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Research Article 研究报告

Genome-wide transcriptional analysis of the recombinant *Escherichia coli* 78-7 carrying a *nif* gene operon of *Paenibacillus polymyxa* WLY78

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Abstract: [Objective] The nitrogen fixation (*nif*) gene operon (*nifBHDKEfNXhesAnifV*) of *Paenibacillus polymyxa* WLY78 encoding the nitrogenase enabled *Escherichia coli* to synthesize functional nitrogenase. The genome-wide transcriptional profiling of the recombinant *E. coli* 78-7 was examined for improving its nitrogenase activity. **[Methods]** The transcriptomic analysis of the recombinant *E. coli* 78-7 cultured under non-N_2-fixing (air and 100 mmol/L NH_4^+) and N_2-fixing (without O_2 and NH_4^+) conditions was implemented. **[Results]** These results reveal that *nif* genes were significantly transcribed under both conditions, indicating that the negative regulation of *nif* gene transcription by O_2 and NH_4^+ is bypassed in heterogeneous *E. coli*. The non-*nif* genes specifically required for nitrogen fixation, such as *mod*, *cys* and *feoAB* encoding transporters of Mo, S, Fe and electron transporters, respectively, were transcribed at different levels in both conditions. The transcription levels of *suf* operon and *isc* system specific for the synthesis of the Fe-S cluster varied greatly. The genes involved in nitrogen metabolism were notably up-regulated in N_2-fixing conditions. **[Conclusion]** These data suggest that the non-*nif* genes specifically required for nitrogen fixation in recombinant *E. coli* had obvious effects on its expression of nitrogenase. Our results will provide valuable exploration regarding the improvement for nitrogenase activity of heterogeneous hosts.

Keywords: *Escherichia coli*, *nif* gene, nitrogen fixation, *Paenibacillus*, transcription

The diazotrophic *Paenibacillus polymyxa* WLY78 possesses a minimal and compact *nif* gene cluster composed of nine genes (*nifBnifHnifDnifKnifEnifNnifXhesAnifV*) which are organized as an operon. In contrast, the diazotrophic models *Klebsiella oxytoca* and *Azotobacter vinelandii* have 20 *nif* genes (*nifJ*, *H*, *D*, *K*, *Y*, *T*, *E*, *N*, *X*, *U*, *S*, *V*, *Z*, *W*, *M*, *F*, *L*, *A*, *B*, *Q*) organized several transcription units[1–2]. Our laboratory has demonstrated that the *Paenibacillus nif* gene operon under the control of its σ^{70}-dependent promoter located in front of the *nifB* gene enabled *Escherichia coli* to synthesize functional nitrogenase[3]. Our recent study has revealed that in *P. polymyxa* WLY78, the *nif*

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genes and the non-*nif* genes encoding transporters for Mo, S and Fe are coordinatively and highly transcribed under N_2-fixing condition compared to non-N_2-fixing condition[4]. Whether the foreign *Paenibacillus nif* genes and the native non-*nif* genes encoding transporters for Mo, Fe and S in the recombinant *E. coli* 78-7 can be coordinatively and highly transcribed under N_2-fixing condition is not known.

Here, we performed a genome-wide transcriptome analysis of the recombinant *E. coli* 78-7 cultured under non-N_2-fixing and N_2-fixing conditions. Our results revealed that the *nif* gene operon composed of *nifBHDKENXhesAnifV* is significantly expressed in *E. coli* in both conditions. The non-*nif* genes encoding transporters of Fe, S, Mo and electrons, the *sufABCDSE* operon and the *isc* system specific for synthesis of the Fe-S cluster are analysed. The transcriptions of genes involved in nitrogen metabolism, ATP synthesis and sigma factors are also analysed. This study will provide valuable information for engineering nitrogenase biosynthetic pathway into non-N_2-fixing-organisms and will also provide guidance for improving nitrogenase activity in the heterologous host.

1 Materials and methods

1.1 Bacterial strains, media and growth conditions

The recombinant *E. coli* 78-7, a derivative carrying the *nif* operon (*nifBHDKENXhesAnifV*) of *P. polymyxa* WLY78, is used in this study[3]. The medium and growth conditions for the strain are used according the reference[3].

1.2 Isolation of RNA and Quantitative real-time RT-PCR

The recombinant *E. coli* 78-7 was grown to OD_{600}=0.30–0.45 at different concentrations of oxygen and ammonium, and cells were then harvested by centrifugation at 4 °C. Isolation of total RNA and Quantitative real-time RT-PCR were performed according to the methods described by Shi et al[4]. The primers used for qRT-PCR reactions are listed in Supplementary Table S1.

1.3 Transcriptomic analysis

The construction of cDNA library and SOLiD sequencing for the total RNA were completed at the Beijing Genomics Institute (Chinese Academy of Sciences), and each sample were carried out three technical replicates. The raw reads were mapped to the reference genome (*E. coli* K12) using the programme BWA as formerly described[5–6]. We used DEGseq to identify differentially expressed genes from RNA-seq data[7]. Transcript level differences with adjusted *P* values of <0.001 were considered to be significant. The RNA-seq sequencing data of the recombinant *E. coli* 78-7 have been deposited in NCBI database under accession number SRP053133.

2 Results

2.1 Genome-wide transcription analysis of the recombinant *E. coli* 78-7

A genome-wide transcription analysis of the recombinant *E. coli* 78-7 cultured under non-N_2-fixing and N_2-fixing conditions is performed. Among 4366 genes of the recombinant *E. coli* 78-7, transcript abundances are increased for 1274 (28%) genes, decreased for 1381 (32%) and not changed for 1711 genes (39%) under the N_2-fixing condition compared to those under the non-N_2-fixing condition control (Figure 1 and Table 1). Based on $\log_2$ fold changes (P<0.05), transcript levels for nearly 5% (204 among 4366 genes) of the recombinant *E. coli* 78-7 genes are changed more than 2-fold under N_2-fixing condition relative to those under the non-N_2-fixing condition control.

2.2 Transcriptional analysis of the nitrogen fixation genes

In *E. coli* 78-7, the expression levels of the nine

genes *nifBHDKENXhesAnifV* within the *nif* operon fall into the highest range in both non-N_2-fixing and N_2-fixing conditions (Figure 2 and Table 1). The mean expression levels of the nine foreign *nif* genes *nifBHDKENXhesAnifV* are much higher than those of the native *E. coli* genes, suggesting that the *nif* promoter is effectively recognized and transcribed by the *E. coli* RNA polymerase.

2.3 Transcriptional analysis of molybdate transporters

Molybdenum is necessary in bacteria for the activity of a limited number of microbial enzymes[8], including nitrogenase[9] and nitrate reductase[10]. As only trace amounts molybdate is present in the environment, bacteria employ an high-affinity energy-dependent molybdate transporter to accumulate it. The *E. coli* molybdate transporter is encoded by the *modABCD* operon, which is negatively regulated by the *modE* gene product in response to the intracellular molybdate concentration. In addition, *E. coli* has the *modF* gene, whose function is unknown.

In *E. coli* 78-7, the expression levels of the *modABCE* genes are similar in both the N_2-fixing condition and the non-N_2-fixing condition (Figure 3-A). However, the expression level of *modF* is significantly up-regulated in the N_2-fixing condition.

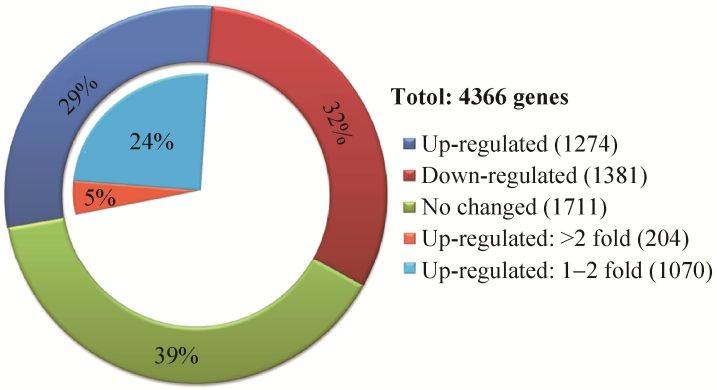

Figure 1. Changes in transcriptional levels of the total genes within the recombinant *E. coli* 78-7 genomes.

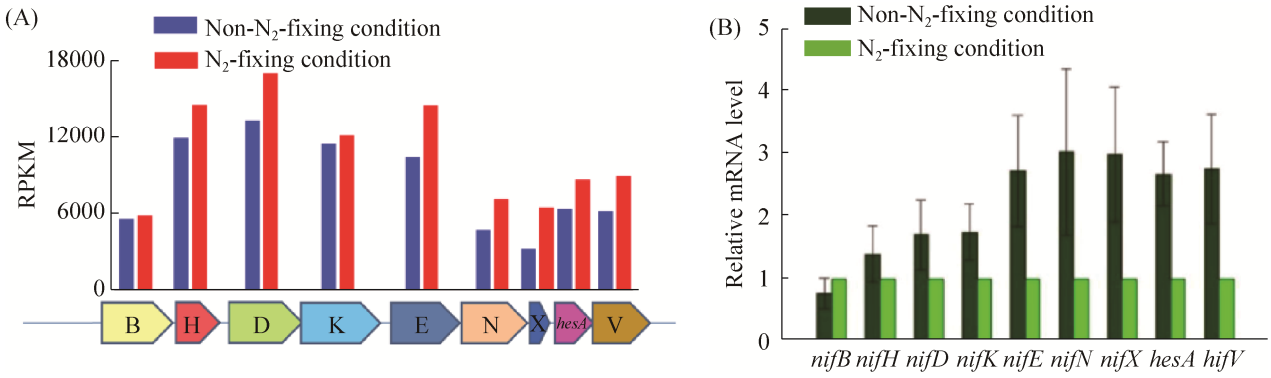

Figure 2. Differential expression of the *nif* gene operon (*nifBHDKENXhesdAnifV*) in N_2-fixing and non-N_2-fixing conditions. A: Transcriptional analysis of the *nif* genes in the recombinant *E. coli* 78-7; B: Quantitative real-time RT-PCR analysis of *nif* gene expression in the recombinant *E. coli* 78-7.

Table 1. Relative expression level (Reads Per Kilobase per Million, RPKM) in the recombinant *E. coli* 78-7

RPKM	Gene numbers/*E. coli* 78-7	
	Non-N_2-fixation (100 mmol/L NH_4^+ and Air) (100 mmol/L NH_4^+ and Air)	N_2-fixation (without NH_4^+ and O_2) (without NH_4^+ and O_2)
0.00	292	301
0.00–0.25(>0)	48	56
0.25–0.50	75	86
0.50–1.00	192	187
1.00–5.00	901	907
5.00–10.00	585	558
10.00–50.00	1405	1399
50.00–100.00	366	390
100.00–500.00	377	361
500.00–1000.00	66	74
1000.00–**	58 [a]	46 [a]

[a] The gene number of this relative expression level includes the *nif* genes.

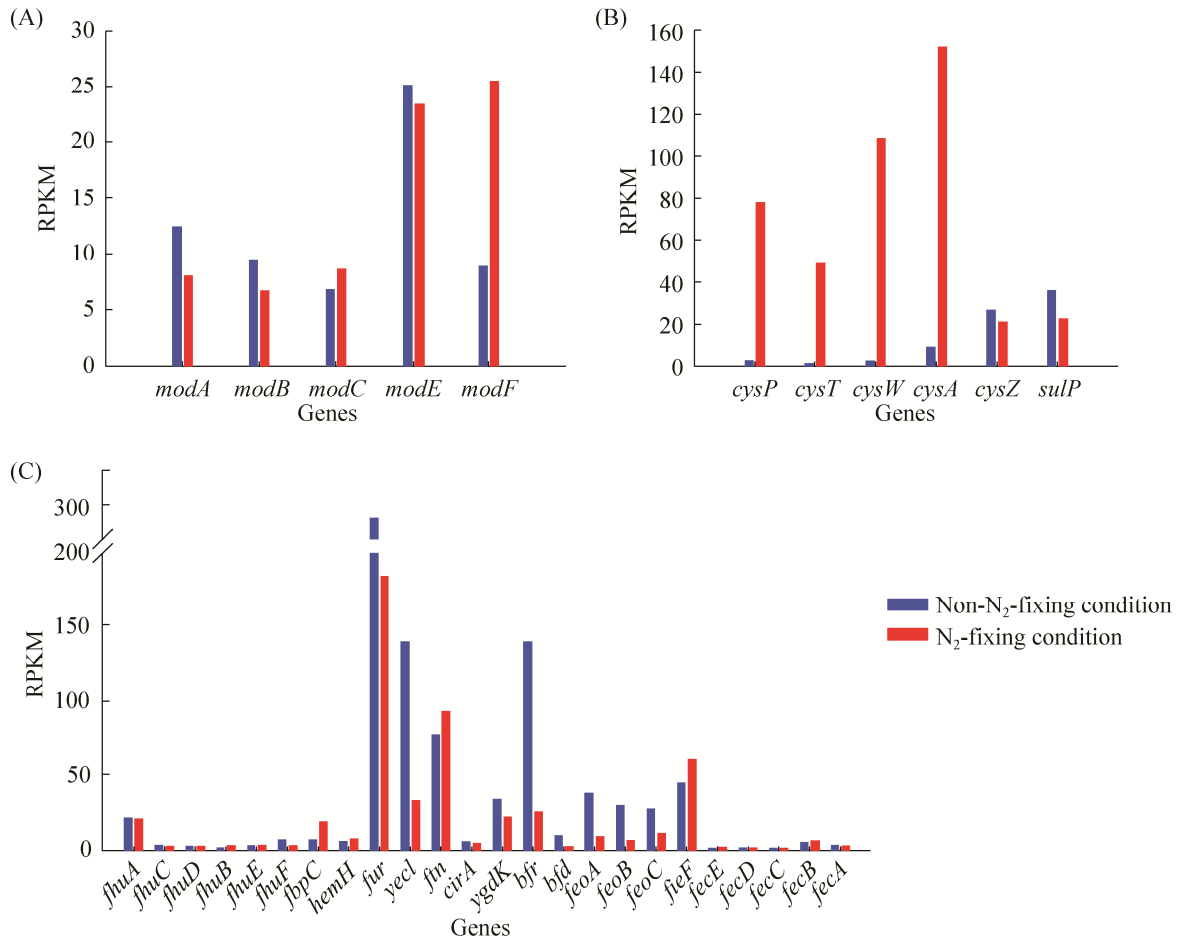

Figure 3. Differential expression of the genes relating to the transport, storage and regulation of molybdenum, sulfate and iron in N_2-fixing and non-N_2-fixing conditions. A: The *mod* genes encoding molybdenum transporters; B: The genes encoding sulfate transporters; C: The genes encoding iron transport, storage and regulation.

2.4 Transcriptional analysis of sulfate transporters

Sulfur is an basic element for microorganisms, peculiarly for diazotrophs whose nitrogenase containing iron–sulfur clusters[2,11–13]. Sulfate and thiosulfate being the preferred sulfur sources for most organisms, are taken in by sulfate permeases through membrane transporters. And sulfate permeases in bacteria belong to the SulT (Sbp/CysPTWA), SulP, CysP/(PiT) and CysZ families[13]. It is reported that sulfate can also be transported by the molybdate transport system, as it is structurally related to the oxyanion molybdate[10].

In *E. coli* 78-7, sulfate permeases include SulT (CysPTWA), SulP and CysZ. The SulT of *E. coli* 78-7 is encoded by the operon *cysPTWA*. *E. coli* also contains a *sbp* gene, which is located in another chromosomal region. Sbp and CysP have partially overlapping activities, and it has been suggested that both proteins interact with membrane proteins CysT and CysW of the SulT permease[13]. The *cysPTWA* cluster is up-regulated from 16-fold to 40-fold, but *cysZ* and *sulP* are down-regulated under the N_2-fixing condition compared to under the non-N_2-fixng condition (Figure 3-B). The *cysK* and *cysM* encoding cysteine synthase and the genes *cysS*, *cysC*, *cysN*, *cysN*, *cysD*, *cysH*, *cysJ*, *cysG* and *cysE* involved in sulfur metabolism are also up-regulated in the N_2-fixing condition compared to in the non-N_2-fixing condition.

2.5 Transcriptional analysis of Fe transporter

Iron (Fe) is also an basic element for almost all organisms and is required in cofactors of many enzymes, involving nitrogenase. under anaerobic conditions or at an acidic pH, Fe is the soluble Fe^{2+} form (ferrous iron). The main route for bacteria to-ferrous-iron uptake would appear to be, in many instances, via Feo (ferrous iron transport)[14]. Three proteins, FeoA, FeoB and FeoC, compose the enterobacterial Feo system. Though the functions of FeoA and FeoC remain unclear, FeoB is responsible for transporting ferrous iron. The *feoABC* genes constitute an operon, but in some bacteria, *feoA* and *feoC* are not always present aside *feoB*[14–15].

In *E. coli* 78-7, the *foeABC* operon is weakly depressed in the N_2-fixing condition compared to in the non-N_2-fixing condition (Figure 3-C). The *fur* gene encoding the ferric uptake regulator Fur as an transcriptional activator, which controls the transcription of genes involved in iron homeostasis as well as its own synthesis, is also weakly depressed in the N_2-fixing condition compared to in the non-N_2-fixing condition (Figure 3-C). The *fecABCDE* operon encoding the Fe^{3+} dicitrate transport system is weakly re-regulated in the N_2-fixing condition. The *fhuACDB* operon, specifying the Fe^{3+} hydroxamate uptake apparatus, is transcribed at similar levels in both the non-N_2-fixing and N_2-fixing conditions. *ftn*, encoding iron storage protein, is up-regulated 1.21-fold in the N_2-fixing condition. The *fhuE*, *fhuF*, and *cirA* genes involved in ferri-coprogen/ rhodotorulic acid, ferrioxamine B, and ferric-dihydroxy benzoate utilization are transcribed at similar levels in both the non-N_2-fixing and N_2-fixing conditions.

2.6 Transcriptional analysis of the iron-sulfur cluster assembly system

Nitrogenase is a complex [Fe-S] enzyme, and its [Fe-S] clusters play a critical role in electron transport and in the substrates reduction driven by the free energy liberated from Mg-ATP hydrolysis[2,11–12]. NifUS (products of *nifU* and *nifS* gene), which mobilizes S and Fe for the assembly of small Fe/S fragments, is commonly considered to be specialized for the assembly of the Fe_4-S_4 cluster of NifH. NifU and NifS also participate in the assembly of the FeMo-co and the P-cluster of the NifDK component of nitrogenase[16]. *nifSU* is widely distributed in diazotrophs, such as *A. vinelandii* and *K. oxytoca*. Except *nifSU*, the *isc* (*iscR*, *iscU*, *iscS*,

iscA, *hscB*, *hscA*, *fdx* and *iscX*) system also plays a critical function in the assembly of the Fe-S cluster in *A. vinelandii*[17]. However, the *Paenibacillus nif* operon does not contain *nifSU*. The synthesis of the Fe-S cluster in *E. coli* 78-7 is possibly provided by *E. coli* iron–sulfur cluster assembly systems. There are two iron–sulfur cluster assembly systems in *E. coli*: the *sufABCDSE* operon and the *isc* system (*iscR*, *iscS*, *iscU*, *iscA*, *hscB*, *hscA*, *fdx* and *orf3*)[18]. This study shows that the *isc* system is highly transcribed in both the non-N_2-fixing and N_2-fixing conditions. However, the transcription level of the *sufABCDSE* operon is very low in both conditions (Figure 4). The data might imply that the assembly of Fe-S clusters of nitrogenase in *E. coli* 78-7 may be due to the main contribution of the *isc* system. Our results are consistent with a report stating that the *suf* operon and *isc* operon were differently expressed when *E. coli* cells were treated with hydrogen peroxide[19].

2.7 Transcriptional analysis of electron transporters

Nitrogen fixation is performed by the nitrogenase, which transfers electrons originating from low-potential electron carriers, such as ferredoxin or flavodoxin molecules, to molecular N_2[20]. In *K. oxytoca*, the physiological electron flow to nitrogenase particularly involves the products of the *nifF* and *nifJ* genes[21]. The product of *nifF* gene, a flavodoxin, mediates electron transfer from the product of *nifJ* gene, a pyruvate–flavodoxin oxidoreductase, to the Fe protein of nitrogenase[22].

In *E. coli* 78-7, there are several genes encoding flavodoxins, including *fldA* and *fldB* (Figure 5-A). The *fldA* is weakly up-regulated 1.07-fold, while the *fldB* is weakly down-regulated 0.8-fold. Other genes encoding electron transport proteins such as *hydN* are also up-regulated in the N_2-fixing condition compared to in the non-N_2-fixing condition. Notably, the *groEL* (encoding chaperonin), which was demonstrated to play an important role in the biogenesis of the MoFe protein in the *E. coli* carrying *K. oxytoca nif* genes[23], is significantly transcribed in both the non-N_2-fixing and N_2-fixing conditions.

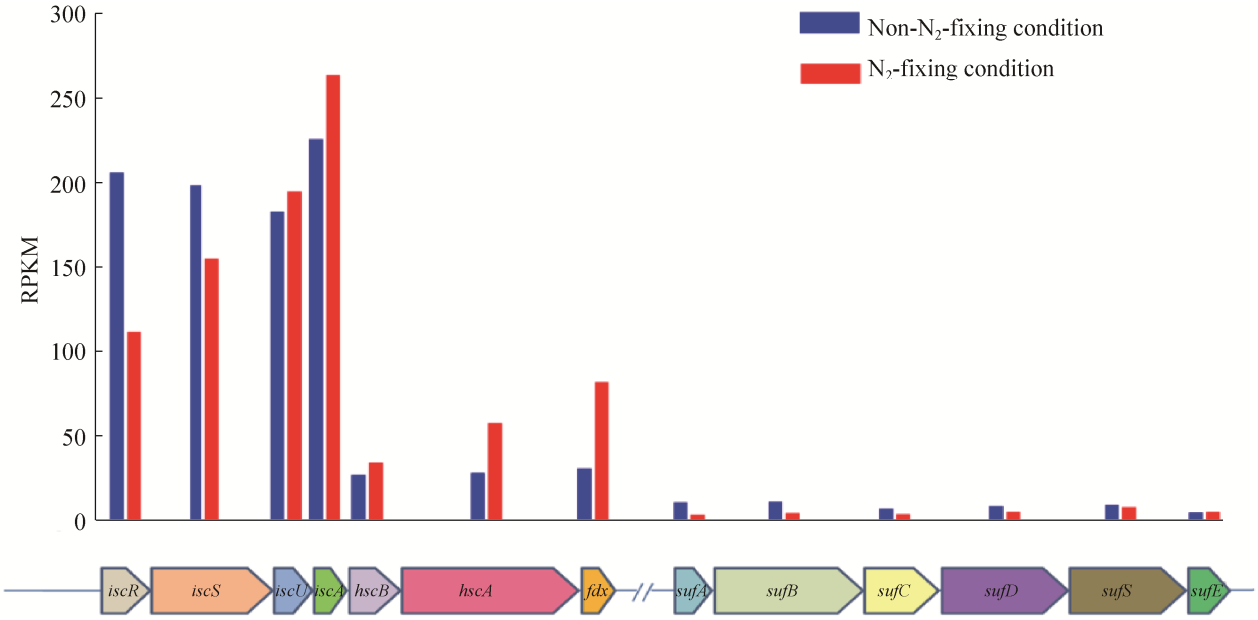

Figure 4. Differential expression of the genes involved in the synthesis of the Fe-S cluster in N_2-fixing and non-N_2-fixing conditions.

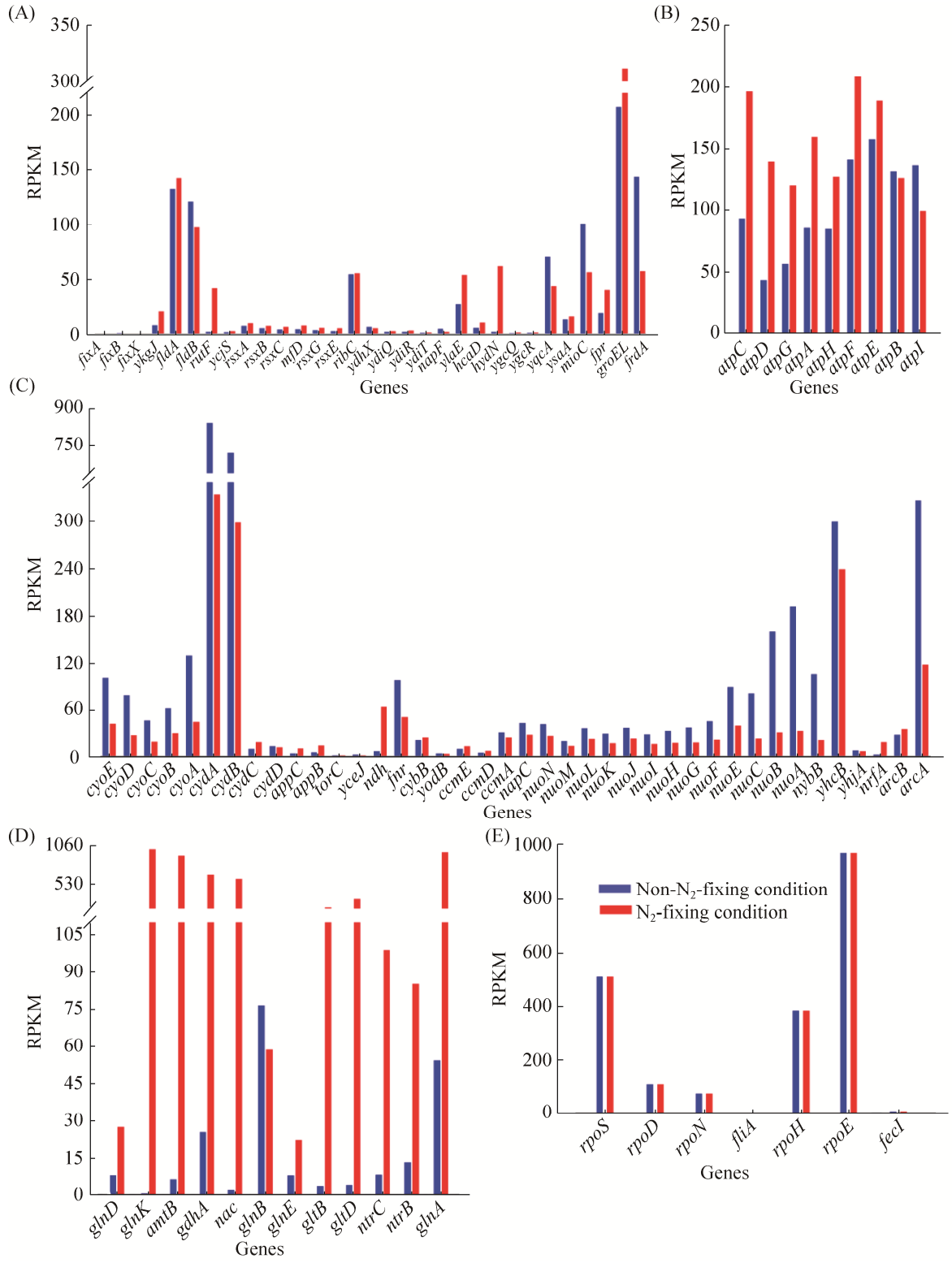

Figure 5. Differential expression of the genes relating to the electron transport, ATP synthase, respiration and energy metabolism, nitrogen metabolism and specifying σ factors in the N_2-fixing and non-N_2-fixing conditions. A: The electron transport genes; B: The *atp* genes encoding ATP synthase; C: The genes involved in respiration and energy metabolism; D: The specific genes for nitrogen metabolism; E: The genes specifying σ factors.

2.8 Transcriptional analysis of ATPase

Most biological nitrogen fixation products are catalysed by the molybdenum nitrogenase enzyme according to the following reaction: $N_2+8e^-+16MgATP+8H^+ \rightarrow 2NH_3+H_2+16MgADP+16Pi$[24]. The nitrogen fixation process is coupled to the hydrolysis of 16 equivalents of ATP. In *E. coli* 78-7, except for *atpB* and *atpI*, the other *atp* genes are highly expressed under the N_2-fixing condition compared to under the non-N_2-fixing condition (Figure 5-B).

2.9 Respiration and energy metabolism

Nitrogen fixation was performed in anaerobic or microanaerobic conditions, as nitrogenase is very sensitive to oxygen. In *E. coli* 78-7, several genes, including *ccmDE* encoding cytochrome c biogenesis protein, *appBC* encoding cytochrome bd-II oxidase subunits and *cybB* encoding *c*ytochrome b561, are up-regulated from 1.15-fold to 2.87-fold. The *cyoABCDE* and *cydAB* are weakly down-regulated in the N_2-fixing condition compared to in the non-N_2-fixing condition. Notably, *ndh* encoding respiratory NADH dehydrogenase is up-regulated 10.38-fold (Figure 5-C). It has been reported that there are two transcriptional regulators controlling independent networks of oxygen-regulated gene expression in *E. coli*[25]. One is a two-component sensor–regulator system (ArcB-A), which represses a wide variety of aerobic enzymes under anaerobic conditions. The other is FNR, the transcriptional regulator essential for expressing anaerobic respiratory processes[26]. In this study, the *E. coli fnr* gene is found to be down-regulated 0.52-fold in the N_2-fixing condition. *arcA* and *arcB* are down-regulated 0.36-fold and 1.7-fold, respectively, in the N_2-fixing condition compared to in the non-N_2-fixing condition. The data are consistent in that *fnr* expression in *E. coli* is weakly repressed by anaerobiosis, while *fnr* gene expression in *B. subtilis* is strongly activated by anaerobiosis[27]. The *narIJHG* encoding nitrate reductase subunits are up-regulated from 2.07-fold to 2.64-fold. The *nirBC* encoding nitrite reductase subunits are up-regulated. The *narK* encoding nitrate/nitrite transporter is weakly down-regulated in the N_2-fixing condition. Our results are consistent with reports that the *nasDE* and *narGHI* were induced by the low oxygen supply in *B. subtilis*[28].

2.10 Transcriptional analysis of nitrogen metabolism

It has been reported that in enteric bacteria, including *E. coli* and *K. oxytoca*, the global transcriptional control of the enzymes involved in nitrogen assimilation was mediated by a two-component nitrogen regulatory (*ntr*) system encoded by the *ntrBC* genes. In *E. coli* 78-7, the *glnAntrBC* operon is up-regulated 17.96-, 6.5- and 11.98-fold, respectively, in the N_2-fixing condition compared to in the non-N_2-fixing condition (Figure 5-D). The first three up-regulated genes in the N_2-fixing condition are *nac*, *glnK* and *amtB* with a 334-fold, 2154-fold and 147-fold increase, respectively. It was reported that binding of GlnK to the ammonia channel AmtB regulates the channel, thereby controlling ammonium influx in response to the intracellular nitrogen status in *E. coli*[29]**.** The nitrogen assimilation control protein (NAC) is a regulatory protein responsible for activating the transcription of operons such as *hutUH*, *putP* and *ureDABCEFG*, whose products supply the cell with ammonia or glutamate from histidine, proline and urea, respectively[30–31]. NAC is also responsible for repressing the transcription of the *gdhA* and *gltBD* operons, whose products are involved in assimilating ammonia under conditions of nitrogen excess or limitation[30]. The expression of the *nac* gene is entirely dependent on (NtrC) and σ^{54}. In *E. coli* 78-7, *nac* is significantly differently expressed, suggesting it plays an important role in the regulation of nitrogen metabolism. *gltB* and *gltD* are up-regulated 60- and 82-fold, respectively and *gdhA* is up-regulated 26-fold. However, *glnB* is weakly

down-regulated, while *glnD* and *glnE* are up-regulated 3.4-fold and 2.7-fold in the N_2-fixing condition compared to in the non-N_2-fixing condition.

2.11 Transcriptional analysis of the sigma factors

In bacteria, the regulation of gene expression occurs basically at the level of transcription. Although repressors and activators can markedly affect the transcriptional efficiency, the interactions between RNA polymerase (RNAP) and the promoters decide the specificity of the transcription reaction[32]. The RNA polymerase holoenzyme (holo RNAP) in bacteria is constituted of core RNAP ($\alpha_2\beta'\beta\sigma\omega$) and the sigma ($\sigma$) factor, and its σ factor recognizes promoter regions and then initiates transcription[33].

There are seven known σ factors in *E. coli*, each regulating a subset of genes with specific functions[32–33]. RpoD (σ^{70}) is the housekeeping σ factor responsible for the expression of essential genes. Our previous results demonstrated that *E. coli* σ^{70} bound to the *Paenibacillus nif* gene promoter[3], indicating that the *Paenibacillus nif* gene operon in *E. coli* is recognized and transcribed by *E. coli* σ^{70}-RNAP. Here, we find that *rpoD* is expressed at similar levels in both the non-N_2-fixing and N_2-fixing conditions (Figure 5-E and Supplementary Table S10). In addition, *rpoN*, which is mainly responsible for the transcription of genes in nitrogen utilization, is expressed at similar levels in both the non-N_2-fixing and N_2-fixing conditions. It was reported that the expression of *rpoN* was induced by nitrogen limitation. Perhaps the combination of limited oxygen and nitrogen here disturbs this regulation and makes the *rpoN* and other σ factors transcribe at similar levels in both the non-N_2-fixing and N_2-fixing conditions. RpoS (σ^S), a σ factor involved in gene expression in the stationary phase and many stress conditions, RpoH (σ^H) acting in heat shock response and RpoE (σ^E) involved in extra cytoplasmic/heat stress are weakly down-regulated by the N_2-fixing condition. *fliA* (σ^F) directing the transcription of flagellar genes and *fecI* acting in iron transport are weakly transcribed in the N_2-fixing condition.

3 Discussion

In this study, the transcriptomic analysis of the whole genome of the recombinant *E. coli* 78-7 cultured under non-N_2-fixing (air and 100 mmol/L NH_4^+) and nitrogen-fixing (without O_2 and NH_4^+) conditions is implemented. Our study reveals that the nine genes *nifB*, *nifH*, *nifD*, *nifK*, *nifE*, *nifN*, *nifX*, *hesA* and *nifV* from *Paenibacillus* are significantly transcribed in *E. coli* under both nitrogen-fixing and non-nitrogen-fixing conditions, indicating that the σ^{70}-dependent *nif* promoter of the *Paenibacillus nif* gene operon was efficiently recognized and transcribed by *E. coli* σ^{70}-RNAP. The data are consistent with our previous demonstration using electrophoretic mobility shift assays (EMSAs) whereby *E. coli* σ^{70}-RNAP bound to the *Paenibacillus nif* gene promoter *in vitro*[3]. However, the result of similar transcription levels of the nine genes *nifBHDKENXhesAnifV* in *E. coli* is very different from that in *P. polymyxa* WLY78 where nine genes *nifBHDKENXhesAnifV* are differently expressed under both condition[4], supporting that the negative regulation of *nif* gene transcription by ammonium and oxygen is bypassed in heterologous *E. coli*.

Furthermore, we investigate the transcription levels of some non-*nif* genes which are required for nitrogen fixation in *E. coli*. The *mod* and *feoAB* encoding transporters of Mo and Fe, respectively, and *fldA* and *fldB* encoding electron transporters are transcribed at low levels in both conditions. The *cys* genes specific for transport of S is highly up-regulated in N_2-fixing condition compared to non- N_2-fixing condition. The *isc* system specific for the synthesis of the Fe-S cluster is transcribed in

medium level in both conditions, whereas *suf* operon specific for the synthesis of the Fe-S cluster is transcribed at very low level in both conditions. Our current results are different from those obtained in *P. polymyxa* WLY78 where those non-*nif* genes are coordinately induced with *nif* genes under N_2-fixing condition[4].

It has been demonstrated that *E. coli groEL* (encoding molecular chaperones) play a role in biogenesis of MoFe protein. The level of MoFe protein is drastically reduced in an *E. coli groEL* mutant and overexpression of the *groE* operon in *K. oxytoca* leads to accumulation of MoFe protein[23]. In this study, we found that *E. coli groEL* are highly expressed in both N_2-fixing and non-N_2-fixing conditions, and are up-regulated by N_2-fixing condition. The highly expressed *groEL* might play important role in nitrogen fixation of *E. coli*.

Most of the genes involved in nitrogen metabolism are significantly up-regulated in N_2-fixing condition compared to non-N_2-fixing condition. Especially, *nac*, *glnK* and *amtB* are significantly up-regulated in N_2-fixing condition. Other genes involved in nitrogen metabolism, such as *glnAntrBC* operon, *gltB*, *gltD* and *gdhA* was up-regulated in the N_2-fixing condition. Our results are consistent with the previous reports that limited nitrogen activated the expression of the specific genes for nitrogen assimilation, such as *ntrBC* and *glnA*[30–31,34].

It is well known that the global level of *nif* regulation in *K. oxytoca* is mediated by the NtrB–NtrC two-component regulatory system[20]. The level of phosphorylated NtrC controls expression of the *nifL-nifA* operon which in turn controls the transcription of other 18 *nif* genes exception of *nifL-nifA*[20]. The genome of *P. polymyxa* WLY78 does not contain *nifA* and *ntrC*, but it has a *glnR* gene whose predicted product is a global regulator of nitrogen metabolism existing in Gram-positive bacteria, including *Paenibacillus* and *Bacillus*. The absence of GlnR in *E. coli* might explain why *Paenibacillus nif* gene transcription in *E. coli* is not regulated by ammonium and oxygen. In addition, the constitutive expression of the foreign *nif* genes suggests that NtrC is not involved in the regulation of *nif* gene transcription in *E. coli*, although NtrC activates the expression of NifA in enteric diazotrophic *K. oxytoca*.

As described above, this study reveals that the *Paenibacillus nif* genes are significantly transcribed in *E. coli* 78-7 in both N_2-fixing and non-N_2-fixing condition, whereas the native non-*nif* genes, such as Fe^{2+}/Fe^{3+} transporters, electron transporters and Fe-S cluster assembly systems required for nitrogen fixation, are transcribed at low level in N_2-fixing condition in *E. coli.* The data suggest that the highly transcribed *nif* genes alone are not enough to maintain high nitrogenase activity of the recombinant *E. coli*. The lower nitrogenase activity in *E. coli* than that in *Paenibacillus* might be caused by the low expression of those native non-*nif* genes of *E. coli.* Thus, we deduce that the nitrogenase activity of the recombinant *E. coli* 78-7 can be enhanced by increasing the transcription levels of the native Fe^{2+}/Fe^{3+} transporters, electron transporters and Fe-S cluster assembly systems of the recombinant *E. coli* 78-7. The hypothesis is supported by our recent reports that *Paenibacillus suf* operon and *pfoAB*, *fldA* and *fer* (the potential electron transport genes), and *K. oxytoca nifSU* (Fe–S cluster assembly) and *nifFJ* (electron transport specific for nitrogenase) can increase nitrogenase activity of the recombinant *E. coli* 78-7[35].

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携带 *Paenibacillus polymyxa* WLY78 固氮基因簇(*nif*)的重组大肠杆菌 *Escherichia coli* 78-7 转录组分析

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摘要：【目的】来自 *Paenibacillus polymyxa WLY78* 的固氮基因簇(*nifBHDKEfNXhesAnifV*)可以转化入 *Escherichia coli* 中表达并使重组大肠杆菌合成有固氮活性的固氮酶。本文拟通过对重组大肠杆菌 *E. coli* 78-7 的转录组分析以提高其固氮能力。【方法】对固氮条件(无氧无 NH_4^+)和非固氮条件(空气和 100 mmol/L NH_4^+)培养的重组大肠杆菌 *E. coli* 78-7 进行转录组分析。【结果】*nif* 基因在两种培养条件下显著表达，说明在重组大肠杆菌中可规避原菌中氧气和 NH_4^+对 *nif* 基因的负调控。对于固氮过程必需的非 *nif* 基因，如参与钼、硫、铁元素转运的 *mod*、*cys* 和 *feoAB*，这些基因在两种培养条件下表达水平有差异。而参与铁硫簇合成的 *suf* 和 *isc* 基因簇在两条件下表达水平差异巨大。此外，参与氮代谢的基因在固氮条件下显著上调。【结论】重组大肠杆菌中与固氮相关的非 *nif* 基因在该菌的固氮过程中具有较大影响，本文对在异源宿主中调高固氮酶活性研究具有重要意义。

关键词：大肠杆菌，*nif* 基因，生物固氮，类芽孢杆菌，转录

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