FinR Regulates Expression of *nicC* and *nicX* Operons, Involved in Nicotinic Acid Degradation in *Pseudomonas putida* KT2440

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ABSTRACT Many proteobacteria harbor FinR homologues in their genomes as putative LysR-type proteins; however, the function of FinR is poorly studied except in the induction of *fpr-1* under superoxide stress conditions in *Pseudomonas putida* and *Pseudomonas aeruginosa*. Here, by analyzing the influence of *finR* deletion on the transcriptomic profile of *P. putida* KT2440 through RNA sequencing and real-time quantitative PCR (RT-qPCR), we found 11 operons that are potentially regulated by FinR. Among them, the expression of *nicC* and *nicX* operons, which were reported to be responsible for the aerobic degradation of nicotinic acid (NA), was significantly decreased in the *finR* mutant, and complementation with intact *finR* restored the expression of the two operons. The results of bacterial NA utilization demonstrated that the deletion of *finR* impaired bacterial growth in minimal medium supplemented with NA/6HNA (6-hydroxynicotinic acid) as the sole carbon source and that complementation with intact *finR* restored the growth of the mutant strain. The expression of *nicC* and *nicX* operons was previously revealed to be repressed by the NicR repressor and induced by NA/6HNA. Our transcriptional assay revealed that the deletion of *finR* weakened the induction of *nicC* and *nicX* by NA/6HNA. Meanwhile, the deletion of *finR* largely decreased the effect of *nicR* deletion on the expression of *nicC* and *nicX* operons. These results suggest that *finR* plays a positive role and cooperates with NicR in the regulation of *nicC* and *nicX* operons. *In vitro* experiments showed that both FinR and NicR bound to *nicX* and *nicC* promoter regions directly. The results of this study deepened our knowledge of FinR function and nicotinic acid degradation in *P. putida*.

IMPORTANCE This study analyzed the influence of *finR* deletion on the transcriptomic profile of *Pseudomonas putida* KT2440. The FinR regulator is widely distributed but poorly studied in diverse proteobacteria. Here, we found 11 operons that potentially are regulated by FinR in KT2440. We further demonstrated that FinR played a positive role and cooperated with the NicR repressor in bacterial nicotinic acid (NA) degradation via regulating the expression of *nicC* and *nicX* operons. Furthermore, a transcriptomic analysis also indicated a potentially negative role of FinR in the expression of the *hut* cluster involved in bacterial histidine utilization. The work deepened our knowledge of FinR function and nicotinic acid degradation in *P. putida*.

KEYWORDS FinR, *Pseudomonas putida* KT2440, *nicC* and *nicX* operons, nicotinic acid, transcriptomic profile

The LysR-type transcriptional regulators (LTTRs) represent the most abundant type of transcriptional regulator with an N-terminal DNA-binding helix-turn-helix motif and a C-terminal coinducer-binding domain as conserved structures (1). LTTRs exhibit negative autoregulation and regulate a diverse set of genes, including those involved in virulence, metabolism, quorum sensing, and cell division (2–6). Many proteobacterial

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genomes contain a LysR-type oxidative stress-sensing transcriptional regulator, FinR, which is located next to *fpr-1*, encoding ferredoxin NADP⁺ reductase, an enzyme catalyzing the reversible electron-transferring reaction between NADPH and one-electron carriers such as ferredoxin or flavodoxin (7–10). Previous studies revealed that FinR binds directly to the promoter region of *fpr-1* and is essential for the induction of *fpr-1* during exposure to superoxide stress in *Pseudomonas putida* KT2440 (8). *Fpr-1* is important in maintaining the cellular NADP⁺/NADPH ratio by mediating a reversible redox reaction between NADP⁺/NADPH and electron carriers such as ferredoxin or flavodoxin (7). An interruption of *finR* or *fpr-1* increased bacterial sensitivity to oxidative stress in *Escherichia coli*, *Salmonella*, and *Pseudomonas* (9–12).

Normally, redox-sensing proteins harbor conserved cysteine residues to control their regulation, as is the case for SoxR and OxyR (13). FinR in *P. putida* has three well-conserved cysteine residues and two additional cysteine residues, but the five cysteines are not associated with FinR activation, since strains with mutations in each of these cysteine residues show transcription activity similar to that of the wild-type strain (8). These results suggest that FinR has a unique redox-sensing mechanism which is different from the well-characterized mechanisms of OxyR and SoxR.

Although FinR is widely distributed in diverse proteobacteria, its function is poorly studied except in the induction of *fpr-1* under superoxide stress conditions in *P. putida* and *P. aeruginosa*. In this study, we analyzed the influence of *finR* deletion on the transcriptomic profile of *P. putida* KT2440 through RNA sequencing and real-time quantitative PCR (RT-qPCR) and found 11 operons potentially regulated by FinR. Of these, *nicC* and *nicX* were positively regulated by FinR. *nicC* and *nicX* operons belong to the *nic* cluster, which is responsible for the aerobic degradation of nicotinic acid (NA). In this study, we mainly focused on characterizing the role of FinR in the expression of *nicC* and *nicX* operons and nicotinic acid utilization.

RESULTS

Transcriptomic analysis revealed genes under the influence of FinR. FinR is a LysR-type transcriptional factor positioned divergently adjacent to *fpr-1* in the *P. putida* KT2440 genome. Many bacteria possess a FinR homologue in their genomes, but except in the induction of *fpr-1* in response to superoxide (9, 10), the function of FinR has been poorly studied. To identify the set of genes under the influence of FinR, we constructed a *finR* deletion mutant and performed transcriptomic analysis. The results of the transcriptomic analysis revealed that expression of 189 genes was statistically significantly different ($\log_2$ fold change of ≥ 1 or ≤ -1) in the *finR* mutant compared to that in the wild-type KT2440 (see Table S1 in the supplemental material). Among the 189 genes, 56 genes exhibited larger differences in expression levels ($\log_2$ fold change of ≥ 2 or ≤ -2), with 16 genes showing decreased expression, including the formerly reported *fpr-1*, and 40 genes showing increased expression (Table 1). The 56 genes belong to 26 operons on the basis of the organization of the KT2440 genome. To verify the results of the transcriptomic analysis, we compared the transcriptions of the 26 operons in a *finR* mutant and the wild-type KT2440 by RT-qPCR. One gene was chosen and tested for each operon. As revealed in Fig. 1, the expression levels of 11 genes were different between the *finR* mutant and the wild type in the RT-qPCR assay, with trends similar to those observed in the transcriptomic analysis, while the expression of the other 15 genes showed no obvious difference in the RT-qPCR assay.

FinR positively regulates *nicC* and *nicX* operons. Among the 11 verified operons, three operons with decreased expression, *PP_3940* (*nicT*), *PP_3942* (*nicE*), and *PP_3945* (*nicX*), were of interest. The three operons belong to the *nic* cluster (Fig. 2A), which consists of 11 genes and is responsible for the aerobic degradation of nicotinic acid (NA) (14). To further test the role of FinR in the expression of the *nic* cluster, we compared the expression levels of all 11 *nic* genes in the *finR* mutant and the wild type by RT-qPCR. The results showed that the expression of eight genes was decreased in the *finR* mutant relative to that in the wild-type strain (Fig. 2B), while the expression of the other three genes, *nicA*, *nicB*, and *nicS*, showed no difference. Complementation

TABLE 1 Genes upregulated or downregulated in *finR* deletion mutant versus in wild-type *P. putida* KT2440^a

Gene ID ^b	Log ₂ fold change	P value	Gene name	Description
PP_0057	−2.207	1.72E−39		Major facilitator family transporter
PP_0137	2.856	1.25E−103	<i>gltP</i>	Glutamate/aspartate-proton DAACS transporter
PP_0596	3.701	0		Omega-amino acid-pyruvate aminotransferase
PP_0597	3.623	1.02E−290	<i>mmsA-I</i>	Methylmalonate-semialdehyde dehydrogenase
PP_0614	2.447	2.58E−08		Bifunctional <i>N</i> -carbamoyl-beta-alanine amidohydrolase/allantoin amidohydrolase
PP_0615	3.250	5.79E−05		ABC transporter ATP-binding protein
PP_0616	3.154	2.77E−10		ABC transporter ATP-binding protein
PP_0617	4.385	3.32E−07		Branched-chain amino acid ABC transporter permease
PP_0618	4.646	3.32E−10		Branched-chain amino acid ABC transporter permease
PP_0619	3.991	1.39E−46		Branched-chain amino acid ABC transporter substrate-binding protein
PP_0620	5.553	3.63E−206		GntR family transcriptional regulator
PP_0817	−2.297	5.46E−33	<i>alaC</i>	Aminotransferase
PP_1039	4.143	0.0075892	<i>tatC-I</i>	Sec-independent protein translocase protein
PP_1638	−3.811	2.01E−149	<i>fpr-I</i>	Ferredoxin-NADP ⁺ reductase
PP_2034	3.046	8.06E−77		Membrane protein
PP_2035	3.295	2.17E−52	<i>benE-I</i>	Benzoate transport protein
PP_2036	3.045	8.77E−101		4-Hydroxy-tetrahydrodipicolinate synthase
PP_2037	2.091	3.92E−10		Aldolase
PP_2052	2.233	3.20E−14		Bifunctional sugar-phosphatase/mannitol-1-phosphate 5-dehydrogenase
PP_2397	4.002	3.31E−68		EF hand domain-containing protein
PP_2404	3.914	1.29E−18		Hypothetical protein
PP_2453	2.024	6.07E−148	<i>ansB</i>	Glutaminase-asparaginase
PP_2454	−2.195	2.45E−119	<i>rbsB</i>	Ribose ABC transporter, periplasmic ribose-binding subunit
PP_3073	−3.332	1.27E−84	<i>hbdH</i>	3-Hydroxybutyrate dehydrogenase
PP_3074	−4.063	3.12E−164	<i>bhbP</i>	D-Beta-hydroxybutyrate permease
PP_3191	−2.213	4.57E−134		Threonine ammonia-lyase/dehydratase
PP_3589	2.618	1.16E−36	<i>sdaC</i>	Serine:H ⁺ symport permease
PP_3661	3.956	1.44E−90		Membrane protein
PP_3745	−3.031	6.86E−37	<i>glcD</i>	Glycolate oxidase FAD-linked subunit
PP_3940	−2.455	3.63E−21	<i>nicT</i>	Metabolite transport protein NicT
PP_3941	−2.753	1.35E−33	<i>nicF</i>	Maleamate amidohydrolase
PP_3942	−2.414	5.88E−22	<i>nicE</i>	Maleate isomerase
PP_3943	−2.694	1.60E−16	<i>nicD</i>	<i>N</i> -Formylmaleamate deformylase
PP_3944	−3.601	2.45E−44	<i>nicC</i>	6-Hydroxynicotinate 3-monooxygenase
PP_3945	−2.474	2.26E−58	<i>nicX</i>	2,5-Dihydroxypyridine 5,6-dioxygenase
PP_4232	2.120	1.93E−14		Cytochrome <i>c</i> family protein
PP_4233	2.640	1.50E−05		Oxidoreductase small subunit
PP_4434	3.476	1.57E−41	<i>dadA-I</i>	D-Amino acid dehydrogenase small subunit
PP_4435	4.100	0		Hypothetical protein
PP_4548	2.585	6.57E−14		Oxidoreductase
PP_4625	3.054	4.80E−34	<i>cidB</i>	Holin CidA anti-holin regulator
PP_4626	3.358	3.29E−12	<i>cidA</i>	Murein hydrolase holin regulator
PP_4735	3.332	0.00E+00	<i>lldP</i>	L-Lactate permease
PP_4736	3.441	4.45E−105	<i>lldD</i>	L-Lactate dehydrogenase
PP_4737	4.059	1.27E−94	<i>lld2</i>	D-Lactate dehydrogenase
PP_5029	4.210	5.79E−145	<i>hutG</i>	<i>N</i> -Formylglutamate deformylase
PP_5030	4.031	1.76E−213	<i>hutI</i>	Imidazolonepropionase
PP_5031	3.454	3.46E−109	<i>proY</i>	Proline (histidine) APC transporter
PP_5032	3.937	0	<i>hutH</i>	Histidine ammonia-lyase
PP_5033	4.132	6.99E−245	<i>hutU</i>	Urocanate hydratase
PP_5036	4.591	0	<i>hutF</i>	Formiminoglutamate deiminase
PP_5073	−3.290	6.77E−50		Hypothetical protein
PP_5269	4.310	0.00E+00	<i>dadX</i>	Alanine racemase
PP_5270	3.167	0	<i>dadA-II</i>	D-Amino acid:quinone oxidoreductase
PP_5338	−2.377	1.80E−156	<i>aspA</i>	Aspartate ammonia-lyase
PP_5549	2.424	1.95E−36		Hypothetical protein

^aGenes with log₂ fold changes of >2 or <−2 are shown.^bID, identifier.

with intact *finR* under the control of its own promoter restored the expression of the eight genes to wild-type levels.

The results of a previous study suggested that the *nic* cluster from *P. putida* is organized in three NA-inducible operons, i.e., *nicAB* under the control of the *Pa* promoter (promoter of *nicA*), *nicXR* under the control of the *Px* promoter (promoter of

FIG 1 Analysis of the relative transcriptional levels of 26 operons in wild-type KT2440 and the *finR* deletion mutant by RT-qPCR. Transcriptional levels in the wild type were used as a reference. The results are the averages from three independent assays. The data values represent mean values with standard deviations. *, $P \leq 0.05$; **, $P \leq 0.01$.

nicX), and *nicCDEF* under the control of the *Pc* promoter (promoter of *nicC*). Furthermore, the NA-independent expression of the *nicS*, *nicTP*, and *nicR* operons suggests the existence of three additional constitutive promoters upstream of the *nicS*, *nicT*, and *nicR* genes, respectively (15). To test the role of FinR in the expression activity of these promoters, we constructed seven *nic* promoter-*lacZ* fusion reporters and introduced them to the *finR* mutant and the wild-type strain. Then, β -galactosidase activity was measured. As shown in Fig. 2C, the promoter activities for *nicC* and *nicX* were significantly lower in the *finR* mutant than in the wild type. Meanwhile, the promoter activities for *nicR*, *nicA*, and *nicS* showed no difference and the promoter activities for *nicT* and *nicP-II* were too low to be detected. Altogether, both results from the RT-qPCR and the promoter-*lacZ* fusion reporters reveal that FinR positively regulates the expression of *nicC* and *nicX* operons.

FinR deletion impaired NA utilization. The initial hydroxylation of NA is catalyzed by two hydroxylases, NicA and NicB, which can convert NA to 6-hydroxynicotinic acid (6HNA). The second enzymatic step for aerobic NA degradation is the oxidative decarboxylation of 6HNA to render 2,5-dihydroxypyridine (2,5DHP) catalyzed by the 6HNA-3-monooxygenase NicC. Then, the Fe^{2+} -dependent dioxygenase NicX converts 2,5DHP to *N*-formylmaleamic acid. Further conversion of *N*-formylmaleamic acid to formic and maleamic acids is catalyzed by the NicD protein (14). Since FinR positively regulates the expression of *nicX* and *nicC* operons, we wondered whether *finR* deletion would influence bacterial NA degradation and lead to impaired growth of KT2440 in minimal medium containing NA or 6HNA as the sole carbon source. The results from previous studies showed that the *finR* mutant had longer lag phases than the wild type in minimal medium containing benzoate (10 mM) or glucose (111 mM) as the carbon source because of the decreased expression of *fpr-1* (10). Thus, to eliminate the influence of the decreased expression of *fpr-1* on the growth of the *finR* mutant in minimal medium, we introduced an *fpr-1* overexpression vector (403-*fpr-1* [see Table S2 in the supplemental material]) into the *finR* mutant. As showed in Fig. 3A, complementation with *fpr-1* restored the growth trend of the *finR* mutant to the wild-type level in minimal medium containing glucose as the sole carbon source. Then, the growth trend of the wild-type strain, the *finR* mutant complemented with *fpr-1* ($\Delta finR + 403-fpr-1$ strain), and the *finR* complementary strain (*cΔfinR* strain) in minimal medium containing NA or 6HNA as the sole carbon source was tested. A noticeably longer growth delay in the *finR* mutant was observed in a liquid growth assay as revealed in Fig. 3B and C, and

FIG 2 FinR regulates expression of *nicC* and *nicX* operons. (A) Schematic representation of the *nic* cluster from *P. putida*. Arrows above the genes represent promoters of indicated operons. (B) Analysis of the relative transcriptional levels of *nic* cluster genes in wild-type KT2440, the *finR* deletion mutant, and the *finR* complemented strain (*c* $\Delta finR$) by RT-qPCR. Transcriptional levels in the wild type were used as a reference. The results are the averages from three independent assays. (C) Promoter activity of *nic* cluster in the wild type and the *finR* mutant. β -Galactosidase activity was measured from stationary-phase (24 h) LB broth cultures. ND, not detected. The results are the averages from three independent assays. The data values represent mean values with standard deviations. *, $P \leq 0.05$; **, $P \leq 0.01$.

the complementation restored the growth to a level close to that of the wild type. Similar results were obtained from a plate growth assay, in which the growth of the *finR* mutant was severely impaired in both NA- and 6HNA-containing plates. Complementation with wild-type *finR* under the control of its own promoter restored the growth (Fig. 3D and E). These results suggest that FinR deletion impaired NA utilization.

FinR cooperates with NicR in the regulation of *nicC* and *nicX* operons. The results from a previous study revealed that NicR, a MarR-like protein, repressed the activity of *nicC* and *nicX* promoters and that 6HNA was the inducer molecule (15). Here, we found that FinR positively regulated the activity of *nicC* and *nicX* promoters. To investigate the relationship of the two regulators in the induction of *nicC* and *nicX* operons in the absence or presence of inducers, we constructed a *nicR* signal mutant and a *nicR finR* double mutant and compared the expression levels of *nic* genes in the presence or absence of NA/6HNA by RT-qPCR. As previously reported, the expression of *nicC*, *nicX*, and *nicA* operons was significantly higher in the *nicR* deletion mutant than in the wild-type strain in the absence of inducers (Fig. 4A). Meanwhile, the deletion of *finR* largely decreased the effect of *nicR* deletion on the expression of *nicC* and *nicX* operons, since the expression of the two operons was lower in the *nicR finR* double mutant than in the *nicR* single mutant in the absence of inducers (Fig. 4A). We also noticed that the expression of the two operons in the double deletion mutant was still approximately 100-fold higher than in the wild-type strain, suggesting that the repressive function of NicR played a chief role in regulating the expression of the two operons, while the positive role of FinR was needed for full induction.

FIG 3 *finR* deletion impaired NA/6HNA utilization. Growth trends of wild-type KT2440, the *finR* mutant, the *finR* mutant complemented with 403-*fpr-1*, and the *finR* complemented strain in liquid minimal medium containing 0.4% glucose (A), 5 mM NA (B), or 5 mM 6HNA (C) as the sole carbon source. Growth of strains on solid minimal medium containing 5 mM NA (D) or 5 mM 6HNA (E) as the sole carbon source after 48 h of incubation.

In the presence of NA/6HNA, the expression of *nicC* and *nicX* operons was induced in the wild type and the *finR* mutant but not in the *nicR* mutant and the *nicR finR* double mutant (Fig. 4B, C, D, and E). *finR* deletion weakened the induction of *nicC* and *nicX* operons in the presence of NA/6HNA (Fig. 4B and C), which might be the reason for the impaired growth of the *finR* mutant in minimal medium containing NA/6HNA. Furthermore, the expression of the *nicA* operon was induced by NA but not 6HNA in all tested strains, which is similar to the result from a previous report (15). These results suggest that *finR* plays a positive role and cooperates with NicR in the induction of *nicC* and *nicX* operons in the presence of NA/6HNA.

FinR and NicR bind *nicX* and *nicC* promoters *in vitro*. FinR is a DNA-binding transcriptional regulator and has been shown to bind to the promoter of the target genes to activate their transcription (8, 9). NicR is a repressor of *nicC* and *nicX* operons, but whether NicR directly binds to *nicC* and *nicX* promoters is unknown. To test whether FinR and NicR bind *nicC* and *nicX* promoters, we purified FinR/NicR and carried out an electrophoretic mobility shift assay (EMSA). Purified FinR/NicR was incubated with a fragment of DNA spanning -134 to $+51$ bp relative to the translational start site of *nicC*. The addition of FinR/NicR to the reaction mixtures caused a shift in the mobility of the *nicC* promoter DNA fragment. A competition experiment with unlabeled DNA

FIG 4 Induction of the *nic* cluster by NA/6HNA. (A) Analysis of the relative transcriptional levels of *nic* cluster genes in wild-type KT2440, the *finR* mutant, the *nicR* mutant, and the *finR nicR* double mutant in the absence of inducer by RT-qPCR. Induction of the *nic* cluster by 5 mM NA/6HNA in wild-type (B), *finR* mutant (C), *nicR* mutant (D), and *finR nicR* double mutant (E) strains. Control check (CK) represents the addition of water instead of NA/6HNA to the culture. The results are the averages from three independent assays. The data values represent mean values with standard deviations. *, $P \leq 0.05$; **, $P \leq 0.01$.

fragments eliminated the shift (Fig. 5A and C). In the case of the *nicX* promoter (−181 to +43 bp relative to the translational start of *nicX*), there was also a shift in the mobility of the *nicX* operon promoter. Similarly, unlabeled DNA from the *nicX* promoter eliminated the shift (Fig. 5B and D). These results indicated there were specific interactions between the FinR or NicR protein and the *nicC* or *nicX* promoter DNA. To further determine the precise binding sites of FinR/NicR in the two promoters, we performed a DNase I footprinting assay. The data obtained indicated that the GATTAGAGCGTA TACGCTA sequence in the *nicC* promoter and the AAGTAGGGTGTACACTAT sequence in the *nicX* promoter were protected by the NicR protein (Fig. 5E and F). In the case of FinR binding, we tried several times but failed to confirm the precise binding site of FinR to the *nicC* and *nicX* promoters.

To map the specific domains of the promoters that interacted with FinR, series of truncated fragments of the *nicC* and *nicX* promoters were amplified and used in the EMSA. As shown in Fig. 5G and Fig. S1 in the supplemental material, *nicCF2* and *nicXF1* DNA fragments produced strong band shifts with FinR on the gel, whereas no such retarded band was observed with the other three fragments, indicating that the binding sites are located between positions −19 and −120 on the *nicC* promoter and −102 and −204 on the *nicX* promoter relative to their translational start codons.

FIG 5 FinR and NicR proteins bind to *nicX* and *nicC* promoters. (A) Binding of FinR protein to *nicC* promoter and competition experiment with the unlabeled DNA fragment. The concentrations of the FinR protein and the amounts of unlabeled DNA used are indicated. (B) Binding of the FinR protein to the *nicX* promoter. (C) Binding of the NicR protein to the *nicC* promoter. (D) Binding of the NicR protein to the *nicX* promoter. (E) DNase I footprinting assay with fragments containing the *nicC* promoter in the presence (blue peaks) and absence (red peaks) of NicR. The protected nucleotides (relative to the translational start codon) are boxed. (F) DNase I footprinting assay with fragments containing the *nicX* promoter in the presence (blue peaks) and absence (red peaks) of NicR. (G) Binding of the truncated promoter fragments to FinR. Schematic diagram of the truncated fragments of *nicC* or *nicX* promoter DNA; a + or - on the right represents the fragment binding to FinR (+) or not (-). The coordinates represent the location relative to the ATG initiation codon. Red boxes represent the position of the truncated fragments bound by FinR in the intergenic region. Green boxes represent the position of the DNA sequences protected by NicR in the intergenic region.

Oxidative stress showed no influence on the expression of *nicX* and *nicC* operons. Since FinR was previously reported to be a redox-sensing transcriptional regulator and to induce *fpr-1* expression under superoxide stress conditions, we wondered whether the genes controlled by FinR revealed in this study were regulated by oxidative stress. We tested the influence of two superoxide-generating agents (hydrogen peroxide and paraquat) on the expression of the 11 FinR-regulated genes in the wild-type strain using RT-qPCR. As shown in Fig. 6, except for the expression of *fpr-1*, which was apparently induced by paraquat as previously described (16), the expression of the other 10 genes, including *nicC* and *nicX*, showed no difference in the presence/absence of hydrogen peroxide and paraquat under our experimental conditions, indicating that the expression of these genes was not regulated by oxidative stress via FinR.

DISCUSSION

NA is a carboxylic derivative of pyridine and is a widely distributed molecule that serves as the sole carbon and nitrogen source for some bacteria and fungi (17, 18). NA

FIG 6 Influence of oxidative stress (1 mM H₂O₂ or 1 mM paraquat [PQ]) on the expression of the 11 FinR-regulated genes in wild-type KT2440. The results are the averages from three independent assays. The data values represent the mean values with standard deviations. **, $P \leq 0.01$.

forms parts of biological molecules such as pyridine cofactors, e.g., NAD(P), and alkaloids, e.g., nicotine and anabasine, and it is essential (in the form of vitamin B₃) for organisms that are not able to carry out its synthesis (17). The degradation of NA in *P. putida* depends on the expression of genes from the *nic* cluster (Fig. 2A). NicR is a repressor of *nicC* and *nicX* operons and 6HNA is the inducer (15). Here, we reveal that FinR is an activator of *nicC* and *nicX* operons. *finR* deletion leads to decreased expression of the two operons and thus results in impaired NA/6HNA utilization (Fig. 2B and C and Fig. 3). The results of a transcriptional assay show that *finR* deletion weakened the induction of *nicC* and *nicX* in the presence of NA/6HNA (Fig. 4B and C), indicating that FinR and NicR cooperate in the regulation of *nicC* and *nicX* operons. Furthermore, the expression of *nicC* and *nicX* operons was significantly increased in the *finR nicR* double mutant relative to that in the wild type, but to a lower level than that in the *nicR* mutant (Fig. 4A), indicating that the repressive function of NicR played a chief role in regulating the expression of the two operons, while a positive role of FinR was needed for full induction.

Apart from the *nicC* and *nicX* operons, the *finR* mutant exhibited increased expression levels of genes that encode enzymes related to histidine utilization, such as *hutG* (*N*-formylglutamate deformylase), *hutI* (imidazolonepropionase), *hutH* (histidine ammonia-lyase), *hutU* (urocanate hydratase), and *hutF* (formiminoglutamate deiminase) (Table 1). The results from previous studies revealed that the histidine utilization (*hut*) loci of *Pseudomonas fluorescens* and *P. putida* SBW25 conferred the ability to utilize histidine as a sole carbon and nitrogen source (19, 20). *hut* genes in *P. putida* are divided into three major transcriptional units: *hutF*, *hutC* (the repressor gene), and *hutUHIG* (see Fig. S2 in the supplemental material) (21). The three *hut* operons are negatively regulated by the *hutC* repressor, with urocanate (the first intermediate of the histidine degradation pathway) as the physiological inducer. CbrB, an enhancer binding protein for σ^{54} RNA polymerase, is required for bacterial histidine utilization, indicating positive control of *hut* gene expression by CbrB (20). Here, our results indicated that FinR was a repressor of *hut* cluster expression. However, more studies are required to demonstrate how FinR regulates the expression of *hut* genes and whether FinR influences bacterial histidine utilization via regulating the expression of the *hut* cluster. Furthermore, we also found that the expression of *PP_4735*, encoding an omega-amino acid pyruvate aminotransferase that catalyzes the reversible transamination of various sulfur-containing ω -amino acids and ω -amino carboxylic acids with pyruvate to yield the corresponding aldehydic acids and L-alanine, was increased in the *finR* mutant.

A transcriptomic analysis indicated that 26 operons were differentially expressed more than 4-fold in the *finR* mutant. However, an RT-qPCR analysis showed that only 15 of them were differentially expressed in the mutant strain. We infer that two reasons exist for this obvious discrepancy between the transcriptomic and RT-qPCR results

obtained in this study. The most important reason is that the three RNA copies of the wild-type strain (or *finR* mutant) used for the transcriptomic assay were extracted from one single sample, while the RNAs used for the RT-qPCR assay were extracted from several individual samples; thus, some discrepancy between RNAs for the two techniques surely existed, and we consider the RT-qPCR result more precise under this condition. Second, the different principles and sensitivities of the two techniques may also lead to some discrepancy in the final expression results.

FinR is a member of the LysR family of transcriptional regulators, which often use palindromic DNA sequences as a binding box to modulate the expression of the target genes (1), but no consensus sequence for the FinR binding box on target gene promoters has been identified so far. The results from EMSAs and site-directed mutagenesis methods in a previous study of *P. aeruginosa* revealed that FinR bound specifically to the palindromic sequence TATCCATATTCTGGATA in the *fpr-1* promoter (9–12). We tried several times to confirm the precise binding sites of FinR on *nicC* and *nicX* promoters by using a DNase I footprinting assay, but all attempts failed to reveal clear DNA protection. To narrow the range of binding sites, we performed EMSAs with truncated promoter fragments and found that the binding sites are located within a 100-bp region for both promoters (Fig. 5G and Fig. S1 in the supplemental material). Further studies on interactions between FinR and target promoters are needed to reveal the precise binding sequences.

In conclusion, we investigated the effect of *finR* deletion on the transcriptomic profile of *P. putida* KT2440 and found 11 operons that are likely regulated by FinR. We further proved that FinR plays a positive role in the expression of *nicC* and *nicX* operons and bacterial nicotinic acid utility. These results deepened our knowledge of FinR function and nicotinic acid degradation in *P. putida* KT2400.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this work are listed in Table S2 in the supplemental material. The *P. putida* KT2440 and *E. coli* strains were routinely cultured at 28°C and 37°C, respectively, in Luria-Bertani (LB) broth, which was solidified with 1.5% agar when necessary. For the NA/6HNA degradation assay, *P. putida* strains were grown at 28°C in M9 minimal medium supplemented with 5 mM NA or 6HNA as the carbon source. Antibiotics were used, when required, at the following concentrations: kanamycin, 50 mg · liter⁻¹; chloramphenicol, 25 mg · liter⁻¹; gentamicin, 20 mg · liter⁻¹ for *E. coli* or 40 mg · liter⁻¹ for *P. putida*.

Plasmid and strain construction. All DNA manipulations were performed according to standard protocols (22). All cloning steps involving PCR were verified by commercial sequencing (Tsingke). Plasmids were transferred to *E. coli* and *P. putida* strains by transformation, biparental mating, or electroporation. Isogenic mutants of *finR* or *nicR*, promoter-*lacZ* reporter plasmids, and complementary plasmids were constructed according to a previously described method (23). The primers used for plasmid and strain construction are listed in Table 2. The strains and constructs are available upon request.

Assays for β -galactosidase activity. β -Galactosidase activity was measured as described previously (24). Overnight cultures were inoculated (1:100 dilution) in LB medium and grown for 1.5 h; cultures were diluted 1:1 three times (every half hour) before the start of sample collection. These steps were performed to ensure proper dilution of β -galactosidase that accumulated after overnight growth. Experiments were repeated at least three times with two technical repeats per sample, and data are given in Miller units.

Library preparation, RNA sequencing, and data analyses. Transcriptome libraries were constructed as previously described (25, 26). The total RNA sample extracted from exponentially growing cells incubated in LB medium was depleted of rRNA with the Ribo-Zero kit for Gram-negative bacteria (Illumina). cDNA libraries were prepared with the TruSeq stranded mRNA sample preparation kit (Illumina) according to the low sample LS protocol. Libraries were validated with a DNA 1000 chip on the Agilent 2100 Bioanalyzer, and the concentration was measured using a Qubit 2.0 fluorometer (Invitrogen, Life Technologies). The concentration of each library was normalized to 10 nM in TE buffer (10 mM Tris-Cl [pH 7.0], 1 mM EDTA), and cDNA libraries were pooled for sequencing on the Illumina HiSeq 2000 platform at Beckman Coulter Genomics. The transcriptome libraries were single-end sequenced with 100-bp reads.

The RNA-seq data were trimmed using Trimmomatic (27) and analyzed with the open source software Rockhopper (version 2.0.3) with the default settings, choosing reverse complement reads and strand-specific analysis (28). The reads were mapped to the sequenced reference *P. putida* KT2440 genome (GenBank accession no. [NC_002947.3](https://ncbi.nlm.nih.gov/nuccore/NC_002947.3)). Using SAMtools (29), the mapped files were merged and the identification of novel transcripts was performed by visual inspection with Integrative Genomics Viewer (30), as Rockhopper detects many false positives. The differential gene expression analysis was carried out with the Web server T-REX (31) using the reads per kilobase per million (RPKM) values

TABLE 2 Primers used for plasmid construction and RT-qPCR in this study

Primer	Sequence (5'→3') ^a
<i>finR</i> _UpS	CCGGAATTCCTCCAGGGCATTGAGGG
<i>finR</i> _UpA	CCGCTCGAGCAAGGTGCTCTTCCCAGTT
<i>finR</i> _DwS	TGCTCTAGACACGTAACCGCGATCTTCC
<i>finR</i> _DwA	CGCGGATCCGCTATGCCGCAACTTCACT
<i>kanS</i>	CGCGGATCCCTCGTGAAGAAGGTGTTG
<i>kanA</i>	CCGGAATTCAGCCACGTTGTGTCTCAA
<i>finRS</i>	TGCTCTAGAACTCGGCGGTGGAGG
<i>finRA</i>	CCGGAATTCCTGGCGAAGCGATGGA
<i>nicR</i> _UpS	CCGCTCGAGCTGCATGTGCGGTCCAA
<i>nicR</i> _UpA	CCGGAATTCACCTGTTCCGGTAACGTCGTAG
<i>nicR</i> _DwS	CCGGAATTCGTTGCCATTTTGTACTGCTG
<i>nicR</i> _DwA	CGCGGATCCGCCAAGGGGATGACGC
<i>nicCproS</i>	AAACTGCACTGTTGCAGCAATGTGGCG
<i>nicCproA</i>	CGGGGTACCATGGCGGTGCGGTGGTT
<i>nicXproS</i>	CGGGGTACCTCAGCTCCAGCTTCTGCTCC
<i>nicXproA</i>	AAACTGCAGGCTCTGCGTTTCTGCTCACTC
<i>nicTproS</i>	AAACTGCAGGATGTCAGTGTGGACCGTGGTT
<i>nicTproA</i>	CGGGGTACCCCGCTGGCAGCCTTTT
<i>nicP-IIproS</i>	AAACTGCAGAGCGGGCGGCAATACAACC
<i>nicP-IIproA</i>	CGGGGTACCCCGGCGACTTTCCTCAA
<i>nicRproS</i>	CGGGGTACCGATGCCCCGTGCGTTCT
<i>nicRproA</i>	AAACTGCAGGGGGGTGTTGTCGAGCG
<i>nicAproS</i>	CGGGGTACCGCCATTGCCGTCACAGATAC
<i>nicAproA</i>	CGTTAGGCACAGGTCGTT
<i>nicSproS</i>	AAACTGCAGACCGGTTTTCGAGTTTG
<i>nicSproA</i>	CGGGGTACCCGGAGGACGCTTCTTCC
qpcrfpr-1s	GTGCGCTATGTGAACGAA
qpcrfpr-1a	CACGGTCGGGTAGTAGAT
qpcr3074s	CTGTGCCCTGCTTACTTA
qpcr3074a	GGATATTGCTCTGACGGA
Qpcr3745s	ACCCTTCCTCGCAAATTG
Qpcr3745a	GTCACCTTGAGCACATT
Qpcr3940s	TGTACTACCTGACCATGTG
Qpcr3940a	CCATGATCCAGCCAGAAA
Qpcr3944s	CGAAGATCCGTGAAGAGT
Qpcr3944a	GGTGTAGTAGACCATCATGT
Qpcr3946s	GATCAGCGAACTGAGCAT
Qpcr3946a	GCTGTCGATCATCTTGCG
Qpcr5073s	TTCAACGACAAGACCAAG
Qpcr5073a	ACAATATCCACATCCACTTC
Qpcr5338s	GATCAAAGGCGATTCCA
Qpcr5338a	GCAGGTAAGTGGTACTCAC
Qpcr0137s	GCCGAAGTACAACATGAG
Qpcr0137a	GAAACAGCTGGAAGGTA
Qpcr0569s	CTGAGTACGCTGGACTAC
Qpcr0569a	GACGTGATTGAGATTGCC
Qpcr0614s	CCTGAGCCACATGGATAT
Qpcr0614a	CATGGCACGAAGATCATC
Qpcr0619s	ATGCAAAGACGTACCCTG
Qpcr0619a	GGACTTGAGCCCATAGAA
Qpcr0620s	GATCGTCGAATCAGTCAAG
Qpcr0620a	CCACTTCGTTGAGGTAGG
Qpcr2035s	CAACGATGGATTCAAGACC
Qpcr2035a	CGACGAGGATGTAACAGA
Qpcr2037s	CACCTACTACCCCAACA
Qpcr2037a	GTGGATGATGCAGTTGAC
Qpcr2397s	GGTGTGTATCGCACTGTA
Qpcr2397a	GGTTTGTCGGGGATGTAT
Qpcr4435s	CATTGACAGGCTTCTTGAG
Qpcr4435a	GGCTTGGCTTGAACATAG
Qpcr4548s	GTACTGCCAGGGTTACTC
Qpcr4548a	CTTCTAAGCATCTGGTAATAC
Qpcr4626s	AGATGCTGCTGTTCTTCA
Qpcr4626a	GCGGTCATATCATCACTA
Qpcr4735s	TACTTCACCTCGAACTTCAT
Qpcr4735a	ACTTTCAGGAACAGGGTAA

(Continued on next page)

TABLE 2 (Continued)

Primer	Sequence (5'→3') ^a
Qpcr5031s	CATCAACAGCGACATCTTT
Qpcr5031a	CAATCAGCAGGAACACATT
Qpcr5033s	CGATTACGGCAACAACAT
Qpcr5033a	TCAGTTCCTTGACCTTGG
Qpcr5036s	GCTTCATCAACTCCACTG
Qpcr5036a	GACTTCCTTCTGCTGTTT
Qpcr5070s	GCATCAATGGCGTGTGGA
Qpcr5270a	GAATAGCCTTTCAGCGGATAC
Qpcr5269s	GTCTGATGGACATGCTCAG
Qpcr5269a	CCGCTTCAGGTTACAGAA
Qpcr3939s	GCTGGCGACTACGATAAC
Qpcr3939a	GCCACATAAAAGGTATGC
Qpcr3943s	GGCATTATTTTCGACACCTAT
Qpcr3943a	GAATATCCGCAGCACAAG
Qpcr3945s	TGACTGAGTACGGCTATG
Qpcr3945a	AATAGTTCTTGAACGGCAG
Qpcr3948s	TGAACCACTCACACAGG
Qpcr3948a	GACCATTGAAGGCGTAGA
Qpcr3949s	CCTGTTCCGTGACGATAT
Qpcr3949a	GGTTGGAGTTATAGAAGTAGCA
3944emsas	TGTCAAGCTACCTGCTGATGCCCCACCCAGACCT
3944emsaa	GGATTTTCGTTTCGATCAGCT
3945emsas	TGTCAAGCTACCTGCTGATTGTTACCGTAAATACTGGTCA
3945emsaa	CGTGCTCGAACATCTGGGTC
3944mids	CTCTTGTCTGATAGGTG
3944mida	CTCTTTTCAAAGGTCATT
3945mids	ATGCGTTTTTCATCTGCAC
3945mida	GCGCACCATGGCAGTGCC

^aThe restriction site regions of the primers are underlined.

generated in the Rockhopper analysis, in which the *finR* mutant was compared to the wild-type control. Differential expression of genes was considered significant with a log₂ fold change of ≥ 1 and an adjusted *P* value of ≤ 0.05 . The Basic Local Alignment Search Tool (BLAST) with search criteria including a query of $>80\%$, identity of $>60\%$, and *E* value of $<10^{-6}$ was used in sequence homology searches.

RNA extraction, preparation of cDNA, and RT-qPCR. Total RNA from exponentially growing cells was extracted with total RNA extraction reagent (Vazyme) as recommended by the manufacturer. Reverse transcription reactions to generate the corresponding cDNA were performed with 1 μ g of RNA using a PrimeScript RT reagent kit (TaKaRa RR047A). RpoD was used as an internal control for normalization. The primers used for RT-qPCR are listed in Table 2. The results were analyzed by means of the comparative threshold cycle method to determine the relative expression of each gene in the mutants with respect to that in the wild type. Three individual replicates were performed with three independent cultures grown on different days. Standard errors were calculated from these independent replicates.

Expression and purification of His-tagged FinR/NicR. An *E. coli* BL21 strain carrying the FinR/NicR expression vector was grown overnight in LB broth with kanamycin, diluted 1:100, and grown for 4 h at 37°C. The expression of His-tagged FinR/NicR protein was induced with the addition of 0.4 mM IPTG (isopropyl- β -D-thiogalactopyranoside), followed by an incubation at 16°C overnight. Cultures were harvested by centrifugation at $6,000 \times g$ for 10 min and resuspended in lysing buffer (10 mM Tris-Cl [pH 8.0], 300 mM KCl, and 10% glycerol). The cells were lysed in a JNBIO pressure cell breaking apparatus, and the extract was clarified by centrifugation at $15,000 \times g$ for 5 min. The soluble fraction was filtered through a 0.22- μ m-pore-size filter. The crude extract was loaded on a NiSO₄ column and then collected from the column in elution buffer (10 mM Tris-Cl [pH 8.0], 300 mM KCl, 10% glycerol, and 200 mM imidazole). The protein concentration was determined with a bicinchoninic acid (BCA) assay.

Electrophoretic mobility shift assay. The EMSA was performed as previously described (32). The fragments of the *nicC* and *nicX* promoters were generated by PCR using 6-FAM (6-carboxyfluorescein phosphoramidite)-tagged primers. Equal amounts of each of these labeled DNA fragments (50 ng) were added to binding reactions with various amounts of FinR/NicR in the binding buffer (10 mM Tris [pH 7.8], 8 mM magnesium acetate, 50 mM KCl, 5% glycerol, 250 ng $\cdot$ ml⁻¹ bovine serum albumin [BSA]; 20 μ l total reaction volume) in each gel. FinR/NicR was incubated with DNA for 25 min at room temperature. All reaction mixtures were loaded onto a 5% acrylamide gel containing 10 mM Tris-Cl (pH 8.0), 400 mM glycine, and 5 mM EDTA and electrophoresed at 100 V at room temperature for 1 to 1.5 h. The gels were dried and exposed to a phosphorimaging screen.

DNase I footprinting assay. A DNase I footprinting assay was performed as described previously (33) with slight modifications. The 6-FAM-labeled promoter DNA sequences of *nicC* and *nicX* were PCR-amplified from genomic DNA using 5'-end 6-FAM-labeled primers and purified by 6% PAGE. In a 600- μ l reaction system, 1,000 ng of labeled DNA fragment was bound to 1,000 μ g FinR/NicR (bovine serum albumin was used instead of FinR/NicR in the control experiment) in buffer containing 10 mM

Tris-HCl (pH 7.8), 10 mM MgCl₂, 1 mM CaCl₂, 0.4 mM dithiothreitol, 100 mM KCl, and 5% glycerol and incubated for 20 min at 25°C. After binding, 0.03 U of RNase-free DNase I (Roche, Basel, Switzerland) was added and allowed to react for 5 min at 25°C. The reaction was stopped and precipitated with ethanol. Samples were analyzed in a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), and the electropherograms were aligned with GeneMapper v3.5 (Applied Biosystems).

Statistical analysis. For analyses of the significance of differences in gene expression, Student's *t* tests were used for comparison of two groups of data. A *P* value of ≤0.05 was considered significantly statistically different; a *P* value of ≤0.01 was considered extremely significantly statistically different.

Accession number(s). RNA-seq data have been deposited in the SRA database under accession numbers SRP139421, SRP139424, SRP139442, SRP139452, SRP139482, and SRP139486.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AEM.01210-18>.

SUPPLEMENTAL FILE 1, PDF file, 0.7 MB.

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We have no competing interests.

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