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

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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-
OmpR-EnvZ plays an important role in cell metabolism of *Salmonella* Typhimurium

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 extracellular 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
OmpR-EnvZ plays an important role in cell metabolism of Salmonella Typhimurium

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

<table>
<thead>
<tr>
<th>Compounds</th>
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<th>TT-13/TT-1</th>
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<td>×</td>
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<tr>
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<td>Urea</td>
<td>×</td>
<td>√</td>
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</tbody>
</table>

√ 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.
OmpR-EnvZ plays an important role in cell metabolism of *Salmonella Typhimurium*. 

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Presence or absence</th>
<th>Fold change</th>
</tr>
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<tr>
<td><strong>Amino acids</strong></td>
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<tr>
<td>L-Alanine</td>
<td>√</td>
<td>√</td>
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<td>L-Valine</td>
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<tr>
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<td>Glycine</td>
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<td>L-Threonine</td>
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</table>

\(\checkmark\) represents the compounds that are 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.

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 strain 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
OmpR-EnvZ plays an important role in cell metabolism of *Salmonella Typhimurium*. 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/T1-1). 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, glycglycine, 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, glycglycine, 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*,

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**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.
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*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*, 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.

**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.

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-
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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.

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As an important source of carbon and nitrogen, ethanolamine can be utilized by *S. Typhimurium* in the presence of vitamin B12 [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 inter-mediate 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,
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L-lysine, L-tryptophan, butanediolic acid, malic acid, phosphoric acid, citric acid, decanediolic acid, glycyglycine, 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

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Disclosure of conflict of interest

None.

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References


OmpR-EnvZ plays an important role in cell metabolism of Salmonella Typhimurium.


[39] Sturgill G and Rather PN. Evidence that putrescine acts as an extracellular signal re-
OmpR-EnvZ plays an important role in cell metabolism of *Salmonella* Typhimurium


[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.
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**Table S1.** Primers for qRT-PCR

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<thead>
<tr>
<th>Primers</th>
<th>Sequences (5' to 3')</th>
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<td>eutB-For</td>
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</tr>
<tr>
<td>eutB-Re</td>
<td>CTGCCCTGCTGTTTAGAGATTAC</td>
</tr>
<tr>
<td>pduP-For</td>
<td>GCCCTGAAGGTTGAAGAGG</td>
</tr>
<tr>
<td>pduP-Re</td>
<td>TGGGTGTCGCTGAGAAGAG</td>
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<tr>
<td>gftA-For</td>
<td>GTCGAGCTCTACCGCACCTGC</td>
</tr>
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<td>gftA-Re</td>
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<tr>
<td>acnB-For</td>
<td>AAGCTTTTCTGCGCTTCTCT</td>
</tr>
<tr>
<td>acnB-Re</td>
<td>ATCTGCTTTCTCGGTGTACTG</td>
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<tr>
<td>cadA-For</td>
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<td>cadA-Re</td>
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