Int J Clin Exp Med 2018;11(8):8075-8084
www.ijcem.com /ISSN:1940-5901/IJCEM0069519

Original Article

The effect of OmpR-EnvZ on metabolite profile of *Salmonella* Typhimurium: a preliminary investigation

Tian Tang[1], Haojiang Zuo[1], Chuan Wang[1], Peibing Zeng[1], Qun Gao[2], Bo Zheng[1], Guoqing Wang[1], Xiaofang Pei[1]

[1]*Department of Sanitary Inspection and Quarantine, West China School of Public Health, Sichuan University, Chengdu, P.R. China;* [2]*Institute of Preventive Veterinary Medicine, Sichuan Agricultural University, Wenjiang, Chengdu, Sichuan 611130, P.R. China*

Received November 21, 2017; Accepted June 29, 2018; Epub August 15, 2018; Published August 30, 2018

Abstract: The OmpR-EnvZ system which consists of a histidine kinase EnvZ and a response regulator OmpR is a global regulator in most gram-negative bacteria. In *Salmonella* Typhimurium (*S.* Typhimurium), OmpR-EnvZ directs the expression of hundreds of genes that are associated with virulence, porin formation, flagellum assembly and curli biosynthesis. In a previous report, we have shown that *ompR* inactivation promotes the growth rate of *S.* Typhimurium *in vitro*. This unusual phenomenon prompts us to investigate the role of OmpR-EnvZ in cell metabolism. By performing gas chromatography (GC) coupled with mass spectrometry (MS), we analyzed the extracellular and intracellular metabolite profiles of a *S.* Typhimurium wild-type (TT-1) strain and an *ompR* deletion mutant (TT-13). A total of 35 extracellular and 34 intracellular substances were identified in both strains, wherein, the levels of 16 extracellular compounds including L-leucine, L-methionine, L-aspartic acid, L-ornithine, L-phenylalanine, L-glutamic acid, L-lysine, L-tryptophan, glycolic acid, butanedioic acid, malic acid, phosphoric acid, citric acid, decanedioic acid, glycylglycine, myo-inositol and uridine were changed in an *ompR* mutant (TT-13) ($P<0.05$). Besides, *ompR* disruption also affects the intracellular biochemical reaction and results in accumulation or overconsumption of 11 endogenous substances, such as L-threonine, D-arabinose, glyceric acid, hexanedioic acid, ethanolamine, uracil, 6-hydroxypurine, serine, citric acid, cadaverine and putrescine. These evidences suggest a great impact of OmpR-EnvZ system on metabolite profile of *S.* Typhimurium.

Keywords: *Salmonella*, OmpR-EnvZ, metabolism

Introduction

In the long term of evolution process, bacteria have developed a set of delicate signal perception and transduction mechanism to deal with the changing environment. The most successful strategy is the two-component regulatory system (TCS) which endows these unicellular organisms to sense and respond to the environmental cues outside the cells [1]. Currently, over 4000 TCS have been found in 145 bacterial genomes, indicating a great impact of these systems on adaptive response [2].

As a TCS, OmpR/EnvZ has been found in virtually every gram-negative bacterium and involves in varieties of physiological process. EnvZ is a histidine kinase, can auto-phosphorylate itself under several environmental signals, such as the fluctuation of osmotic pressure and reduction of pH [3, 4]. OmpR is a response regulator which can be activated by the phosphorylated EnvZ [5, 6]. Once being activated, OmpR upregulates or downregulates the transcription 329 genes in *S.* Typhimurium [7]. Some of those are located in *Salmonella* pathogenicity island I (SPI1), such as *hilC* and *hilD* [8]. SPI1 encodes a type III secretion system (T3SS) which is required for colonization and invasiveness of intestinal epithelium [9, 10]. OmpR also modulates the function of *Salmonella* pathogenicity island 2 (SPI2) by regulating the expression of SsrA/SsrB in mRNA level [11, 12]. SsrA/SsrB is also a TCS which facilitates the intracellular proliferation and systemic infection of *S.* Typhimurium [13-15]. Other genes regulated by the OmpR/EnvZ includes the porin-encoding genes *ompF* and *ompC* [16], the curli biosynthe-

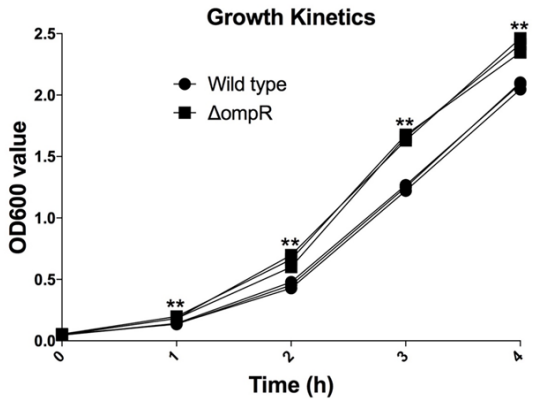

Figure 1. The *in vitro* growth kinetics measurement over a period of 4 h. Overnight cultures of *Salmonella* wild-type strain (TT-1) or the ompR mutant (TT-13) were sub-cultured in fresh LB broth. At hourly intervals, 1 ml sample was taken for OD measurement. Differences analysis between groups were determined using one-way ANOVA and unpaired *t* tests. P<0.05 (**) was considered as significant difference.

sis related gene cluster *csgDEFG* [17] as well as the tripeptide permease encoding gene *tppB* [18].

In a previous study, we showed that the *S.* Typhimurium *ompR* mutant presents a growth advantage over the wild-type strain [19]. This unusual phenomenon prompts us to hypothesize a role of OmpR/EnvZ in cell metabolism. In the current study, we compared the metabolite profile of a *S.* Typhimurium wild-type strain (TT-1) with that of an *ompR* mutant (TT-13) by using gas chromatography coupled with mass spectrometry (GC-MS). The data suggest that the OmpR/EnvZ severely affects the cell metabolism of *S.* Typhimurium, as indicated by the significant differences on levels of 16 extra- and 11 intracellular compounds between the wild-type strain and the *ompR* mutant.

Materials and methods

Media and chemicals

Tryptone, yeast extract and agar were purchased from Oxoid (Basingstoke, Hampshire, UK). *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA), methanol, hexane, and adonitol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pyridine was purchased from J&K Scientific (Beijing, China). Other chemicals were obtained from Aladdin (Shanghai, China). All chemicals used were of chromatography grade.

Bacterial strains

S. Typhimurium wild-type strain TT-1 and the *ompR* deletion mutant TT-13 (Δ*ompR* TT-1) used in this study were described previously [20]. Cells were routinely cultured in Lennox broth (LB) (Trptone 10 g/l, Yeast Extract 5 g/l, NaCl 5 g/l). To prepare samples for compounds extraction, overnight culture of TT-1 (wild-type) or TT-13 (Δ*ompR* TT-1) was sub-inoculated 1:100 in 25 mL of fresh LB and incubated at 37°C at 180 rev/min. When the OD600 value reached at 0.6 (mid-logarithmic phase), culture supernatants and cell pellets were separated by centrifugation at 7500 rev/min at 0°C for 5 min, then subjected to metabolites extraction, respectively.

In vitro growth kinetics measurement

Overnight culture of *S.* Typhimurium wild-type strain (TT-1) and the *ompR* mutant (TT-13) were inoculated 1:100 in 10 ml of fresh LB broth, followed by incubation at 37°C at 180 rev/min. At hourly intervals, 1 ml culture was taken for OD measurement. The experiment was performed in triplicate.

Metabolites extraction and GC-MS analysis

The culture supernatants of the test strains were designated as the extracellular metabolites. The compounds within the cells were designated as the intracellular metabolites. Both extra- and intracellular metabolites as well as the medium control (LB) were prepared as described previously [20]. Samples were stored at -80°C before use. Prior to GC-MS analysis, a batch of samples were treated with 80 μl of methoxyamine hydrochloride (20 mg/mL in pyridine) at 37°C for 90 min, then supplemented with 80 μl of MSTFA and incubated at 60°C for 60 min. An aliquot of 100 μl derivatives diluted with 900 μl of hexane was analyzed on an Agilent 7890A GC coupled with 5975C quadrupole mass detector (Palo Alto, CA, USA). DB-5MS capillary column (J&W Scientific, Folsom, CA) (30 m × 0.25 mm × 0.25 μm) was used to separate the components. The inlet temperature of the GC was 230°C. The ion source and interface temperature of the MS were 230°C and 290°C, respectively. 1 μl of sample was injected splitlessly. Helium was used as carrier gas with a constant flow rate of 1.3 ml/min. The GC oven temperature was held at 70°C for the initial 3 min, then raised

Table 1. The fold change of metabolites detected in extracellular extracts of S. Typhimurium wild-type strain (TT-1) and the *ompR* mutant (TT-13)

Compounds	Presence or absence			Fold change
	LB Control	TT-13	TT-1	TT-13/TT-1
Amino acids				
L-Alanine	√	√	√	
L-Valine	√	√	√	
L-Leucine	√	√	√	↓5.15
L-Isoleucine	√	√	√	
Glycine	√	√	√	
Serine	√	√	√	
L-Threonine	√	√	√	
L-Methionine	√	√	√	↑1.08
L-Aspartic acid	√	√	√	↑1.24
L-Histidine	√	√	√	
L-Ornithine	√	√	√	↑1.33
L-Phenylalanine	√	√	√	↑1.15
L-Glutamic acid	√	√	√	↓1.81
Asparagine	√	√	√	
L-Lysine	√	√	√	↑1.07
L-Tyrosine	√	√	√	
L-Tryptophan	√	√	√	↑1.48
Sugar or sugar derivatives				
D-Gluconic acid	√	×	×	
D-Trehalose	√	√	√	
Glucopyranose	√	×	×	
Mannitol	√	×	×	
Organic acids				
Lactic acid	√	√	√	
Glycolic acid	√	×	×	
Butanedioic acid	√	√	√	↑1.08
Glyceric acid	√	√	√	
Butanoic acid	√	√	√	
2-Butenedioic acid	√	√	√	
Malic acid	√	√	√	↑1.12
4-Aminobutanoic acid	√	√	√	
Phosphoric acid	√	√	√	↑1.54
Citric acid	√	√	√	↑1.25
Decanedioic acid	√	√	√	↑1.20
Polyamines and polyols				
Ethanolamine	√	√	√	
Glycylglycine	√	√	√	↑1.34
Glycerol	√	√	√	
Uracil	√	√	√	
Myo-inositol	√	√	√	↑1.15
Uridine	√	√	√	↑4.69
Urea	×	√	√	

√represents compounds that are detected; ×means compounds that are not detected. The fold change values only present the levels of compounds in TT-13 that are significantly different from that in TT-1 ($P<0.05$). The up arrow means increase; the down arrow means decrease.

with a gradient of 2°C/min until it reached 280°C, where it is held for 3 min. The scanning range of the MS was set at m/z 50-500. The retention time and mass spectral profile of each chromatogram peak were compared with those in NIST14 (NIST, MD, USA) and Golm metabolome database [21]. The GC peaks were collected when their areas were more than 50,000. The compound was identified when its matching value to a known standard in the database was more than 800. To allow comparison between groups, we normalized all data to the internal standard. The raw data of GC-MS was subject to an Automated Mass Deconvolution Identification System (AMDIS) for deconvolution.

RNA isolation, cDNA synthesis and quantitative real-time polymerase chain reaction analysis (qRT-PCR)

To investigate the relative expression of genes that are associated with compounds metabolism, 250 μl overnight cultures of TT-1 (wild-type) and TT-13 (Δ*ompR* TT-1) were individually seeded in 25 ml of fresh LB and incubated at 37°C at 180 rev/min. Cells were harvested by centrifugation when the OD600 reached at 0.6. RNA isolation and cDNA synthesis were performed using a commercial bacterial total RNA extraction kit (Product ID: DP430) (Tiangen, Beijing, China) and a first-strand cDNA synthesis kit (Product ID: KR-116) (Tiangen, Beijing, China), respectively. The cDNA samples containing three biological replicates each from an independent culture of an *ompR* mutant (TT-13) or a wild-type strain (TT-1) were then subject to quantitative real-time polymerase chain reaction (qRT-PCR) analysis. The target genes of qRT-PCR were *udK*, *speC*, *gltA*, *pduP*, *eutB*, *cadA* and *acnB*. The qRT-PCRs were performed on a CFX96 real-time PCR instrument (Bio-Rad). Thermal cycling conditions were as follows: 15 min incubation at 95°C for pre-denatur-

Table 2. The fold change of metabolites detected in intracellular extracts of S. Typhimurium wild-type strain (TT-1) and the *ompR* mutant (TT-13)

Compounds	Presence or absence		Fold change
	TT-13	TT-1	TT-13/TT-1
Amino acids			
L-Alanine	√	√	
L-Valine	√	√	
L-Isoleucine	√	√	
Glycine	√	√	
Serine	√	√	↓1.38
L-Threonine	√	√	↑1.40
L-Methionine	√	√	
L-Aspartic acid	√	√	
L-Phenylalanine	√	√	
L-Glutamic acid	√	√	
L-Lysine	√	√	
L-Tyrosine	√	√	
L-Tryptophan	√	√	
Sugars or sugar derivatives			
D-Trehalose	√	√	
D-Arabinose	√	√	↑2.74
D-Ribofuranose	√	√	
Organic acids			
Lactic acid	√	√	
Butanedioic acid	√	√	
Glyceric acid	√	√	↑2.32
2-Butenedioic acid	√	√	
Malic acid	√	√	
Hexanedioic acid	√	√	↑1.82
Phosphoric acid	√	√	
Citric acid	√	√	↓4.09
Terephthalic acid	√	√	
Polyamines and polyols			
Ethanolamine	√	√	↑1.39
Glycerol	√	√	
Uracil	√	√	↑2.13
Myo-inositol	√	√	
Urea	√	√	
Cadaverine	√	√	↓2.01
Putrescine	√	√	↓2.06
Phosphorylethanolamine	√	√	
6-Hydroxypurine	√	√	↑3.53

√represents the compounds that are detected. The fold change values only presents the levels of compounds in TT-13 that are significantly different from that in TT-1 ($P<0.05$). The up arrow means increase; the down arrow means decrease.

ation and activation of the polymerase, followed by denaturation 40 cycles of 3 s at 95°C and 32 s annealing plus elongation at 60°C. The fluorescence intensity was recorded at each elongation step. The specificity of the PCR products was validated via dissociation curve. The relative change in mRNA level of a target gene was calculated using the $2^{-\Delta\Delta CT}$ method [22], and normalized against the transcript levels of the housekeeping genes *gyrB*, *rpoD* and *gmk*, respectively. The PCR primers used are listed in supplemented materials (Table S1).

Statistical analysis

Both the extracellular metabolome data (four biological replicates each from a single colony) and intracellular data (six biological replicates each from a single colony) were analyzed with unpaired *t* test at 95% confidence intervals by using IBM SPSS statistical software (IBM, UY, USA). The heat maps with hierarchical clustering were generated using the Heml Heatmap Illustrator software (Wuhan, Hubei, China).

Results

The in vitro growth kinetics of the wild-type S. Typhimurium strain and the ompR mutant

We have shown that *ompR* disruption promote the growth of S. Typhimurium *in vitro* [20]. Such conclusion is obtained when cells of the *ompR* mutant are cultured in trypticase soy broth (TSB). As TSB is a nutrient-rich medium, we sought to reinvestigate the growth kinetics of S. Typhimurium *ompR* mutant (TT-13) in LB, a routinely culture medium, to exclude the impact of culture medium to cell growth. The S. Typhimurium *ompR* mutant (TT-13) still presented a growth advantage comparing the wild-type stain in LB, as indicated by a higher OD value at each sampling time point (**Figure 1**), suggesting that the effect of *ompR* disruption on cell growth of S. Typhimurium is irrelevant to the culture medium.

Metabolite profiling

By performing GC-MS, 38, 35 and 34 compounds were identified in medium control, extra- and intracellular extracts, respectively (**Tables 1** and **2**). Urea was not detected in

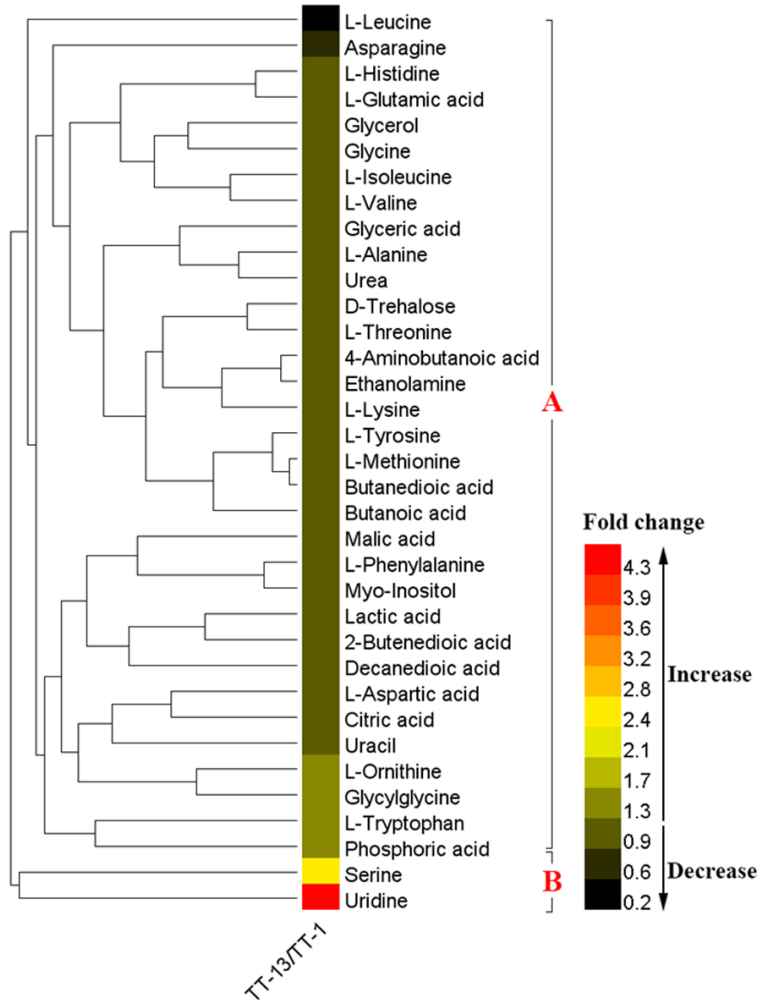

Figure 2. Clustering heat map of the extracellular metabolites. Colors of red and black represent increase and decrease of a metabolite in the absence of *ompR*, respectively (see color key). The dendrogram for metabolite clustering is shown on the left of the heat map. Letters A and B showing on the right of the heat map represent cluster A and cluster B, respectively.

medium control but shown in extracellular extracts, indicating that urea is a secreted compound and not a medium component. D-arabinose, D-ribofuranose, hexanedioic acid, terephthalic acid, cadaverine, putrescine, phosphorylethanolamine and 6-hydroxypurine were detected only in endogenous specimens, suggesting that these compounds were bacterial metabolites.

To visualize the change of metabolite fingerprint between the wild-type S. Typhimurium strain (TT-1) and the *ompR* mutant (TT-13), two clustering heat maps were generated to arrange the extra- and intracellular metabolites based on their fold change between strains (TT-13/TT-1). The levels of most extra- and intracellular metabolites detected in TT-13 (Δ*ompR* TT-1) were changed in comparison with that in the wild-type strain (TT-1), and are shown by different color keys in **Figures 2** and **3**. Two major clusters, namely, A and B, showing on the right of each heat map represents the compounds whose levels are decreased and increased in the *ompR* mutant (TT-13), respectively (**Figures 2** and **3**). The fold change value >1 means a compound whose level is elevated in the *ompR* mutant (TT-13), otherwise is reduced.

The statistical difference of each compound between the wild-type (TT-1) and the *ompR* mutant (TT-13) was determined by unpaired *t* test. The levels of 16 extracellular compounds including L-leucine, L-methionine, L-aspartic acid, L-ornithine, L-phenylalanine, L-glutamic acid, L-lysine, L-tryptophan, butanedioic acid, malic acid, phosphoric acid, citric acid, decanedioic acid, glycylglycine, myo-inositol and uridine were changed between strains ($P<0.05$), wherein, 14 compounds enhanced and 2 compounds declined in the absence of *ompR* (**Table 1**). In regards to the endogenous metabolome, the levels of 11 compounds showed statistical differences between strains ($P<0.05$) (**Table 2**). The levels of L-threonine, D-arabinose, glyceric acid, hexanedioic acid, ethanolamine, uracil and 6-hydroxypurine increased in TT-13 (Δ*ompR* TT-1), whereas, serine, citric acid, cadaverine and putrescine decreased.

qRT-PCR analysis

The mRNA levels of 7 genes between the wild-type S. Typhimurium strain (TT-1) and the *ompR* mutant (TT-13), including *udK*, *speC*,

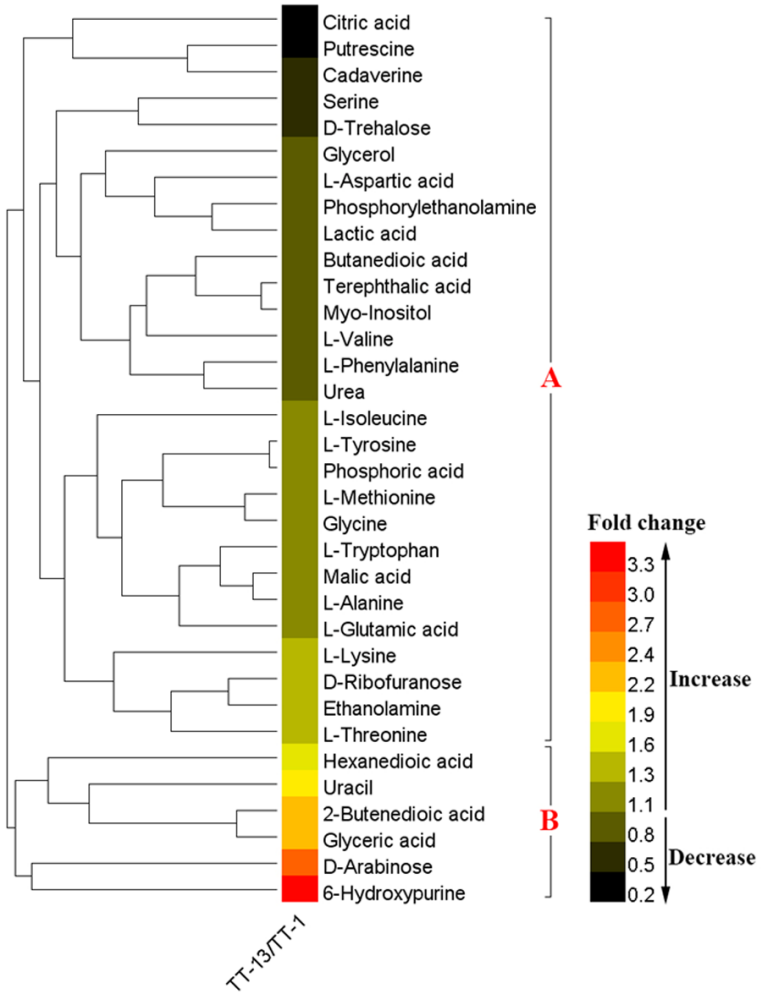

Figure 3. Clustering heat map of the intracellular metabolites. Colors of red and black represent increase and decrease of a metabolite in the absence of *ompR*, respectively (see color key). The dendrogram for metabolite clustering is shown on the left of the heat map. Letters A and B showing on the right of the heat map represent cluster A and cluster B, respectively.

mutant (TT-13), since TSB is a nutrient-rich medium. In the present study, we used LB, a routinely culture medium, to reinvestigate the growth kinetics of the *ompR* mutant (TT-13). The data excluded the effect of culture medium on cell growth, because the *ompR* mutant (TT-13) still presented a growth advantage over the wild-type strain (TT-1) when growing in LB medium (**Figure 1**). This result not only highlights the effect of OmpR-EnvZ on cell growth per se, but also leads us to hypothesize a role of OmpR-EnvZ in cell metabolism of S. *Typhimurium*. By performing GC-MS, we compared the metabolite profile of a *Salmonella* wild-type strain (TT-1) with that of an *ompR* mutant (TT-13). GC-MS was chosen because of its high separation efficiency and capability to detect hundreds of small molecules in a single analysis [23, 24]. After GC-MS, 35 and 34 compounds were individually detected in extra- and intracellular extracts, wherein, 16/35 extracellular and 11/34 intracellular compounds were statistically changed in the absence of *ompR* (**Tables 1** and **2**).

gltA, *pduP*, *eutB*, *cadA* and *acnB*, were determined by *qRT-PCR*. The fold change value >1.5 or <0.5 presents the transcriptional level a gene which is upregulated or downregulated in the absence of *ompR*. As shown in **Figure 4**, *ompR* disruption dramatically impaired the expression of *udk*, *speC* and *gltA*, but promoted the expression of *eutB* and *cadA*.

Discussion

We have shown that inactivation of *ompR* promotes the growth rate of S. Typhimurium *in vitro* [20]. Such conclusion is obtained when cells are cultured in TSB, we therefore suspect that the culture medium may play a potential role in accelerating the growth of the *ompR*

To explain the variance caused by *ompR* disruption, we measured the transcriptional level of several genes that are associated with the metabolism of certain compounds. The uridine kinase, encoded by *udK*, plays an important role in uridine transport [25, 26]. We suggested that disruption of *ompR* significantly diminished the expression of *udK* (**Figure 4**). Such decline in mRNA level may reduce the production of uridine kinase and repress the transport of uridine. Consistent with this point, the level of extracellular uridine in the *ompR* mutant (TT-13) was 4-fold higher than that in the wild-type strain (**Table 1**). In addition, the uptake of L-leucine was increased in the *ompR* mutant (TT-13). The Facultative anaerobic bacteria such as *E. coli* possesses three different leu-

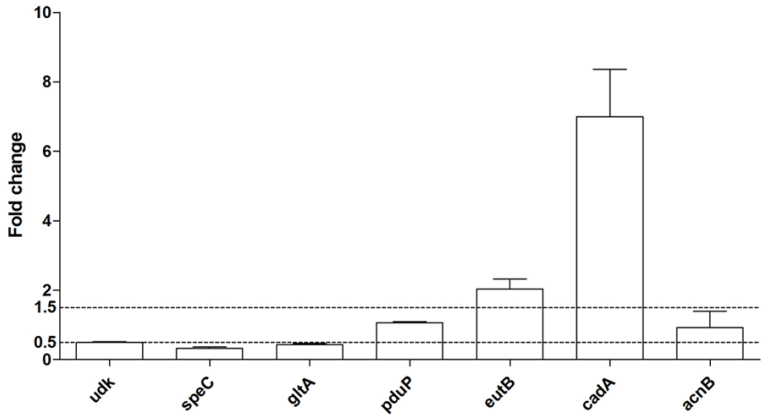

Figure 4. The relative expression of target genes in the *ompR* mutant (TT-13) versus that in the wild-type strain (TT-1). The effect of *ompR* disruption on target genes expression were monitored in independent experiments using total mRNA of three independent biological samples. The fold change value >1.5 and <0.5 represent a target gene that are upregulated and downregulated in the absence of *ompR*, respectively.

cine transport systems, known as LIV-I, LIV-II and L-systems. The former two systems facilitate the transport of all branch amino acids, while the L-system transports leucine exclusively [27]. The *ompR* disruption probably affects the absorption of L-luecine via activating the L-system. The levels of L-isoleucine and L-valine barely changed in the *ompR* mutant (TT-13) supports this idea, because such compounds can be transported by either the LIV-I or the LIV-II system rather than the L-system.

As an important source of carbon and nitrogen, ethanolamine can be utilized by *S*. Typhimurium in the presence of vitamin B_{12} [28, 29]. The ethanolamine ammonia lyase and acetaldehyde dehydrogenase, encoded by *eutBC* and *pduP*, respectively [30, 31], are two essential enzymes which in turn hydrolyze ethanolamine to produce acetyl coenzyme [29]. We observed that the expression of *eutB* was increased in the absence of *ompR* (**Figure 4**). Such shift in mRNA level is supposed to result in more production of ethanolamine ammonia lyase and overconsumption of ethanolamine. The accumulation of ethanolamine in the *ompR* mutant (TT-13) suggest that a translational or post-translational regulation of OmpR-EnvZ on ethanolamine catabolism may exist. It has been demonstrated that the succinate dehydrogenase genes *sdhCDA* are upregulated in the absence of *ompR* [32]. We showed that *ompR* disruption did not alter the level of succinate but the level of citric acid (**Table 2**). As an intermediate in the tricarboxylic acid (TCA) cycle, the turnover of citric acid depends on two critical enzymes, known as citrate synthase and aconitase. The citrate synthase, a pace-making enzyme in the TCA cycle, encoded by *gltA* [33], is able to catalyze the condensation of acetyl coenzyme A and oxaloacetate to form citric acid [34]. While the aconitase, encoded by *acnB* [35], can catalyze the reversible isomerization of citric acid to isocitric acid via *cis*-aconitate [36]. The down-regulation of *gltA* and the reduction of citric acid in the *ompR* mutant (TT-13) indicate that *ompR* inactivation affects the turnover of citric acid by repressing the transcription of *gltA* (**Table 2** and **Figure 4**).

Putrescine are found in virtually every living cell, and associated with multiple functions such as the biosynthesis of siderophores [37], acid resistance [38] and cellular differentiation [39]. In bacteria, putrescine primarily forms via the decarboxylation of L-arginine or/and L-ornithine [40]. In this study, a more than 2-fold reduction of putrescine level was observed in the *ompR* mutant (TT-13). We conclude that *ompR* disruption inhibits the metabolism of L-ornithine by reducing the production of ornithine decarboxylase. Because, the *speC* gene (encoding the ornithine decarboxylase [41]) was downregulated in the absence of *ompR* (**Figure 4**).

Conclusion

Although the effect of OmpR-EnvZ on regulation of gene expression has been extensively studied, its role in regulation of cell metabolism, especially the anabolism and catabolism of small organic molecules, remains to be elucidated. In the current investigation, we compared the metabolome of a *S*. Typhimurium wild-type strain with that of an *ompR* mutant. The data suggest that *ompR* disruption affects the transport of 16 compounds, including L-leucine, L-methionine, L-aspartic acid, L-ornithine, L-phenylalanine, L-glutamic acid,

L-lysine, L-tryptophan, butanedioic acid, malic acid, phosphoric acid, citric acid, decanedioic acid, glycylglycine, myo-inositol and uridine. Besides, *ompR* inactivation also affects the intracellular biochemical reaction and results in accumulation or overconsumption of 11 substances, such as L-threonine, D-arabinose, glyceric acid, hexanedioic acid, ethanolamine, uracil, 6-hydroxypurine, serine, citric acid, cadaverine and putrescine. These evidences shed lights on a broad effect of OmpR-EnvZ on cell metabolism of S. Typhimurium.

Acknowledgements

This research was supported by National Mega Projects of Science and Technology in 13th Five-Year Plan of China: Technical Platform for Communicable Disease Surveillance (No. 2017ZX10103010-002), Sichuan Science & Technology Department Foundation (No. 2017-JY0240), China Postdoctoral Science Foundation (No. 2016M602691) and Young Scholar Research Grants of Sichuan University (No. 2016SCU11005).

Disclosure of conflict of interest

None.

Address correspondence to: Xiaofang Pei, Department of Sanitary Inspection and Quarantine, West China School of Public Health, Sichuan University, 16#, Section 3, South Renmin Road, Chengdu 610031, P.R. China. Tel: +86 02885503679; E-mail: xxpei@scu.edu.cn

References

[1] Wuichet K, Cantwell BJ and Zhulin IB. Evolution and phyletic distribution of two-component signal transduction systems. Curr Opin Microbiol 2010; 13: 219-225.

[2] Ulrich LE, Koonin EV and Zhulin IB. One-component systems dominate signal transduction in prokaryotes. Trends Microbiol 2005; 13: 52-56.

[3] Kenney LJ, Bauer MD and Silhavy TJ. Phosphorylation-dependent conformational changes in OmpR, an osmoregulatory DNA-binding protein of Escherichia coli. Proc Natl Acad Sci U S A 1995; 92: 8866-8870.

[4] Bang IS, Kim BH, Foster JW and Park YK. OmpR regulates the stationary-phase acid tolerance response of Salmonella enterica serovar typhimurium. J Bacteriol 2000; 182: 2245-2252.

[5] Delgado J, Forst S, Harlocker S and Inouye M. Identification of a phosphorylation site and functional analysis of conserved aspartic acid residues of OmpR, a transcriptional activator for ompF and ompC in Escherichia coli. Mol Microbiol 1993; 10: 1037-1047.

[6] Roberts DL, Bennett DW and Forst SA. Identification of the site of phosphorylation on the osmosensor, EnvZ, of Escherichia coli. J Biol Chem 1994; 269: 8728-8733.

[7] Perkins TT, Davies MR, Klemm EJ, Rowley G, Wileman T, James K, Keane T, Maskell D, Hinton JC, Dougan G and Kingsley RA. ChIP-seq and transcriptome analysis of the OmpR regulon of Salmonella enterica serovars typhi and Typhimurium reveals accessory genes implicated in host colonization. Mol Microbiol 2013; 87: 526-538.

[8] Cameron AD, Dorman CJ. A fundamental regulatory mechanism operating through OmpR and DNA topology controls expression of Salmonella pathogenicity islands SPI-1 and SPI-2. PLoS Genet 2012; 8: e1002615.

[9] Hapfelmeier S, Stecher B, Barthel M, Kremer M, Muller AJ, Heikenwalder M, Stallmach T, Hensel M, Pfeffer K, Akira S and Hardt WD. The Salmonella pathogenicity island (SPI)-2 and SPI-1 type III secretion systems allow Salmonella serovar typhimurium to trigger colitis via MyD88-dependent and MyD88-independent mechanisms. J Immunol 2005; 174: 1675-1685.

[10] Lostroh CP and Lee CA. The Salmonella pathogenicity island-1 type III secretion system. Microbes Infect 2001; 3: 1281-1291.

[11] Lee AK, Detweiler CS and Falkow S. OmpR regulates the two-component system SsrA-ssrB in Salmonella pathogenicity island 2. J Bacteriol 2000; 182: 771-781.

[12] Garmendia J, Beuzon CR, Ruiz-Albert J and Holden DW. The roles of SsrA-SsrB and OmpR-EnvZ in the regulation of genes encoding the Salmonella typhimurium SPI-2 type III secretion system. Microbiology 2003; 149: 2385-2396.

[13] Hensel M, Shea JE, Waterman SR, Mundy R, Nikolaus T, Banks G, Vazquez-Torres A, Gleeson C, Fang FC and Holden DW. Genes encoding putative effector proteins of the type III secretion system of Salmonella pathogenicity island 2 are required for bacterial virulence and proliferation in macrophages. Mol Microbiol 1998; 30: 163-174.

[14] Shea JE, Hensel M, Gleeson C and Holden DW. Identification of a virulence locus encoding a second type III secretion system in Salmonella typhimurium. Proc Natl Acad Sci U S A 1996; 93: 2593-2597.

[15] Hensel M. Salmonella pathogenicity island 2. Mol Microbiol 2000; 36: 1015-1023.

[16] Chatfield SN, Dorman CJ, Hayward C and Dougan G. Role of ompR-dependent genes in Salmonella Typhimurium virulence: mutants deficient in both ompC and ompF are attenuated in vivo. Infect Immun 1991; 59: 449-452.
[17] Prigent-Combaret C, Brombacher E, Vidal O, Ambert A, Lejeune P, Landini P and Dorel C. Complex regulatory network controls initial adhesion and biofilm formation in Escherichia coli via regulation of the csgD gene. J Bacteriol 2001; 183: 7213-7223.
[18] Gibson MM, Ellis EM, Graeme-Cook KA and Higgins CF. OmpR and EnvZ are pleiotropic regulatory proteins: positive regulation of the tripeptide permease (tppB) of Salmonella typhimurium. Mol Gen Genet 1987; 207: 120-129.
[19] Tang T, Gao Q, Barrow P, Wang M, Cheng A, Jia R, Zhu D, Chen S, Liu M, Sun K, Yang Q and Chen X. Development and evaluation of live attenuated Salmonella vaccines in newly hatched duckings. Vaccine 2015; 33: 5564-5571.
[20] Tang T, Gao Q, Lin H, Biville F, Xiong J, Pei X, Zheng B, Zou X and Wang C. ClpXP affects the cell metabolism of Salmonella typhimurium partially in an RpoS-dependent manner. Metabolomics 2017; 13: 157.
[21] Hummel J, Selbig J, Walther D and Kopka J. The golm metabolome database: a database for GC-MS based metabolite profiling. In: editors. Metabolomics. Springer; 2007. pp. 75-95.
[22] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 2001; 25: 402-408.
[23] Smart KF, Aggio RB, Van Houtte JR and Villas-Boas SG. Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography-mass spectrometry. Nat Protoc 2010; 5: 1709-1729.
[24] Villas-Boas SG, Mas S, Akesson M, Smedsgaard J and Nielsen J. Mass spectrometry in metabolome analysis. Mass Spectrom Rev 2005; 24: 613-646.
[25] Mygind B and Munch P. Transport of pyrimidine nucleosides in cells of Escherichia coli K 12. Eur J Biochem 1975; 59: 365-372.
[26] Bremer H and Yuan D. Uridine transport and incorporation into nucleic acids in Escherichia coli. Biochim Biophys Acta 1968; 169: 21-34.
[27] Rahmanian M, Claus DR and Oxender DL. Multiplicity of leucine transport systems in Escherichia coli K-12. J Bacteriol 1973; 116: 1258-1266.
[28] Brinsmade SR, Paldon T and Escalante-Semerena JC. Minimal functions and physiological conditions required for growth of salmonella enterica on ethanolamine in the absence of the metabolosome. J Bacteriol 2005; 187: 8039-8046.
[29] Roof DM and Roth JR. Ethanolamine utilization in Salmonella typhimurium. J Bacteriol 1988; 170: 3855-3863.
[30] Faust L, Connor JA, Roof DM, Hoch JA and Babior BM. Cloning, sequencing, and expression of the genes encoding the adenosylcobalamin-dependent ethanolamine ammonia-lyase of Salmonella typhimurium. J Biol Chem 1990; 265: 12462-12466.
[31] McClelland M, Sanderson KE, Spieth J, Clifton SW, Latreille P, Courtney L, Porwollik S, Ali J, Dante M, Du F, Hou S, Layman D, Leonard S, Nguyen C, Scott K, Holmes A, Grewal N, Mulvaney E, Ryan E, Sun H, Florea L, Miller W, Stoneking T, Nhan M, Waterston R and Wilson RK. Complete genome sequence of Salmonella enterica serovar Typhimurium LT2. Nature 2001; 413: 852-856.
[32] Perkins TT, Davies MR, Klemm EJ, Rowley G, Wileman T, James K, Keane T, Maskell D, Hinton JC, Dougan G and Kingsley RA. ChIP-seq and transcriptome analysis of the OmpR regulon of Salmonella enterica serovars Typhi and Typhimurium reveals accessory genes implicated in host colonization. Mol Microbiol 2013; 87: 526-538.
[33] Horswill AR, Dudding AR and Escalante-Semerena JC. Studies of propionate toxicity in Salmonella enterica identify 2-methylcitrate as a potent inhibitor of cell growth. J Biol Chem 2001; 276: 19094-19101.
[34] Wiegand G and Remington SJ. Citrate synthase: structure, control, and mechanism. Annual review of biophysics and biophysical chemistry. Annu Rev Biophys Biophys Chem 1986; 15: 97-117.
[35] Horswill AR and Escalante-Semerena JC. In vitro conversion of propionate to pyruvate by Salmonella enterica enzymes: 2-methylcitrate dehydratase (PrpD) and aconitase Enzymes catalyze the conversion of 2-methylcitrate to 2-methylisocitrate. Biochemistry 2001; 40: 4703-4713.
[36] Beinert H and Kennedy MC. Aconitase, a two-faced protein: enzyme and iron regulatory factor. FASEB J 1993; 7: 1442-1449.
[37] Brickman TJ and Armstrong SK. The ornithine decarboxylase gene odc is required for alcaligin siderophore biosynthesis in Bordetella spp.: putrescine is a precursor of alcaligin. J Bacteriol 1996; 178: 54-60.
[38] Foster JW. Escherichia coli acid resistance: tales of an amateur acidophile. Nat Rev Microbiol 2004; 2: 898-907.
[39] Sturgill G and Rather PN. Evidence that putrescine acts as an extracellular signal re-

quired for swarming in proteus mirabilis. Mol Microbiol 2004; 51: 437-446.

[40] Schneider J and Wendisch VF. Biotechnological production of polyamines by bacteria: recent achievements and future perspectives. Appl Microbiol Biotechnol 2011; 91: 17-30.

[41] Hafner EW, Tabor CW and Tabor H. Mutants of Escherichia coli that do not contain 1, 4-diaminobutane (putrescine) or spermidine. J Biol Chem 1979; 254: 12419-12426.

Table S1. Primers for qRT-PCR

Primers	Sequences (5' to 3')
eutB-For	GGGAAAACTGCCTGTACTTTG
eutB-Re	CTGCCTGTCGTTGTAGAGATAC
pduP-For	GCCCTGAAGGTTGAAGAGG
pduP-Re	TTGGTGTGGCGATAGTGAAG
gltA-For	GTTTCACCTCTACCGCATCC
gltA-Re	GAACTCGTCATACTCTTCCTGC
acnB-For	AACGTCTTCCTGGCTTCTG
acnB-Re	ATCTGCTTTCTCGGTGTACTG
cadA-For	AGGGTAAATGCGGTATGAGC
cadA-Re	TCAGTAGACGCTACGATACCG
speC-For	ACGTAGTGGCGCTGGACAGT
speC-Re	ATTCCTGCGCGTTCCCGACT
udk-For	CGTTTGCGTGAAGAGATGAAC
udk-Re	CGCATATTGTTTGGAAGGCTC
gmk-For	TGTATGACACTCAGGTTTCCG
gmk-Re	CACTTGCTCAATGGTTTCGC
rpoD-For	TGTTGAGTCTGAAATCGGTCG
rpoD-Re	CAGATAGGTAATGGCTTCCGG
gyrB-For	CCGATCCACCCGAATATCTTC
gyrB-Re	TTGTCCATGTAGGCGTTCAG