A Thesis for the Degree of Master of Science

Enzyme IIA^{Ntr} Is Associated with Both Regulation of Propionate Metabolism and the Ability of Invasion in *Salmonella enterica* serovar Typhimurium

***Salmonella enterica* serovar Typhimurium**에서 프로피오네이트 대사 조절 및 병원성에 관여하는 **EIIA^{Ntr}** 기능에 관한 연구

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석사학위논문

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ABSTRACT

Salmonella Typhimurium is one of the major food-borne pathogens which can infect a wide range of animals and cause food-borne gastroenteritis in millions of people worldwide. The ability of *Salmonella* to cause disease depends on metabolic activities and various virulence factors. Many Gram-negative bacteria including *Salmonella* have two parallel phosphotransferase systems and these are also known to be associated with bacterial virulence as well as metabolic and regulatory mechanisms. One system is phosphoenolpyruvate:carbohydrate phosphotransferase system (carbohydrate PTS) and the other is nitrogen-related phosphotransferase system (nitrogen-related PTS; PTS^{Ntr}). Enzyme IIA^{Ntr} ($EIIA^{Ntr}$), one component of PTS^{Ntr}, has been reported to show varieties of regulations associated with metabolism of carbon and nitrogen, potassium homeostasis, and virulence of some pathogens. In order to understand roles of $EIIA^{Ntr}$ further, I selected many genes showing noticeable differences of expression levels from RNA-sequencing data of wild-type and a mutant strain lacking *ptsN*. The expression levels of targeted genes are verified by quantitative real-time PCR and β-galactosidase assay. Interestingly, one of the highly down-regulated genes in the *ptsN* mutant was the propionate catabolism operon (*prpBCDE*). Even

though *prpR* encoding regulator protein and *rpoN* encoding sigma factor 54 (σ^{54}) are required for the expression of the propionate operon, the transcriptional levels of *prpR* and *rpoN* were not down-regulated by the *ptsN* mutation. The *hilA* expression was increased but the invasion ability of *Salmonella* was reduced in the *ptsN* mutant under SPI-1 inducing conditions. These results suggest that $EIIA^{Ntr}$ is required for the virulence of *Salmonella* as well as metabolism of alternative carbon source.

Keywords: *Salmonella enterica* serovar Typhimurium, nitrogen-related EIIA ($EIIA^{Ntr}$), propionate metabolism, *Salmonella* virulence

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Ⅰ. INTRODUCTION

Salmonella enterica serovar Typhimurium (here referred to as *Salmonella*) is a Gram-negative, facultative anaerobic, and intracellular pathogen which can infect a wide range of animals and cause food-borne gastroenteritis in millions of people worldwide (1, 2). *Salmonella* pathogenesis and the colonization of host tissues are mediated by a specialized apparatus, named as the type III secretion system (T3SS). *Salmonella* encodes two distinct T3SSs within *Salmonella* pathogenicity islands 1 and 2 (SPI-1 and SPI-2). The SPI-1 system is required for invasion of non-phagocytic cells, whereas the SPI-2 system is expressed within the phagosome for intracellular survival and establishment of systemic disease (1-3).

In the animal intestinal tract, *Salmonella* invasion is mediated by various environmental signals. One of them is short chain fatty acid (SCFA) such as acetate, propionate, and butyrate. Since SCFAs can be present in high concentrations in the animal intestine, many microbes including *Salmonella* have evolved the enzymatic machinery to catabolize these SCFAs. The SCFAs are produced as fermentation products by normal flora, and also show antibacterial activities (4). So some SCFAs such as acetate and propionate are widely used as preservatives of some food products with very high

concentrations (100-300 mM). Interestingly, recent studies demonstrated that propionate repressed the SPI-1 system of *Salmonella*, reducing expression of SPI-1 transcriptional regulators and consequently leading to reduced bacterial penetration of cultured epithelial cells (5, 6). Chien-Che Hung *et al.* examined that the propionyl-CoA, the intermediate of propionate metabolism pathway, destabilizes HilD protein, the apex of the regulatory cascade within SPI-1 (6). These results suggest that *Salmonella* uses propionate as an environmental cue, as well as a source of carbon and energy.

In Proteobacteria, the utilization of glucose and alternative carbon sources is modulated by the phosphoenolpyruvate (PEP)-dependent carbohydrate phosphotransferase system (carbohydrate PTS). The carbohydrate PTS consists of enzyme I (EI), HPr, and Enzyme II (EII) in which PEP provides phosphoryl group (Figure 1). In the end of the cascade, the various sugar-specific EII transporters phosphorylate and uptake their substrates such as monosaccharides, disaccharides, and other sugar derivatives (7). The carbohydrate PTS has important roles in carbohydrate uptake and signaling (7). For example, regulation of secondary carbon sources, named as carbon catabolite repression (CCR) is mediated by $EIIA^{Glc}$ protein of the glucose-specific PTS in enterobacteria (7-9). Briefly, the phosphorylation state of $EIIA^{Glc}$ by the presence of glucose can decide whether or not the utilization

of secondary carbon source is switched on. In *Escherichia coli*, phospho-$EIIA^{Glc}$ can bind and activate adenylyl cyclase (AC), which leads to the synthesis of cyclic AMP (cAMP). Since high cAMP concentrations result in the formation of cAMP-CRP complexes, the promoters of catabolic genes are activated by the binding of cAMP-CRP complex. On the other hand, the presence of glucose results in dephosphorylation of $EIIA^{Glc}$. In its non-phosphorylated form, $EIIA^{Glc}$ binds and inactivates metabolic enzymes (induction prevention) and inhibit transporters of secondary carbon sources (inducer exclusion).

In addition to the carbohydrate PTS, many Proteobacteria have a parallel system named as nitrogen-related phosphotransferase system (nitrogen-related PTS; PTS^{Ntr}) (10, 11). PTS^{Ntr} is composed of Enzyme I^{Ntr} (EI^{Ntr}, encoded by *ptsP*), NPr (encoded by *ptsO*), and Enzyme IIA^{Ntr} ($EIIA^{Ntr}$, encoded by *ptsN*) (Figure 1). Three components are paralogues of EI, HPr and EIIA of the carbohydrate PTS respectively. Unlike the carbohydrate PTS, the final receptor of phosphoryl group has not yet been identified. Since a specific substrate transferred by the PTS^{Ntr} is yet to be determined, PTS^{Ntr} has been investigated in its regulatory functions. Especially, $EIIA^{Ntr}$ encoded by *ptsN* shows its regulatory function by binding to other proteins. In *Escherichia coli*, $EIIA^{Ntr}$ regulates potassium homeostasis using interactions with the potassium

transporter TrkA and sensor kinase KdpD. In *Ralstonia eutropha*, the role of $EIIA^{Ntr}$ is related with the stringent response controlling cellular poly(3-hydroxybutyrate) (PHB) content under conditions of nitrogen deprivation. In *Salmonella* Typhimurium, $EIIA^{Ntr}$ negatively affects the expression of SPI-2 genes by direct interaction with SsrB protein which is master regulator of SPI-2. Since this interaction prevents the SsrB protein from binding to its target promoter, *Salmonella* lacking the $EIIA^{Ntr}$ protein cannot replicate within macrophages. To sum up, the $EIIA^{Ntr}$ protein of nitrogen-related PTS plays regulatory roles in potassium homeostasis, metabolism of carbon and nitrogen, and virulence of pathogens.

To gain insight into the role of $EIIA^{Ntr}$ in *Salmonella* Typhimurim, I analyzed RNA sequencing data presenting trascriptome of wild-type and a mutant *Salmonella* strain lacking *ptsN*. Many genes showing noticeable differences of expression levels were selected and verified by quantitative real-time PCR (qRT-PCR) and β-galactosidase assay. Among them, one of the highly down-regulated genes in the *ptsN* mutant strain was the propionate catabolism operon (*prpBCDE*). Additional β-galactosidase assay demonstrated that the expression levels of important transcriptional factors related with the propionate operon were not influenced by the deletion of *ptsN* gene. Since propionate metabolism is associated with the regulation of

Salmonella invasion through inhibiting SPI-1 regulator, the possibility that the *ptsN* gene may affect invasiveness of *Salmonella* was raised. I show here that the *ptsN* gene influences both the expression of *hilA*, another regulator of SPI-1, and *Salmonella* invasion of epithelial cells.

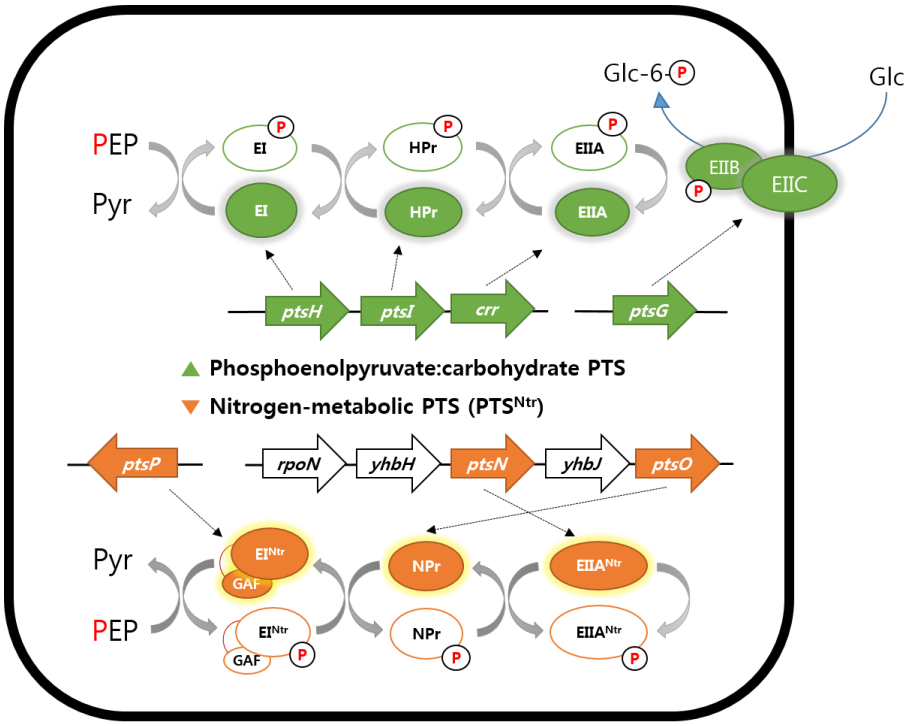


Figure 1. Organization of parallel phosphotransferase systems in *Salmonella* Typhimurium The carbohydrate PTS (top) is presented in many bacteria and dedicated to carbohydrate uptake. Transport is driven by translocation of phosphoryl groups derived from phosphoenolpyruvate (PEP) to the incoming sugar. In addition to the carbohydrate PTS, many Proteobacteria possess the paralogous PTS^{Ntr} (bottom). The final receptor of phosphoryl group has not yet been identified. (Enzyme $I^{(Ntr)}$, $EI^{(Ntr)}$; Enzyme $IIA^{(Ntr)}$, $EIIA^{(Ntr)}$; GAF domain, GAF; Pyruvate, Pyr; Phosphoryl group, P; glucose, Glc; Glucose-6-phosphate, Glc-6-P)

Ⅱ. MATERIALS AND METHODS

2.1. Bacterial strains and growth condition

All strains are isogenic to *Salmonella enterica* serovar Typhimurium SL1344. Cultures were grown aerobically at 37℃ in Luria-Bertani (LB) broth medium (Difco). Anaerobic cultures were incubated overnight standing at 37℃ in LB. Appropriate antibiotics were used in the following concentration if necessary; ampicillin (Ap) 50 μg/ml, kanamycin (Km) 50 μg/ml, and chloramphenicol (Cm) 25 μg/ml. All strains and plasmids used in this study are listed in Table 1.

2.2. Construction of bacterial strains and plasmids

All gene deletions were created using the one-step inactivation method (12). At first, PCR products were generated from the kanamycin resistance gene of pKD13 using primers carrying at their 5' ends 40 base pair (bp) of homology to the regions flanking the start and stop codons of the gene to be deleted . A *Salmonella* strain carrying pKD46 was transformed with the resultant PCR products. Recombinant strains were selected by plating on LB plate containing kanamycin and homologous recombination site was also checked by gel electrophoresis using diagnostic PCR with primer sets of each

genes as listed Table 2. Bacteriophage P22 transduction was used to transfer marked deletions and to create multiple mutations in strains. To create unmarked deletions, the FLP recombinase was used to remove resistance marker using the plasmid pCP20 (12). The strains carrying a *lacZ* fusion to *prpB* were constructed using the plasmid pCE70 into the FRT site (13).

pRPD1 plasmid was constructed by cloning *prpR* gene into pET-28a (Novagen). *prpR* gene was amplified by PCR using primers containing enzyme site of NdeI and SalI. In case of pRPD2, *prpR* gene was amplified by PCR using BamHI-HisTag-prpR-F and HisTag-prpR-ptsI-R primers from pRPD1 and then cloned into pUHE21-2*lacI*^{q} (17).

pFTCC-K plasmid was created by using pZC320 (14) and pCE40 (15) for promoter activity assay. First, promoterless *lacZ* gene was inserted into pZC320 between EcoRI and SalI sites. To change the resistant marker from ampicillin to kanamycin, aminoclyciside phosphotransferase (Km^{R}) was amplified from pCE40 using pCE40-t-his-F-ScaI and pCE40-kan-R-BamHI primers and then cloned into the pZC320 plasmid containing the *lacZ* gene. For constructing pRPD15 plasmid, the promoter region of *prpR* was amplified by PCR using SalI-PprpBCDE-F and PprpBCDE-SalI-R primers. Finally, resulting construct was digested with enzyme SalI and cloned into pFTCC-K. Based on the direction of the promoter region upstream *lacZ* gene, construction

of pRPD15 was determined using Y0464 and prpB-confirm-F primers.

pRPD17 plasmid was constructed by cloning the promoter region of *rpoN* into pRS415 (16). The promoter region was amplified by PCR using the primer set: PrpoN-F-EcoRI and PrpoN-R-BamHI. Plasmid for complementation of the *ptsN* mutant was constructed by cloning the *ptsN* gene into pBAD24 (17). The *ptsN* gene was amplified by PCR using primers pBAD24-ptsN-NcoI-F and pBAD24-ptsN-HindIII-R. Resulted products of *ptsN* was cloned between NcoI and HindIII.

2.3. RNA isolation and qRT-PCR analysis

Salmonella strains were grown in LB medium for 3 hr 30 min. Total RNA was isolated using an RNeasy mini kit (Qiagen). The isolated RNA was treated with DNase and cDNA was synthesized (Clontech). cDNA was used as templete for real-time PCR using 2× iQ SYBR Green Supermix (Bio-rad), and the CFX Connect real-time PCR detection system (Bio-rad) with cycling once at 95 for 5 min followed by 40 cycles at 95℃ for 15 sec, 55℃ for 15 sec, and 72 ℃ for 20 sec. The relative expression of target genes was normalized to that of *gyrB* using Bio-Rad CFX Manager 3.1. The primers used for the detection of genes are listed in Table 2.

2.4. β-galactosidase assay

Salmonella strains were grown in LB medium with or without 30 mM propionic acid and β-galactosidase activity was determined. Detailed description of the procedure can be found in Miller (18). When described briefly, β-galactosidase expressed by the *lacZ* gene can be assayed by measuring hydrolysis of the chromogenic substrate, o-nitrophenyl- β-D-galactoside (ONPG). The end-product, o-nitrophenol, can be measured by determining the absorbance at 420 nm. The reaction can be run using whole cells that have been permeabilized to allow ONPG to enter the cytoplasm. And then the reaction is stopped by adding Na_2CO_3 which shifts the reaction mixture to pH 11. At this pH most of the o-nitrophenol is converted to the yellow colored form and β-galactosidase is inactivated.

2.5. HeLa cell invasion assays

HeLa cells were cultivated in 24-well plates to obtain a monolayer of the cells. Each well was seeded with 2×10^5 cells suspended in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and incubated for 1 day at 37 ℃ under 5% CO_2. Bacterial cells were grown overnight and arabinose was added to the media at a concentration of 0.01%. The wells were washed with PBS before bacterial cells were added. Bacterial

cells were also washed with PBS, diluted in pre-warmed DMEM, and then added to the HeLa cell with a multiplicity of infection of 10. Plate was then centrifuged for 5 min at 500 *g* and incubated for 30 min at 37 ℃ under 5% CO_2. The cells were washed two times with PBS, and the medium was replaced by medium supplemented with 100 μg ml^{-1} gentamicin to kill extracellular bacteria. Cells were incubated for 30 min additionally and washed three times with PBS. The cells were lysed with 1% Triton X-100 for 15 min and then diluted with PBS. A dilution of the suspension was plated on LB agar medium to count colony forming units. Triplicates were tested for each strain under the same condition.

Table 1. Bacterial strains and plasmids used in this study

Strains or plasmids	Relevant characteristics[a]	Source or reference
***Salmonella enterica* serovar Typhimurium**		
SL1344	WT *S. enterica* serovar Typhimurium	ATCC
SR3203	△*ptsN*	(19)
	△*prpB*::*lacZ*	This study
	△*prpB*::*lacZ*, △*ptsN*	This study
	P*hilA*::*lacZ*	This study
	P*hilA*::*lacZ*, △*ptsN*	This study
	P*hilA*::*lacZ*, △*prpR*	This study
Escherichia coli		
DH5α	F-supE44 *hsd*R17 *rec*A1 *gyr*A96 thi-1 *rel*A1	
Plasmids		
pRPD1	pET-28a::*prpR*	This study
pRPD2	pUHE21-2*lacI*^{q}::(His tag-*prpR*)	This study
pRPD15	pFTCC-K::(P*prpR-lacZ*)	This study
pRPD17	pRS415::P*rpoN*	This study
pBD01	pBAD24::*ptsN*	This study
pJJ11	pACYC184::*ptsN*	(19)

pKD13	Ap^R FRT Km^R FRT PS1 PS4 oriR6Kψ	(12)
pKD46	Ap^R; P_{BAD}-gam-beta-exo oriR101	(12)
pCP20	Ap^R; oriR101 *repA101*^{ts}	(12)
pCE70	Km^R; FRT *tnpR lacZY*$^+$; oriR6Kγ	(13)
pCE40	*ahp* FRT '*lacZ lacY*$^+$ t_{his} oriR6K	(15)
pET-28a	Km^R; *lacI*; pBR322 ori	(Novagen)
pACYC184	Cloning vector; Cm^R Tet^R p15A ori	(20)
pUHE21-2*lacI*^{q}	rep_{pMB1} Ap^R *lacI*^{q}	(21)
pZC320	A derivative of F plasmid; ori2; Ap^R	(14)
pFTCC-K	Km^R; promoterless *lacZ* transcriptional fusion plasmid from pZC320	This study
pRS415	Ap^R; *lacZYA*	(16)
pBAD24	P_{BAD}; pBR322 ori; Ap^R	(17)

a. Ap^R, ampicillin resistant; Km^R, kanamycin sesisant; Cm^R, chloramphenicol resistant; Tet^R, tetracycline resistant

Table 2. Primers used for the construction of bacterial strains and plasmids

Primers	DNA sequence from 5' to 3'
pKD13-k2-R	TTG TCA AGA CCG ACC TGT CC
LacZ-R	TGA GGG GAC GAC GAC AGT AT
prpR-lambda-F	TCC GCG CGA TAA TAG CGA TAA ACC GGT GAT CTG GAC GGT CGT GTA GGC TGG AGC TGC TTC
prpR-lambda-R	CAA AGC CGA GTT GAA TAG GCG TGA TGG TCG CCA GAT GAT CCT GTC AAA CAT GAG AAT TAA
prpR-confirm-F	AGG CAA AGA GGC AGC GAG AA
prpR-confirm-R	CAG CGC CTG TAA TAC ATC GA
prpB-lambda-red-R	AAT CCC CAG ATC CGG CAG TCC GAG CGA GCC CGC CGC TAC GCT GTC AAA CAT GAG AAT TAA
prpB-lambda-red-F	CCC AAC GGG CCG GGT ATC AGG CTA TCT ATC TTT CTG GCG GGT GTA GGC TGG AGC TGC TTC
prpB-confirm-F	AAG TCC ATA GCC AAA GCG GG

pCE40-t-his-F-ScaI	AAA AGT ACT ATC CGA CCA AAA AAA CCG GG
pCE40-kan-R-BamHI	AAA GGA TCC AGC CAT GAG AGC TTA GTA CG
pET-28a-prpR-F-NdeI	AAA CAT ATG ACG ACT GCC CAC AGC
pET-28a-prpR-R-SalI	AAA GTC GAC TTA ATT ATC CGA CTG GTC TTT G
pET-28a-seq-F	TCC CGC GAA ATT AAT ACG AC
pET-28a-seq-R	GCT AGT TAT TGC TCA GCG G
BamHI-HisTag-prpR-F	AAA GGA TCC GGC AGC AGC CAT CAT CAT CA
HisTag-prpR-ptsI-R	AAA CTG CAG TTA ATT ATC CGA CTG GTC TTT G
SalI-PprpBCDE-F	TTT GTC GAC CGC AGT AGA TTC ATC TTT AAG
PprpBCDE-SalI-R	TTT GTC GAC ATC TCG TCC TCA TGT TAG TA
Y0464	GGT TAT GTG GAC AAA ATA CCT G
PrpoN-F-EcoRI	AAA GAA TTC GCC GCT AAT CCC

	AAA TTT AT
PrpoN-R-BamHI	AAA GGA TCC AGC GTA GAC AAC TGC AAC AG
pBAD24-ptsN-NcoI-F	AAA CCA TGG TAA ATA ACG ATA CGA CTC
pBAD24-ptsN-HindIII-R	AAA AAG CTT CCA TTG AGT TGT TAT GCC TCA

Table 3. Primers used for qRT-PCR

Primers	DNA sequence from 5’ to 3’
zur-RT-F	GAT TTG CTC GAT TTG CTG CG
zur-RT-R	ATG CAT GAT GTC TTC CAC GC
znuA-RT-F	TTA AAC CGC TTG GGT TCA TC
znuA-RT-R	AAC GGT TTT ACA TCG GCA AG
znuC-RT-F	AAC GTC TCG GTC TCA TTT GG
znuC-RT-R	GTG CTT GAT CAC CCC TTC AT
znuB-RT-F	TGA AGT TAC AGC GCG TGA AG
znuB-RT-R	AGG CTG AGA AGG TCA GTC CA
yodA-RT-F	CTT CTG GGA ATG CTG TTG GT
yodA-RT-R	CTG AAG ACA GGG TCC AGC TC
hha-RT-F	ACC AGG GCC AAT CCT GTT AG
hha-RT-R	CGC TCC CCT TGG TGG ATA TT
ybaJ-RT-F	AAC CAT GGC TGG GTT AAT GA
ybaJ-RT-R	TTG ACG AAA CAA CGG AAC AG
hns-RT-F	AAG TGG AAG AAC GCA CTC GT
hns-RT-R	TTT CAC CGT TTT CGT CAA CA
prpR-RT-F	CGG CTT TAC TTG CCT TTC AG
prpR-RT-R	TGT CAG ACG GGT CAT ATC CA

prpB-RT-F	GGG ATT TCT ACG CTG GAT GA
prpB-RT-R	CCA TCT CCT CTT TCG AGA C
prpD-RT-F	TCC GGT ACA GGC AGC TTT TA
prpD-RT-R	CGA TGC AGC CCT GAA TTT CA
prpC-RT-F	TTC AAC GCC TCG ACG TTT AC
prpC-RT-R	CCA AAG CCA ATC ACC ACC TC
ptsN-RT-F	TTA ACC AGG AGT GTA CGC GT
ptsN-RT-R	CTT CTT CCA GTT TCC CGT GC
gyrB-RT-F	TCA CCG TCA GAT CTA CGA GC
gyrB-RT-R	CCG CCT TCG TAG TGG AAA TG
rpoD-RT-F	CAA TGA CCA TCT GCC GGA AG
rpoD-RT-R	CAT CTT CAT CGG TGC TGG TG
rpoN-RT-F1	GCT GGA ACT TCA GCA GGA AC
rpoN-RT-R1	TCT TCC GGC ATC TCT TTT TG

Ⅲ. RESULTS

3.1. Several genes selected from RNA-sequencing result were confirmed with qRT-PCR.

It has been reported that $EIIA^{Ntr}$ encoded by *ptsN* shows varieties of regulations associated with metabolism of carbon and nitrogen, potassium homeostasis, and virulence of some pathogens. In order to understand roles of $EIIA^{Ntr}$ further, senior researcher analyzed transcriptome of wild-type and a mutant *Salmonella* strain lacking *ptsN* by RNA-sequencing. Strains were grown in LB media and total RNA was extracted at mid-logarithmic phase. Among many genes showing noticeable differences of expression levels, I selected three groups of genes; propionate metabolism, global regulatory factors, and zinc uptake. First, propionate catabolism operon (*prpBCDE*) encodes the enzymes for propionate metabolism which is regulated by activator PrpR. Based on the RNA-sequencing data, expression levels of *prpBCDE* and *prpR* were down-regulated from three-fold to seven-fold compared to wild-type. When these were confirmed with qRT-PCR, expression levels of *prpBCDE* showed corresponding down-regulation by *ptsN* mutation. On the contrary, results of qRT-PCR demonstrated that the expression level of *prpR* was not affected by *ptsN* mutation (Figure 2).

In case of *hha* and *ybaJ*, these genes were induced at least fivefold by

ptsN mutation according to RNA-sequencing data. It has been known that Hha can form a complex with H-NS encoded by *hns* and the complex is able to bind DNA and mediate some genetic regulations (22). However, qRT-PCR showed that the expression of these global regulators was not influenced by *ptsN* mutation (Figure 2). For third group, RNA-sequencing data presented that the expression of *zur*, a regulator of zinc uptake, increased twofold compared to wild-type. When I confirmed several genes involved in zinc uptake such as *yodA* and *znuACB* (23), expression levels between wild-type and the *ptsN* mutant hardly showed any differences (Figure 2).

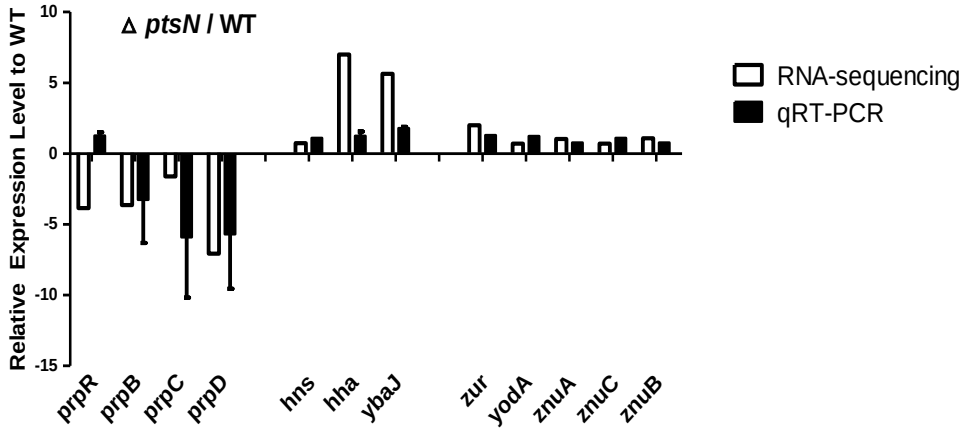


Figure 2. Relative expression levels of selected genes in wild-type and *ptsN* mutant measured by RNA-sequencing and qRT-PCR.

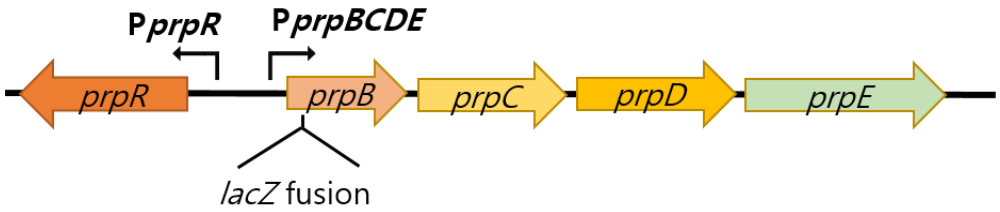
RNA-sequencing was performed in wild-type and a mutant *Salmonella* Typhimurium SL1344 lacking *ptsN*. Total RNA was prepared at mid-log phase in LB media. Three groups of twelve genes presenting noticeable differences of expression levels were selected and verified by qRT-PCR at the same condition.

3.2. The expression levels of propionate catabolism operon (*prpBCDE*) are positively regulated by *ptsN*.

In order to determine the *ptsN* regulation on the propionate catabolism operon (*prpBCDE*), I employed *lacZY* reporter fusions to the operon. In *Escherichia coli* and *Salmonella enterica*, it has been shown that *prpR* and *prpBCDE* have bidirectional promoter region (Figure 3A) (24). For the transcriptional activation of *prpBCDE*, PrpR protein needs 2-methylcitrate as a co-activator which is the intermediate of propionate metabolism (25). *Salmonella* degrades propionate into succinate and pyruvate using 2-methylcitric acid cycle. When *Salmonella* uptake propionate into the cell, propionate is converted to 2-methylcitrate by PrpE and PrpC (25). 2-methylcitrate is degraded to succinate and pyruvate via 2-mehylisocitrate in which PrpD, AcnA/AcnB, and PrpB catalyze each step (26). Even though *prpB* gene is destroyed by *lacZY* reporter fusions, the function of PrpE and PrpC has to be normal to provide the co-activator for the transcriptional activation of *prpBCDE*. When I checked whether *prpBCDE* is induced by 30 mM propionate, the transcriptional expression of *prpBCDE* increased tenfold. In contrast, *prpBCDE* expression in the mutant of *ptsN* was reduced around one third at the same conditions. To demonstrate the effect of *ptsN* deletion, I tested the *ptsN* mutant strain harboring pJJ11 plasmid which encodes *ptsN* gene. As

a result, *prpBCDE* expression in the *ptsN* mutant strain was recovered by pJJ11 plasmid in the presence of 30 mM propionate (Figure 3B). These results show that *ptsN* gene is required for the full activation of the propionate catabolism operon *prpBCDE*.

(A)


P*prpR*
P*prpBCDE*
prpR
prpB
prpC
prpD
prpE
lacZ fusion

(B)

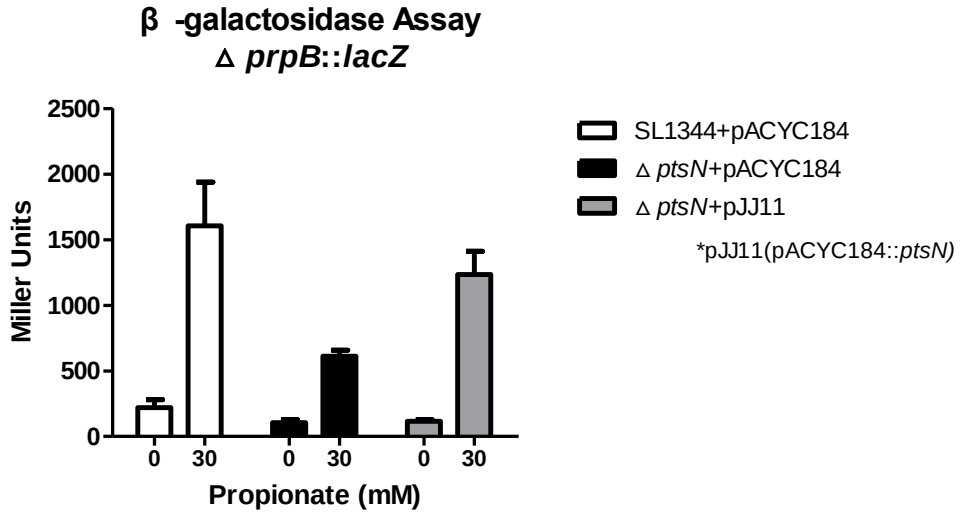

β -galactosidase Assay
Δ *prpB*::*lacZ*
Miller Units
2500
2000
1500
1000
500
0
0
30
0
30
0
30
Propionate (mM)
SL1344+pACYC184
Δ *ptsN*+pACYC184
Δ *ptsN*+pJJ11
*pJJ11(pACYC184::*ptsN)*

Figure 3. Expression levels of the *prpBCDE* operon were determined by β-galactosidase activities expressed from strains harboring a *lacZ* transcriptional fusion to the *prpBCDE* operon.

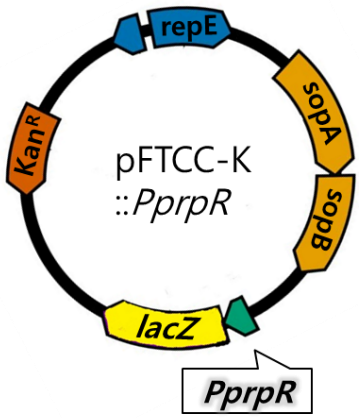
(A) Schematic representation of the propionate operon of *Salmonella* Typhimurium and a *lacZ* fusion (*PprpR*, promoter of *prpR*; *PprpBCDE*, promoter of *prpBCDE*). (B) β-galactosidase activities (Miller units) of the *prpBCDE* were determined in the presence or absence of propionate. Wild-type, *ptsN* mutant strain and *ptsN* mutant strain conveying pJJ11 were incubated in LB media. At mid-logarithmic phase, 30 mM propionate was added to induce the propionate operon.

3.3. The important transcriptional factors of propionate operon, *prpR* and *rpoN*, are not down-regulated by *ptsN*.

It was demonstrated that PrpR, transcription factor sigma-54 (σ^{54}) and IHF protein are essential for the transcription of the *prpBCDE* operon (27). PrpR protein is a σ^{54}–dependent transcriptional activator which belong to the AAA+ superfamily of ATPases (28, 29). Before the transcription starts, σ^{54} binds to specific promoter sequences in which together with core RNA polymerase (RNAP) it forms a closed σ^{54}-RNAP-promoter complex. Activator contacts the σ^{54}-RNAP-promoter complex via DNA looping, which is often aided by IHF proteins and then ATP hydrolysis of the activator AAA+ domain results in an open complex to initiate the transcription (30). When I tested the strains lacking *prpR* or *rpoN*, which encodes σ^{54}, both mutant strains showed no expression of *prpBCDE* (data not shown). Moreover, to understand the effect of *ptsN* on the *prpBCDE* operon I determined the transcription levels of *prpR* and *rpoN* by *ptsN* mutation. First, I constructed the pRPD15 plasmid and pRPD17 which convey the promoter region of *prpR* and *rpoN*, respectively (Figure 4A and Figure 5A). Each plasmid is containing *lacZ* gene downstream the promoter region to measure the transcriptional intensity using β-galactosidase assay. In case of *prpR*, the expression level was low in wild-type strain and *ptsN* mutant strain had the same expression level of *prpR*. When

prpR expression was induced by propionate, there was no expression change in both strains (Figure 4B). The expression level of *rpoN* increased about twofold in the *ptsN* mutant strain (Figure 5B). However, it is difficult to explain the decreased expression of *prpBCDE* by *ptsN* mutation since *rpoN* has positive regulation on *prpBCDE*. Taken together, even though *prpR* and *rpoN* are important for the *prpBCDE* expression, their expressions are not down-regulated by the deletion of *ptsN* gene.

A


repE
sopA
sopB
lacZ
Kan^R
pFTCC-K
::PprpR
PprpR

B

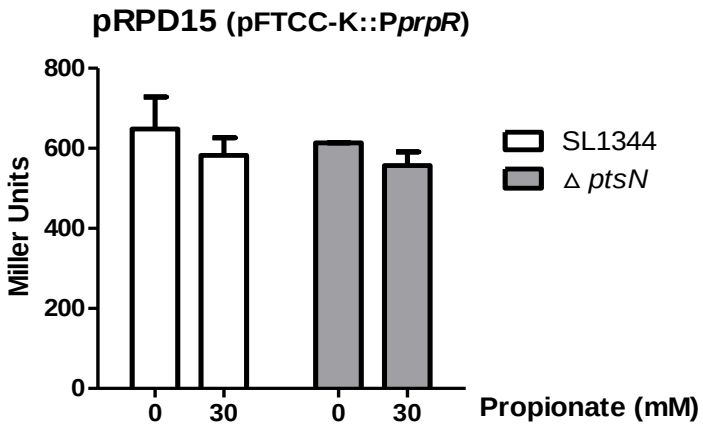
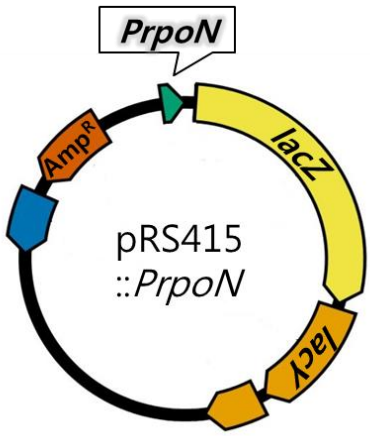

pRPD15 (pFTCC-K::PprpR)
800
600
400
200
0
Miller Units
0
30
0
30
Propionate (mM)
SL1344
Δ ptsN

Figure 4. The expression levels of *prpR* in wild-type and the *ptsN* mutant strain were examined by β-galactosidase assay. (A) Schematic representation of pRPD15, a derivative of F plasmid pZC320 expressing *lacZ* gene downstream of the *prpR*-directional promoter region (*PprpR*). (B) Wild-type and the *ptsN* mutant strain were incubated in LB media. At mid-logarithmic phase, 30 mM propionate was added or not. Transcriptional levels of the *prpR* gene were determined in the wild-type and △*ptsN* strain carrying pRPD15.

A


PrpoN
Amp^R
lacZ
pRS415
::PrpoN
lacY

B

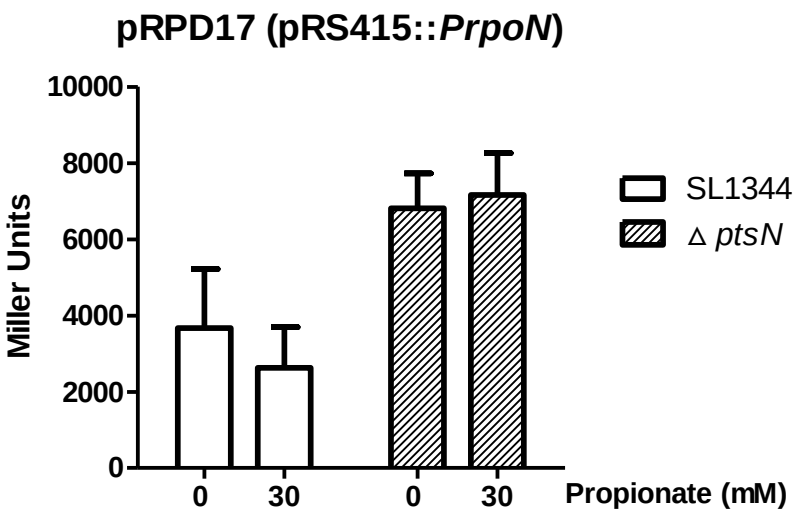

pRPD17 (pRS415::PrpoN)
Miller Units
10000
8000
6000
4000
2000
0
0
30
0
30
Propionate (mM)
SL1344
Δ ptsN

Figure 5. The expression levels of *rpoN* in wild-type and the *ptsN* mutant strain were examined by β-galactosidase assay. (A) Schematic representation of pPRD17 harboring the *rpoN* promoter region (*PrpoN*) upstream *lacZYA* gene. (B) Wild-type and the *ptsN* mutant strain were incubated in LB media. At mid-logarithmic phase, 30 mM propionate was added or not. β-galactosidase activities of pRPD17 plasmid were determined in both wild-type and *ptsN* mutant strain.

3.4. Both *prpR* and *ptsN* influence expression of *hilA*, the central regulator of *Salmonella* invasion.

Propionate is one of the major SCFA produced as fermentation end products by intestinal microflora (31, 32). SCFA are known to have various effects upon the host, and especially can inhibit the growth of some bacterial species (4). Furthermore, major SCFA have been investigated to affect *Salmonella* invasion in complex ways (6). *Salmonella* invasion is mediated by the gene encoded within *Salmonella* Pathogenicity Island 1 (SPI-1) and further study demonstrated that the intermediate of propionate metabolism represses HilD protein, the central regulator of SPI-1, post-translationally (6). To better understand the effect of the *ptsN* gene on propionate metabolism, I tested the SPI-1 regulator *hilA* which is regulated by HilD downstream of the invasion regulatory cascade. Invasion ability of *Salmonella* is known to be induced by certain environmental and bacterial growth conditions such as low oxygen tension, high osmolarity, slightly alkaline pH, and exponential phase growth (33-36). To provide low oxygen tension and exponential phase growth, overnight standing culture was adopted to induce SPI-1 regulations. As expected, *prpR* mutation made *hilA* expression increase compared with wild-type under static culture condition (Figure 6). The deletion of the *ptsN* gene also showed the same effect on *hilA* expression. Using pBAD24 plasmid

encoding the *ptsN* gene (pBD01), I confirmed that *hilA* expression in the *ptsN* mutant strain was recovered. Interestingly, *hilA* expression in the *prpR* mutant strain was also recovered by pBD01 plasmid. Therefore, these results imply that *hilA* expression is modulated by both propionate metabolism and the *ptsN* gene.

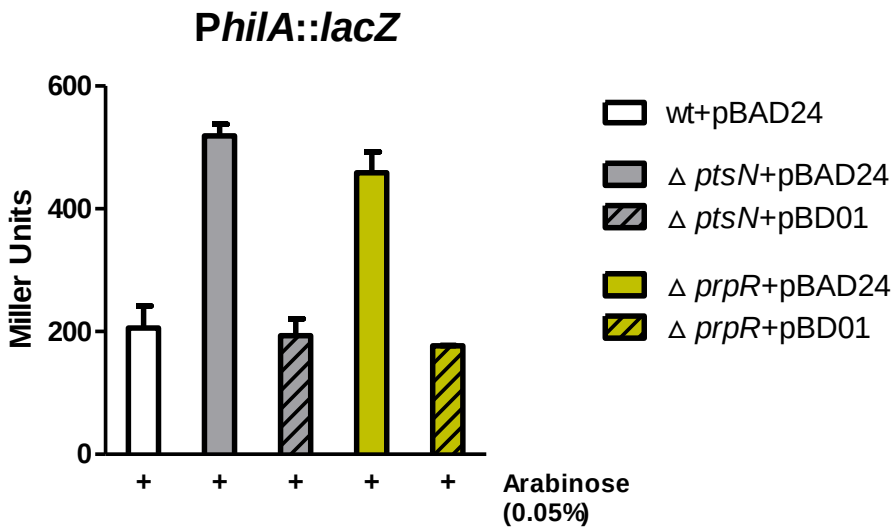


Figure 6. The *hilA* expression is regulated by *prpR* and *ptsN*. Transcriptional levels of the *hilA* were determined in the wild-type, *ptsN* mutant strain and *prpR* mutant strain harboring a backbone plasmid pBAD24 or the *ptsN* expression vector pBAD24::*ptsN* (pBD01). β-galactosidase activities were measured after 12 hr of incubation at LB media, 37℃, and static condition. In order to induce P_{BAD} promoter of arabinose operon, arabinose was used at a concentration of 0.05%.

3.5. The *ptsN* gene is required for displaying the intact ability of *Salmonella* invasion.

HilA is center for SPI-1 transcriptional regulation because this protein activates directly the invasion genes encoded within SPI-1, as well as invasion genes found at other chromosomal loci (34). Based on the fact that *hilA* expression increases in the *ptsN* mutant strain, I tested the ability of *Salmonella* invasion of HeLa cell model (37). I found that the deletion of *ptsN* gene significantly decreased invasion of cultured HeLa cells about threefold (Figure 7). This phenotypic defect of the *ptsN* mutant was due to the lack of $EIIA^{Ntr}$ because expression of the *ptsN* gene from pBAD24 plasmid (pBD01) enabled the mutant strain to recover its invasiveness (Figure 7). Therefore, these data suggest that the *ptsN* gene can affect the ability of *Salmonella* invasion under the standing culture condition.

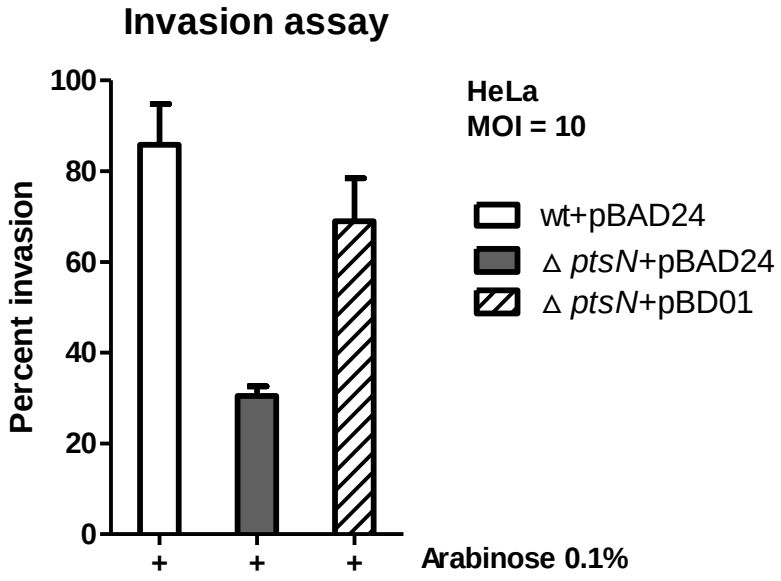


Figure 7. The *ptsN* gene is required for *Salmonella* invasion. Deletion of the *ptsN* gene decreases *Salmonella* invasion of HeLa cells. The wild-type and *ptsN* deletion strains carrying the empty plasmid pBAD24 or the *ptsN* expression vector (pBD01) were grown in LB media statically at 37℃. Arabinose was added to the media in order to induce P_{BAD} promoter of pBAD24 plasmid. Invasion is shown relative to the wild-type harboring the empty plasmid, which was set to 100%.

Ⅳ. DISCUSSION

Previous research demonstrated that $EIIA^{Ntr}$ encoded by *ptsN* has many regulatory roles which are related with metabolism of carbon and nitrogen, potassium homeostasis, and virulence of pathogen (19, 38-41). In order to understand roles of $EIIA^{Ntr}$ further, from transcriptome analysis of wild-type and a mutant strain lacking *ptsN* I selected three groups of genes showing noticeable differences of expression levels: the propionate metabolism, global regulator, and zinc uptake. When I examined the transcriptional levels using qRT-PCR, expression levels of the propionate catabolism operon showed consistent results with RNA-sequencing although other targeted genes did not (Figure 2). Propionate can be present in high concentrations in the animal intestine (6). *Salmonella* degrades propionate into pyruvate via 2-methylcitric acid cycle when the environment lacks preferred carbon sources. Components of 2-methylcitric acid cycle are encoded by the *prpBCDE* operon whose expression is regulated by PrpR protein (27). I confirmed that the expression level of regulator *prpR* was not affect by the *ptsN* mutation using qRT-PCR and β-galactosidase assay (Figure 2 and Figure 4B). Furthermore, σ^{54} transcriptional factor encoded by *rpoN* is also involved in the regulation of the propionate operon (27). σ^{54} is widely distributed protein

in bacteria and utilized in many different physiological mechanisms such as nitrogen assimilation, utilization of alternative carbon sources, assembly of motility organs, and production of extracellular alginate (42). Interestingly, *rpoN* operon comprises *rpoN, yhbH, ptsN, yhbJ and ptsO,* which are co-transcribed from a single promoter upstream of *rpoN* (11). In the *ptsN* mutant strain, some stimuli to induce the transcription of *ptsN* gene may increase. This feasible process can explain why *rpoN* expression also increased in the *ptsN* mutant strain (Figure 5B). Nevertheless, the expression of *prpBCDE* was down-regulated by the *ptsN* mutation (Figure 3B). These results imply that the regulatory effect of *ptsN* gene on the expression of *prpBCDE* is mediated not by *prpR* and *rpoN* at the transcriptional level but by unrevealed mechanisms. Additionally, the interaction between $EIIA^{Ntr}$ and PrpR was examined by the bacterial two-hybrid system (43) since $EIIA^{Ntr}$ carrries out its regulatory role through protein-protein interaction. The result indicated that there is no interaction between $EIIA^{Ntr}$ and PrpR (data not shown). In addition to these transcriptional factors, the integration host factor (IHF) and cAMP-CRP complex are also involved in regulations of the propionate operon (24, 27). Further studies for the relation between the *ptsN* gene and other transcriptional factors remain to be progressed.

It has been shown that the levels of SsrB-regulated gene transcription increase abnormally in the *ptsN* mutant under SPI-2-inducing conditions, consequently attenuating *Salmonella* survival in macrophages (19). Similarly, in the *ptsN* mutant, the transcriptional level of *hilA* increased under SPI-1-inducing conditions whereas invasion activated by HilA protein was repressed (Figure 6, 7). When expression levels of *hilA* were examined under stationary phase with aerobic culture, there was no remarkable difference between wild-type and mutant strain lacking *ptsN* (data not shown). These results suggest that low oxygen tension may be related with the *ptsN* effect on *hilA* expression. On the other hand, *Salmonella* invasion was repressed in the *ptsN* mutant strain (Figure 7) even though HilA protein is a crucial activator for the invasive phenotype (34). Similar results were also observed and discussed on the previous studies (44, 45). T. F. Fahlen *et al.* discussed that strains showing very high levels of *hilA* expression have growth defects based on their observations (45). In my experiments, however, *ptsN* mutant strain did not show noticeable growth defects compared to wild-type at both aerobic and standing culture conditions. Jennifer D. Boddicker and Bradley D. Jones reported that the expression SPI-1 genes is down-regulated after invasion for the intracellular survival of bacteria (44). So excessive expression of *hilA* genes in the *ptsN* mutant may result in co-expression of SPI-1 and SPI-2 after invasion,

consequently preventing the intracellular survival of bacteria. Taken together, $EIIA^{Ntr}$ of nitrogen-related PTS has regulatory functions on *Salmonella* virulence through modulating expression of genes related with SPI-1.

In conclusion, $EIIA^{Ntr}$ encoded by *ptsN* gene, one component of nitrogen-related PTS, influences on propionate metabolism and the ability of invasion. Since propionate is one of the alternative carbon sources present in high concentrations in the animal intestine, this study provides the evidence that the function of $EIIA^{Ntr}$ may important role in both carbon and nitrogen metabolism. Moreover, the effect of the *ptsN* gene on *Salmonella* invasion implies the connection between the utilization of carbon sources and bacterial virulence. To understand the function of $EIIA^{Ntr}$ exactly, further study has to be performed about each regulatory mechanism.

Ⅴ. REFERENCES

1. Ibarra JA & Steele-Mortimer O (2009) *Salmonella*–the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. *Cellular microbiology* 11(11):1579-1586.

2. Haraga A, Ohlson MB, & Miller SI (2008) *Salmonellae* interplay with host cells. *Nature Reviews Microbiology* 6(1):53-66.

3. Coburn B, Grassl GA, & Finlay B (2006) *Salmonella*, the host and disease: a brief review. *Immunology and cell biology* 85(2):112-118.

4. Kwon Y, Park S, Birkhold S, & Ricke S (2000) Induction of Resistance of *Salmonella* Typhimurium to Environmental Stresses by Exposure to Short-Chain Fatty Acids. *Journal of food science* 65(6):1037-1040.

5. Lawhon SD, Maurer R, Suyemoto M, & Altier C (2002) Intestinal short-chain fatty acids alter *Salmonella* Typhimurium invasion gene expression and virulence through BarA/SirA. *Molecular microbiology* 46(5):1451-1464.

6. Hung CC, *et al.* (2013) The intestinal fatty acid propionate inhibits *Salmonella* invasion through the post-translational control of HilD. *Molecular microbiology* 87(5):1045-1060.

7. Deutscher J, Francke C, & Postma PW (2006) How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. *Microbiology and Molecular Biology Reviews* 70(4):939-1031.

8. Görke B & Stülke J (2008) Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. *Nature Reviews Microbiology* 6(8):613-624.

9. Park Y-H, Lee BR, Seok Y-J, & Peterkofsky A (2006) In vitro reconstitution of catabolite repression in *Escherichia coli*. *Journal of Biological Chemistry* 281(10):6448-6454.

10. Peterkofsky A, Wang G, & Seok Y-J (2006) Parallel PTS systems.

Archives of biochemistry and biophysics 453(1):101-107.

11. Pflüger-Grau K & Görke B (2010) Regulatory roles of the bacterial nitrogen-related phosphotransferase system. *Trends in microbiology* 18(5):205-214.

12. Datsenko KA & Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences* 97(12):6640-6645.

13. Merighi M, Ellermeier CD, Slauch JM, & Gunn JS (2005) Resolvase-in vivo expression technology analysis of the *Salmonella enterica* serovar Typhimurium PhoP and PmrA regulons in BALB/c mice. *Journal of bacteriology* 187(21):7407-7416.

14. Shi J & Biek DP (1995) A versatile low-copy-number cloning vector derived from plasmid F. *Gene* 164(1):55-58.

15. Ellermeier CD, Janakiraman A, & Slauch JM (2002) Construction of targeted single copy *lac* fusions using λ Red and FLP-mediated site-specific recombination in bacteria. *Gene* 290(1):153-161.

16. Simons R, Houman F, & Kleckner N (1987) Improved single and multicopy lac-based cloning vectors for protein and operon fusions. *Gene* 53(1):85-96.

17. Guzman L-M, Belin D, Carson MJ, & Beckwith J (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. *Journal of bacteriology* 177(14):4121-4130.

18. Miller J (1993) A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. *Trends in Biochemical Sciences-Library Compendium* 18:193.

19. Choi J, *et al.* (2010) *Salmonella* pathogenicity island 2 expression negatively controlled by $EIIA^{Ntr}$–SsrB interaction is required for *Salmonella* virulence. *Proceedings of the National Academy of Sciences* 107(47):20506-20511.

20. Chang AC & Cohen SN (1978) Construction and characterization of

amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. *Journal of bacteriology* 134(3):1141-1156.

21. Soncini FC, Véscovi EG, & Groisman EA (1995) Transcriptional autoregulation of the *Salmonella* Typhimurium *phoPQ* operon. *Journal of bacteriology* 177(15):4364-4371.

22. Olekhnovich IN & Kadner RJ (2007) Role of nucleoid-associated proteins Hha and H-NS in expression of *Salmonella enterica* activators HilD, HilC, and RtsA required for cell invasion. *Journal of bacteriology* 189(19):6882-6890.

23. Ilari A, *et al.* (2014) The *Salmonella enterica* ZinT structure, zinc affinity and interaction with the high-affinity uptake protein ZnuA provide insight into the management of periplasmic zinc. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1840(1):535-544.

24. Lee SK, Newman JD, & Keasling JD (2005) Catabolite repression of the propionate catabolic genes in *Escherichia coli* and *Salmonella enterica*: evidence for involvement of the cyclic AMP receptor protein. *Journal of bacteriology* 187(8):2793-2800.

25. Palacios S & Escalante-Semerena JC (2004) 2-Methylcitrate-dependent activation of the propionate catabolic operon (*prpBCDE*) of *Salmonella enterica* by the PrpR protein. *Microbiology* 150(11):3877-3887.

26. Palacios S, Starai VJ, & Escalante-Semerena JC (2003) Propionyl coenzyme A is a common intermediate in the 1, 2-propanediol and propionate catabolic pathways needed for expression of the *prpBCDE* operon during growth of *Salmonella enterica* on 1, 2-propanediol. *Journal of bacteriology* 185(9):2802-2810.

27. Palacios S & Escalante-Semerena JC (2000) *prpR, ntrA, and ihf* functions are required for expression of the *prpBCDE* operon, encoding enzymes that catabolize propionate in *Salmonella enterica* serovar Typhimurium LT2. *Journal of bacteriology* 182(4):905-910.

28. Horswill AR & Escalante-Semerena JC (1997) Propionate catabolism in *Salmonella* Typhimurium LT2: two divergently transcribed units comprise the *prp* locus at 8.5 centisomes, *prpR* encodes a member of

the sigma-54 family of activators, and the *prpBCDE* genes constitute an operon. *Journal of bacteriology* 179(3):928-940.

29. Zhang X, *et al.* (2002) Mechanochemical ATPases and transcriptional activation. *Molecular microbiology* 45(4):895-903.

30. Schumacher J, Joly N, Rappas M, Zhang X, & Buck M (2006) Structures and organisation of AAA+ enhancer binding proteins in transcriptional activation. *Journal of structural biology* 156(1):190-199.

31. Louis P, Scott K, Duncan S, & Flint H (2007) Understanding the effects of diet on bacterial metabolism in the large intestine. *Journal of applied microbiology* 102(5):1197-1208.

32. Hooper LV, Midtvedt T, & Gordon JI (2002) How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annual review of nutrition* 22(1):283-307.

33. Bajaj V, Lucas RL, Hwang C, & Lee CA (1996) Co-ordinate regulation of *Salmonella* Typhimurium invasion genes by environmental and regulatory factors is mediated by control of *hilA* expression. *Molecular microbiology* 22(4):703-714.

34. Jones BD (2005) *Salmonella* invasion gene regulation: a story of environmental awareness. *J. Microbiol* 43:110-117.

35. Lee CA & Falkow S (1990) The ability of *Salmonella* to enter mammalian cells is affected by bacterial growth state. *Proceedings of the National Academy of Sciences* 87(11):4304-4308.

36. Rodriguez CR, Schechter LM, & Lee CA (2002) Detection and characterization of the *S.* Typhimurium HilA protein. *BMC microbiology* 2(1):31.

37. Giannella RA, Washington O, Gemski P, & Formal SB (1973) Invasion of HeLa cells by *Salmonella* Typhimurium: a model for study of invasiveness of *Salmonella*. *Journal of Infectious Diseases* 128(1):69-75.

38. Lee CR, *et al.* (2013) Reciprocal regulation of the autophosphorylation

of enzyme I^{Ntr} by glutamine and α-ketoglutarate in *Escherichia coli*. *Molecular microbiology* 88(3):473-485.

39. Lee C-R, Cho S-H, Yoon M-J, Peterkofsky A, & Seok Y-J (2007) *Escherichia coli* enzyme IIA^{Ntr} regulates the K+ transporter TrkA. *Proceedings of the National Academy of Sciences* 104(10):4124-4129.

40. Lüttmann D, Göpel Y, & Görke B (2012) The phosphotransferase protein $EIIA^{Ntr}$ modulates the phosphate starvation response through interaction with histidine kinase PhoR in *Escherichia coli*. *Molecular microbiology* 86(1):96-110.

41. Lüttmann D, *et al.* (2009) Stimulation of the potassium sensor KdpD kinase activity by interaction with the phosphotransferase protein IIA^{Ntr} in *Escherichia coli*. *Molecular microbiology* 72(4):978-994.

42. Ö sterberg S, Peso-Santos Td, & Shingler V (2011) Regulation of alternative sigma factor use. *Annual review of microbiology* 65:37-55.

43. Battesti A & Bouveret E (2012) The bacterial two-hybrid system based on adenylate cyclase reconstitution in *Escherichia coli*. *Methods* 58(4):325-334.

44. Boddicker JD & Jones BD (2004) Lon protease activity causes down-regulation of *Salmonella* pathogenicity island 1 invasion gene expression after infection of epithelial cells. *Infection and immunity* 72(4):2002-2013.

45. Fahlen TF, Mathur N, & Jones BD (2000) Identification and characterization of mutants with increased expression of *hilA*, the invasion gene transcriptional activator of *Salmonella* Typhimurium. *FEMS Immunology & Medical Microbiology* 28(1):25-35.

국문 초록

살모넬라 티피뮤리움(*Salmonella* Typhimurium)은 주요 식중독 원인균 중 하나로서 다양한 동물을 감염시킬 수 있을 뿐만 아니라 세계적으로 매년 수백만 명의 사람에게서 식중독을 유발한다. 식중독 유발은 살모넬라의 대사 활성과 여러 가지 독성 요소에 기인한다고 알려져 있다. 살모넬라를 포함한 많은 그람 음성균은 대사 및 조절 메커니즘에 관여하는 두 종류의 시스템을 갖고 있는데, 하나는 탄소대사 PTS(phosphoenolpyruvate:carbohydrate phosphotransferase system; carbohydrate PTS)이며 다른 하나는 질소대사 PTS(nitrogen-related phosphotransferase system; PTS^{Ntr})이다. 그 중에서 질소대사 PTS를 구성하고 있는 Enzyme IIA^{Ntr}($EIIA^{Ntr}$) 단백질은 탄소 및 질소대사, 칼륨 항상성 조절, 병원성에 걸쳐 다양한 조절 메커니즘을 지닌 것으로 알려져 있다.

이에 본 연구에서는 $EIIA^{Ntr}$를 생산하는 *ptsN* 유전자가 결여된 균주의 전사체와 정상 균주 (wild-type)의 전사체를 비교 분석하였다. 유전자 전사에서 큰 차이를 보이는 후보 유전자군을 선별하여 추가 분석한 결과 프로피오네이트(propionate) 대사 조절

오페론의 발현이 *ptsN* 유전자 돌연변이체에서 정상 균주보다 감소하는 것으로 나타났다. *ptsN* 유전자가 결여된 균주에서 프로피오네이트 대사 조절 오페론의 상위 조절자인 *prpR* 유전자와 *rpoN* 유전자의 발현은 감소하지 않는 것으로 나타났다. 한편 살모넬라의 병원성과 관련된 *Salmonella* Pathogenicity Island-1(SPI-1)을 유도하는 조건에서 실험하였을 때, *ptsN* 유전자가 결여된 균주에서 SPI-1의 중요한 조절자를 생산하는 *hilA* 유전자의 발현이 증가하였고, 이 균주의 숙주세포 감염 능력은 저하되었다. 이러한 실험결과들은 $EIIA^{Ntr}$ 단백질이 대체 에너지원인 프로피오네이트의 대사뿐만 아니라 살모넬라의 병원성에도 관여하고 있음을 시사하고 있다.

주요어: 살모넬라 티피뮤리움(*Salmonella* Typhimurium), 질소 대사 PTS(nitrogen-related PTS; PTS^{Ntr}), 프로피오네이트 대사 조절(propionate metabolism), 살모넬라 병원성(virulence)

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