gradient concentration of FeEnt complex, overexpression of *fepDGC* were also conducted but any clues could not be shown. There would be more complex interaction between Fep system

and other systems when SPC32N infected to the cells rather than direct cause-and-effect relationship because iron ion is used in multiple systems.

Meanwhile, treatment of Fe^{3+} chelating agents such as 2, 2'-dipyridyl and deferiprone for induction of up-regulated transcription of genes associated with Fep system delayed the emergence of phage significantly and this pattern disappeared at Δ*fepDGC* while at Δ*fhuB*, it did not. *fhuB* encodes FhuB, an inner membrane transporter of Fhu system delivering Fe^{3+} into cytoplasm which has the same function compared to FepDGC in Fep system. So, Fep system is thought to be more associated with this pattern.

This tendency means that one of the limitations of phage biocontrol would be overcome. Host bacteria can regulate modifications of cell surface components which are one of phages receptors within a few generation if they are exposed at phage infection. Thus, just treatment of these agents helps more consistent biocontrol and if they are edible, it could be also applied at food industry. Treatment of 1.2 mM of deferiprone didn't affect bacterial cell's growth but significantly enhanced the biocontrol of SPC32N because 2 log reduction of *S.* Typhimurium was maintained for 24 hrs while cells were fully recovered in groups which treated with phage only. Consequently, the use of more economic, effective and edible Fe^{3+} chelators may have powerful synergistic effect with phage treatment which is applicable in broad range of food industries. Meanwhile, considering the resistance to SPC32N did not

show for 11 hr under the 200 μM 2, 2'-dipyridyl (Figure 8) and 1.2 mM deferiprone (Figure 9.A) at the bacterial challenge assay at LB medium, what the cells became resistant to SPC32N was thought to be due to more complex environment in foods. In addition, at milk, there was weak shaking condition (100 rpm) to mimic the transportation process, so faster recovery of CFU was observed compared to the cucumber condition which was incubated statically.

In conclusion, to control pathogenic bacteria more effectively in food and agricultural industries without the concerns of emergence of super bacteria, phage treatment would be a good alternative method. In this study, using total RNA sequencing method, it was found that iron uptake system may have critical role in phage infection. In addition, it was possible to overcome the most challenging hurdle, the emergence of phage resistance, by treating iron-chelating agent to the food. Therefore, in this study, more consistent and powerful biocontrol method using phage was suggested, advancing the phage treatment as alternative antibiotics.

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국문 초록

박테리오파지의 감염에 대한 숙주 박테리아의 반응 체계를 연구, 이해하는 것은 박테리오파지를 이용하여 식중독균을 제어하는 데에 있어 보다 심화되고 통찰력 있는 해결책을 제시하게 한다. 본 연구에서는 숙주 박테리아가 박테리오파지에 감염되었을 때의 전사체를 추출, 분석을 통하여 해당 조건에서 숙주의 전반적인 유전자 발현 변화를 이해하고자 하였으며 이를 통해 박테리오파지와 숙주 사이의 상호작용에 연관되어 있는 새로운 기능적인 유전자를 찾고자 하였다. 살모넬라 타이피뮤리움 LT2 를 숙주로 하는 용균성 박테리오파지 SPC32N 을 해당 균에 감염시킨 결과 숙주 내 존재하는 Fep 체계와 연관 되어 있는 유전자들이 유의미하게 발현량이 증가하였다. 해당 체계는 3 가 철 이온을 외부로부터 유입하는데 역할을 하며 분석을 통해 걸러진 유전자 중 *fepDGC* 유전자가 다른 유전자에 비해 큰 발현량 변화폭을 보였으므로 해당 유전자를 타겟유전자로 선정하였다. 해당 유전자는 Fep 체계에 속해 있는 세포 내막운송체로 이 유전자를 숙주로부터 제거 (Δ*fepDGC*)한 뒤, SPC32N 을 감염시켜 감염성 표현형을 확인하였다. 그 결과, Δ*fepDGC* 는 해당 박테리오파지로부터 확연하게 저항성이 나타났으며 이는 흡착 과정을 확인해본 결과 Δ*fepDGC* 에서 SPC32N 의 흡착률이 유의미하게 감소하는 것과 연관이 있다고 판단된다.

한편 Fep 체계와 관련된 유전자의 발현량을 전사체분석 결과와 유사하게 임의로 모방하고자 3 가 철 이온을 킬레이트하는 화학물질을 배양하는 과정에서 처리, 해당 물질이 숙주세포가 박테리오파지에 대해 저항성을 갖지 못하게 한다는 것을 결과로 얻었으며 이러한 현상을 임의로 오염시킨 식품에서 관찰하였을 때, 마찬가지로 박테리오파지의 제어능을 향상시키는 것을 확인하였다. 결과적으로 전사체분석을 통해 Fep 체계를 이용하면 박테리오파지의 균제어능을 향상시킬 수 있다는 것을 밝힐 수 있었다.

주요어: 전사체 분석, 박테리오파지-숙주간 상호작용, 철 이온 유입 체계

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