The nitrite reductase encoded by nirBDs in Pseudomonas putida Y-9 influences ammonium transformation

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It is unknown whether nirBDs, which conventionally encode an NADH nitrite reductase, play other novel roles in nitrogen cycling. In this study, we explored the role of nirBDs in the nitrogen cycling of Pseudomonas putida Y-9. nirBDs had no effect on organic nitrogen transformation by strain Y-9. The △nirBD strain exhibited higher ammonium removal efficiency (90.7%) than the wild-type strain (76.1%; P < 0.05) and lower end gaseous nitrogen (N2O) production. Moreover, the expression of glnA (control of the ammonium assimilation) in the △nirBD strain was higher than that in the wild-type strain (P < 0.05) after being cultured in ammonium-containing medium. Furthermore, nitrite noticeably inhibited the ammonium elimination of the wild-type strain, with a corresponding removal rate decreasing to 44.8%. However, no similar impact on ammonium transformation was observed for the △nirBD strain, with removal efficiency reaching 97.5%. In conclusion, nirBDs in strain Y-9 decreased the ammonium assimilation and increased the ammonium oxidation to nitrous oxide.

KEYWORDS
Pseudomonas putida Y-9, nirBDs, glnA, ammonium assimilation, ammonium oxidation

Introduction

Microorganisms show multiple nitrogen transformation pathways, contributing to the natural nitrogen-cycling balance (Canfield et al., 2010; Kuyper et al., 2018). Ammonium (NH4+) is the preferred nitrogen source for most bacteria and archaea (Burkovski, 2003; Muro-Pastor et al., 2005). Ammonium assimilation by
microorganisms leads to high ammonium removal efficiency and mitigation of harmful effects on the environment. However, the role of ammonium assimilation in NH$_4^+$ removal is often neglected.

Simultaneous nitrification and denitrification (SND) has been widely applied in wastewater treatment plants as an attractive biological approach to nitrogen removal, owing to its low investment costs and high efficiency (Jin et al., 2015; Lei et al., 2019). Usually, the SND pathway by bacteria is identifying, and characterizing nitrogen removal associated with SND strains (Jin et al., 2015; Chen et al., 2016, 2021; Lei et al., 2019). Usually, the SND pathway by bacteria is NH$_4^+$→NH$_3$OH→NO$_2^-$→NO$_3^-$→NO→N$_2$O→N$_2$. Ammonia monoxygenase catalyzes NH$_4^+$ oxidation to NH$_3$OH and is encoded by amoa, amob, or amoc (Kuyper et al., 2018). Nitrate reductase encoded by narG, narH, napA, or nasA can catalyze NO$_3^-$ reduction to NO$_2^-$. Nitrite reductase catalyzing NO$_2^-$ reduction to NO or NH$_4^+$ is encoded by nirK, nirS, nirB, or nirD (Kuyper et al., 2018; Yang L. et al., 2019; Xia et al., 2020; Zhang et al., 2020).

*nirBDs*, which conventionally encode an NADH nitrite reductase in many bacteria (Jackson et al., 1981; Lin and Stewart, 1996), show different characteristics under different conditions. For example, dissimilatory nitrite reductase encoded by *nirBDs* in *Escherichia coli* and other enterobacteria is only expressed under anaerobic conditions (Macdonald et al., 1985; Gennis and Stewart, 1996), while *nirBDs* in *Streptomyces coelicolor* only encode the assimilatory nitrite reductase under aerobic conditions (Tiffert et al., 2008; Fischer et al., 2012). Recent studies have shown that *nirBDs* in *S. coelicolor* also play an integral role in the nitric oxide (NO) homeostatic regulation system that eliminates nitrite (NO$_2^-$) from cultures during NO$_3^-$ reduction (Yukioka et al., 2017).

We previously observed that the SND strain *Pseudomonas putida* Y-9 exhibited excellent removal ability for NH$_4^+$ and NO$_3^-$ (Xu et al., 2017). Further studies clarified that strain Y-9 could transform NH$_4^+$ into nitrous oxide (N$_2$O) under aerobic conditions (Huang et al., 2019) and remove NH$_4^+$ mainly through assimilation (Huang et al., 2021a). In addition, strain Y-9 can remove NO$_3^-$ via simultaneous nitrate assimilation, dissimilatory nitrate reduction to ammonium (DNRA), and denitrification under aerobic conditions. Within these contexts, the enzyme encoded by *nirBDs* catalyzes NO$_3^-$ reduction to NH$_4^+$ during assimilation and DNRA (Huang et al., 2020). We hypothesized that *nirBDs* in strain Y-9 might be functional in other roles than encoding the traditional nitrite reductase. In this study, the effects of *nirBDs* on different nitrogen transformation pathways in strain Y-9 were explored. Results revealed that knocking out *nirBDs* promoted ammonium assimilation and weakened the emission of nitrous oxide. These findings provide a new understanding on how to use strain Y-9 for the treatment of ammonium nitrogen polluted water.

### Materials and methods

#### Strain and culture media

The SND bacterium *P. putida* Y-9 (GenBank No. KP410740) used here was obtained from our previous study (Xu et al., 2017). *nirBDs* were knocked out from the genome of strain Y-9 using homologous recombination technology, as previously described (Huang et al., 2020), mediated via plasmid pLP12. The primer sequences for *nirBDs* knockout are shown in **Supplementary Table 1. Supplementary Figure 1** shows the successful construction of the *nirBD* deletion mutants.

Lysogeny broth (LB) liquid medium consisting of (per liter) 10.0 g Tryptone, 5.00 g Yeast extract, and 10.0 g NaCl (pH adjusted to 7.0–7.2) was used for strain enrichment.

Nitrification medium (NM) comprised (per liter) 7.00 g K$_2$HPO$_4$, 3.00 g KH$_2$PO$_4$, 0.10 g MgSO$_4$·7H$_2$O, 0.50 g (NH$_4$)$_2$SO$_4$, 0.05 g FeSO$_4$·7H$_2$O, and 5.13 g CH$_3$COONa (pH adjusted to 7.2). NM was used to determine the ammonium transformation characteristics of strain Y-9.

The composition of the denitrification medium (DM) was (per liter) 7.00 g K$_2$HPO$_4$, 3.00 g KH$_2$PO$_4$, 0.10 g MgSO$_4$·7H$_2$O, 0.72 g KNO$_3$ (DM-1) or 0.49 g NaNO$_2$ (DM-2), 0.05 g FeSO$_4$·7H$_2$O, and 5.13 g CH$_3$COONa (pH adjusted to 7.2). The two DM formulae were used to evaluate the nitrate or nitrite transformation ability of strain Y-9.

The organic nitrogen medium (OM) was composed of (per liter, pH 7.2) 7.00 g K$_2$HPO$_4$, 3.00 g KH$_2$PO$_4$, 0.10 g MgSO$_4$·7H$_2$O, 0.79 g tryptone, 0.05 g FeSO$_4$·7H$_2$O, and 0.788 g peptone (pH adjusted to 7.2). OM was used to evaluate the organic nitrogen conversion ability of strain Y-9.

The SND medium contained 7.00 g K$_2$HPO$_4$, 3.00 g KH$_2$PO$_4$, 0.10 g MgSO$_4$·7H$_2$O, 0.79 g tryptone, 0.05 g FeSO$_4$·7H$_2$O, and 0.788 g peptone (pH adjusted to 7.2). The two types of SND media were used to assess the nitrogen transformation ability of strain Y-9 with ammonium and either nitrate or nitrite.

Solid plates were prepared using 2.0% (w/v) agar added into the above liquid media. Before use, all of the above media were autoclaved for 30 min at 0.11 MPa and 121°C.

#### Estimation of the role of *nirBDs* in nitrogen transformation by strain Y-9

Single colonies of the Y-9 and Δ*nirBD* strains were enriched for 36 h using LB liquid medium. Preculture (8 mL) was harvested and washed twice with sterile pure water by centrifugation (4,000 rpm, 8 min), inoculated into 100 mL of NM, DM-1, DM-2, OM, SND-1, or SND-2, and then cultivated at 15°C with shaking at 150 rpm. No strains were added for control treatments. Three replicates were performed for each experiment. Culture samples in different nitrogen media were
taken out to measure the optical density of the strain (OD\textsubscript{600}) and different types of nitrogen using a spectrophotometer (Huang et al., 2019).

Detection of N\textsubscript{2}O and N\textsubscript{2} after Y-9 and \textit{\textDelta nirBD} strains cultured in ammonium medium

The precultures of the Y-9 and the \textit{\textDelta nirBD} strains were inoculated into media containing (1\textsuperscript{5}NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (10 atom%) in 250 mL serum bottles. Then, the serum bottles were sealed with a rubber septum, aerated with oxygen, and incubated at 15°C for 48 h with shaking at 150 rpm. Finally, the 15\textsubscript{N}N\textsubscript{2}O and 15\textsubscript{N}N\textsubscript{2} present in the headspace were collected with a needle and detected using GC-MS (Agilent, USA) and GC-IRMS (Thermo Fisher Scientific, USA), respectively (Ai et al., 2011; Ye et al., 2016; Huang et al., 2019).

Monitoring the expression of \textit{glnA}

DNA fragments of the Y-9 and \textit{\textDelta nirBD} strains containing upstream regions of \textit{glnA} were PCR-amplified and ligated into the upstream region of the promoter-less \textit{lacZ} in pRG970Km (Table 1) to generate the reporter plasmids p970 Km-glnA. Then, the Y-9 and \textit{\textDelta nirBD} strains containing the reporter plasmids were cultured in NM medium with shaking at 150 rpm, and aliquots were collected after 2 days of incubation. The activity of β-galactosidase was measured as described by Miller (1972). Relative expression of glutamine synthetase encoded by \textit{glnA} was represented by OD\textsubscript{420}−galactosidase measured as described by Miller (1972).

Analytical methods

Total nitrogen (TN, including cells) content in the suspension was estimated using the alkaline potassium persulfate digestion-UV spectrophotometric method. The contents of NH\textsubscript{4}+, NO\textsubscript{2}−, and NO\textsubscript{3}− in the supernatant were quantified using the indophenol blue method, the hydrochloric acid photometry method, and the N-(1-naphthalene)-diaminoethane spectrophotometry method, respectively, after samples were centrifuged at 8,000 rpm for 5 min. The above analyses were carried out according to the guidelines set by the State Environmental Protection Administration of China (2002). The decline rate of nitrogen (TN, NH\textsubscript{4}+, NO\textsubscript{2}−, and NO\textsubscript{3}−) was calculated using the equation: \( R_v = (T_1-T_2)/T_1 \times 100\% \), where \( R_v \) represents the nitrogen decrease efficiency, and \( T_1 \) and \( T_2 \) are the original and eventual contents of nitrogen in the system (mg L\textsuperscript{-1}). Culture pH was measured by a pH meter.

The SPSS Statistics program (version 22) and Microsoft Excel 2010 were used for statistical analysis, and Origin 8.6 was used to produce the graphics.

Results and discussion

Impact of \textit{nirBDs} on organic nitrogen transformation by strain Y-9

Both the wild-type and \textit{\textDelta nirBD} strains grew vigorously in the OM and did not reach the stationary phase until 4 days (Figure 1), consistent with data from Fischer et al. (2012), who reported that strain \textit{S. coelicolor} A3(2) (\textit{\textDelta nirBD}) grew well on the plate containing casamino acids. The TN concentrations in the \textit{\textDelta nirBD} and wild-type strain culture systems decreased gradually throughout the experiments and finally only dropped by 18.0 mg L\textsuperscript{-1} without NO\textsubscript{3}− accumulation. This phenomenon

| Strain or plasmid | Description | References or source |
|-------------------|-------------|---------------------|
| \textit{\textDelta nirBD Y-9} | nirBD genes in-frame deletion in strain Y-9; Km\textsuperscript{r} | Huang et al., 2020 |
| \textit{E. coli} | DH5\textalpha | Lab stock |
| \textit{Pseudomonas putida} Y-9 | Wild type | Xu et al., 2017 |
| Plasmids | | |
| pRG970Km | Cloning vector containing promoterless lacZ/\textit{lacZYA-argF}-U69 recA1 endA1 hsdR17(rK::mK- supE44 thi-1 gyrA relA1) | Yan et al., 2009 |
| p970Km-glnA | pRG970 Km containing a glnA transcriptional fusion, Km\textsuperscript{r} | This study |
suggested that strain Y-9 preferred to utilize organic nitrogen for cellular growth rather than converted it into gaseous nitrogen. Besides, variations in all of the measured nitrogen species within the culture medium of the wild-type and ΔnirBD strains were consistent across the entire incubation period. These results indicated that knocking out nirBDs did not affect organic nitrogen transformation by strain Y-9.

Impact of nirBDs on ammonium transformation by strain Y-9

To explore the effects of nirBD on the ammonium transformation process, the Y-9 and ΔnirBD strains were cultured in NM. Both strains grew vigorously (Figure 2), consistent with a previous study (Fischer et al., 2012), with the ΔnirBD strain of S. coelicolor growing similar to the wild-type strain on glucose minimal medium agar plates supplemented with NH$_4^+$ as the nitrogen source. These results illustrated that nirBD was not essential for the utilization of ammonium by strain Y-9. Intriguingly, the NH$_4^+$ removal efficiency by ΔnirBD strain (90.7%) was higher than that by the wild-type strain (76.1%) after 2 days of incubation ($P < 0.05$), which might be attributed to the stronger assimilation or ammonium oxidation ability of the ΔnirBD strain compared to the wild-type strain (Li et al., 2017; Jin et al., 2019).

The gas produced during the NH$_4^+$ removal process was N$_2$O and not N$_2$, according to the results of the GC test, consistent with our previous studies (Huang et al., 2019). Moreover, the δ$^{15}$N/δ$^{14}$N ratio of N$_2$O in the ΔnirBD strain culture system (2.46) was lower than that in the wild-type strain culture system (3.63). Similarly, the decrease in TN in the ΔnirBD strain culture system (15.7 mg L$^{-1}$) was lower than that in the wild-type strain culture system (21.5 mg L$^{-1}$; $P < 0.05$; Figure 2). These results illustrated that knocking out nirBDs reduced the production of N$_2$O, suggesting that the knockout accelerated ammonium assimilation instead of the ammonium oxidation by strain Y-9.

Previous studies have proven that the glutamine synthetase encoded by glnA gene plays an important role in the ammonium assimilation process (Gupta et al., 2012; Van Heeswijk et al., 2013). In this study, glnA was found in strain Y-9 according to the results of the genome-wide scan. Considering that knocking out the nirBDs accelerated ammonium assimilation by strain Y-9, we speculated that the expression of nirBDs might influence the expression of glnA. Thus, the expression of glnA in the ΔnirBD strain was further detected. β-Galactosidase was utilized as a reporter to examine glnA promoter activity. The results showed that the β-galactosidase activity in the ΔnirBD strain was obviously higher than that in the wild-type strain ($P < 0.05$; Figure 3A). Moreover, qPCR results showed that the expression of glnA in the ΔnirBD strain was higher than that in wild-type strain Y-9 (Figure 3B). These findings suggested that knocking out nirBDs would promote the expression of glnA, accelerating ammonium assimilation.

Impact of nirBDs on nitrate transformation by strain Y-9

The OD$_{600}$ of the wild-type strain increased significantly from 0.17 to 1.23, while the ΔnirBD strain exhibited a slower growth trend when culturing in SND-1 medium ($P < 0.05$; Figures 4A,B). These results are consistent with those observed when the two strains grew on agar plates supplemented with NO$_3^-$ as the sole nitrogen source but differed from those when strain NM7 (ΔnirBD) failed to grow under similar
FIGURE 2
The growth curve and nitrogen transformation performance of strain Y-9 in nitrification medium at 15°C. (A) The wild-type strain Y-9. (B) The \( \Delta \text{nirBD} \) strain Y-9.

FIGURE 3
GlnA expression in strain Y-9 and \( \Delta \text{nirBD} \) strain Y-9. (A) \( \beta \)-Galactosidase activity. (B) Relative expression abundance of glnA. The different lowercase letters above the bars indicate significant differences among treatments (\( P < 0.05 \)).

The NirBD protein in strain Y-9 was previously shown to catalyze \( \text{NO}_2^- \) reduction to \( \text{NH}_4^+ \) (Huang et al., 2020). Accordingly, a little amount of \( \text{NO}_2^- \) was detected in our experiments, while \( \text{NH}_4^+ \) gradually increased during the cultivation of the wild type strain Y-9 (Figure 4A). In contrast, the accumulation of \( \text{NO}_2^- \) was nearly equivalent to the decrease in \( \text{NO}_3^- \), but \( \text{NH}_4^+ \) was undetectable during the entire \( \text{NO}_3^- \) transformation process of the \( \Delta \text{nirBD} \) strain (Figure 4B). These findings suggest that \( \text{NO}_2^- \) converted from \( \text{NO}_3^- \) could not be further reduced to \( \text{NH}_4^+ \) by strain Y-9 when \( \text{nirBDs} \) was knocked out, resulting in \( \text{NO}_2^- \) accumulation in the cultures.

After cultivating the wild-type strain for 4 days, the decrease in \( \text{NO}_3^- \) and TN reached 93.7 mg L\(^{-1}\) and 26.4 mg L\(^{-1}\), respectively. Moreover, culture pH increased over the whole cultivation period for the wild-type strain (Figure 4A). These dynamics were due to a small amount of \( \text{NO}_3^- \) being removed by strain Y-9 via weak denitrification (Huang et al., 2020). The \( \Delta \text{nirBD} \) strain achieved a total \( \text{NO}_3^- \) reduction of 105.6 mg L\(^{-1}\), while TN was barely diminished after 4 d of incubation (Figure 4B). This finding could be because the knocking out of \( \text{nirBDs} \) resulted in \( \text{NO}_2^- \) accumulation in the medium, finally inhibiting the growth of strain Y-9 and its TN degrading ability.

Impact of \( \text{nirBDs} \) on nitrite transformation by strain Y-9

Growth of the wild-type and \( \Delta \text{nirBD} \) strains increased slowly during the initial 3 days of cultivation in the \( \text{NO}_2^- \) - containing medium (Figures 4C,D). The probable reason for the slow growth was the high content of free nitrous acid (FNA, > 0.021 mg HNO\(_2\)-N L\(^{-1}\) at 3 days) released due to
FIGURE 4
The growth curve and nitrogen transformation performance of strain Y-9 in denitrification medium at 15°C. (A) The wild-type strain Y-9 in DM-1. (B) The △nirBD strain Y-9 in DM-1. (C) The wild-type strain Y-9 in DM-2. (D) The △nirBD strain Y-9 in DM-2.

the NO$_2^-$ inhibition in strain metabolism (Vadivelu et al., 2006). The wild-type strain grew quickly, with a concomitant considerable reduction of NO$_2^-$ and TN between days 3 and 4. After cultivation, the decrease in TN in suspension (44.2 mg L$^{-1}$) was lower than the reduced amount of NO$_2^-$ in the supernatant (82.6 mg L$^{-1}$; $P < 0.05$). Thus, some amount of NO$_2^-$ (44.2 mg L$^{-1}$) might have been lost from the system through denitrification, while the remainder (38.4 mg L$^{-1}$) could have been assimilated by the wild-type strain Y-9. NO$_2^-$ has well-documented toxicity to bacterial cells (Zemke et al., 2017), and strain Y-9 cannot directly absorb NO$_2^-$.

Moreover, NH$_4^+$ accumulation was tracked throughout the NO$_2^-$ transformation process (Figure 4C). Therefore, it is possible that most of the NO$_2^-$ that had not been removed through denitrification could have been reduced to NH$_4^+$ through assimilation and DNRA by the wild-type strain Y-9, in accordance with our previous results (Huang et al., 2020). The △nirBD strain still grew at a slow rate after 3 days and could seldom remove NO$_2^-$.

Concomitantly, the transformation of NO$_2^-$ in the supernatant only decreased by 10.0 mg L$^{-1}$, which was nearly equal to the decreased TN levels in the culture suspension (8.8 mg L$^{-1}$), indicating that the △nirBD strain also conducted weak denitrification. Additionally, NH$_4^+$ was undetectable throughout the cultivation of the △nirBD strain (Figure 4D). Taken together, these results show that knocking out nirBDs does not allow noxious NO$_2^-$ to be reduced to NH$_4^+$, thereby inhibiting cellular growth and denitrification ability.

Influence of nirBDs on nitrogen transformation of strain Y-9 in SND-1 medium

Knocking out nirBDs accelerated the assimilation of NH$_4^+$ by strain Y-9 (Figure 2) and led to the near complete conversion of NO$_3^-$ into NO$_2^-$ while inhibiting the transformation of NO$_2^-$ (Figures 4B,D). We further evaluated the impact of nirBDs on nitrogen transformation when NH$_4^+$ and NO$_3^-$ coexisted in the medium. The wild-type and △nirBD strains grew vigorously after a 1-day lag phase and reached the stationary phase on days 3 and 2, respectively (Figures 5A,B).
strain was faster than that of the wild-type strain \((P < 0.05)\), consistent with the results when using \(\text{NH}_4^+\) as the sole nitrogen source (Figure 2). These results were attributed to \textit{nirBD} knockout that accelerated the assimilation of \(\text{NH}_4^+\). The final \(\text{NH}_4^+\) removal efficiency by the wild-type and \(\Delta\text{nirBD}\) strains was 92.13 and 95.87\%, respectively, which were both slightly lower than the \(\text{NH}_4^+\) removal efficiency when incubating the two strains in NM (both approximately 100\%) (Figure 2). Consequently, the existence of \(\text{NO}_3^-\) led to little inhibition of \(\text{NH}_4^+\) transformation. Moreover, the contents of TN in suspension both dropped down by approximately 20 mg L\(^{-1}\) in the two systems containing the wild type or \(\Delta\text{nirBD}\) strain, consistent with the decrease in TN in suspension when \(\text{NH}_4^+\) was used as the sole nitrogen source (Figure 2). Thus, no denitrification occurred in strain Y-9 when \(\text{NH}_4^+\) and \(\text{NO}_3^-\) coexisted in the system.

The decrease in \(\text{NO}_3^-\) content in suspension reached only 26.4 mg L\(^{-1}\), and the \(\text{NO}_2^-\) was undetected after 4 days of wild type strain cultivation, indicating that strain Y-9 utilized \(\text{NH}_4^+\) preferentially when \(\text{NH}_4^+\) and \(\text{NO}_3^-\) coexisted in the medium. Similar results were observed by Xu et al. (2017), who used 200 mg L\(^{-1}\) of \(\text{NH}_4^+\) and \(\text{NO}_3^-\) to cultivate strain Y-9. Yang J. R. et al. (2019) and Zhang et al. (2022) also reported that \textit{Acinetobacter} sp. JR1 and \textit{P. taiwanensis} EN-F2 preferred to remove \(\text{NH}_4^+\) from a medium containing both \(\text{NH}_4^+\) and \(\text{NO}_3^-\). Nevertheless, the contents of \(\text{NO}_3^-\) in the \(\Delta\text{nirBD}\) strain cultures dropped by 72.3 mg L\(^{-1}\) and the accumulation of \(\text{NO}_2^-\) reached 61.5 mg L\(^{-1}\) at the end of the experiment (Figure 5B). The above results combined with variation in \(\text{NO}_3^-\) and \(\text{NO}_2^-\) concentrations when cultivating the \(\Delta\text{nirBD}\) strain in DM-1 medium (Figure 4B) suggested that \textit{nirBDs} reduced the \(\text{NO}_2^-\) resulting from \(\text{NO}_3^-\) respiration to \(\text{NH}_4^+\), and the denitrification ability of the \(\Delta\text{nirBD}\) strain was weak. In addition, when using \(\text{NH}_4^+\) or \(\text{NO}_3^-\) as the sole nitrogen source, the pH increased over the entire incubation process of the wild-type strain Y-9 but fluctuated during \(\Delta\text{nirBD}\) strain growth (Figures 2, 4A,B). Intriguingly, the pH increased during the entire cultivation period of the wild-type and \(\Delta\text{nirBD}\) strains when \(\text{NH}_4^+\) and \(\text{NO}_3^-\) were both available (Figures 5A,B). Thus, the coexistence of \(\text{NH}_4^+\) and \(\text{NO}_3^-\) might counteract the effects of \(\textit{nirBDs}\) knockout with regard to culture pH. Nevertheless, these dynamics require further investigation.

![FIGURE 5](image-url) The growth curve and nitrogen transformation performance of strain Y-9 in simultaneous nitrification and denitrification medium. (A) The wild-type strain Y-9 in SND-1. (B) The \(\Delta\text{nirBD}\) strain Y-9 in SND-1. (C) The wild-type strain Y-9 in SND-2. (D) The \(\Delta\text{nirBD}\) strain Y-9 in SND-2.
Influence of nirBDs on the nitrogen transformation of strain Y-9 in the SND-2 medium

When NH$_4^+$ and NO$_2^-$ coexisted in the medium, the wild-type and $\Delta$nirBD strains both barely grew within the first 2 days. Nevertheless, the $\Delta$nirBD strain exhibited higher growth than the wild type strain over the entire cultivation period ($P < 0.05$; Figures 5C,D), owing to the acceleration of NH$_4^+$ assimilation by strain Y-9, due to nirBDs knockout (Figure 2). The NH$_4^+$ removal rate by the $\Delta$nirBD strain (97.5%) was considerably higher than that of the wild-type strain (47.7%), but the decrease in TN in the $\Delta$nirBD strain culture system (9.1 mg L$^{-1}$) was lower than that in the wild-type strain culture system (13.2 mg L$^{-1}$; $P < 0.05$). This finding could be attributed to the denitrification that was inhibited when nirBDs were knocked out (Figures 4A,B). The decrease in NO$_2^-$ in the wild-type strain culture system was consistently greater than that of the $\Delta$nirBD strain culture system over the entire incubation period ($P < 0.05$), which might have been due to the stronger denitrification rate of the wild-type strain compared to the $\Delta$nirBD strain. However, the high concentration of NO$_2^-$ in the system could still inhibit the utilization of NH$_4^+$ by the wild-type strain, with residual NH$_4^+$ levels reaching 47.8 mg L$^{-1}$ at the end of the experiment (Figure 5C), consistent with the reports that the addition of NO$_2^-$ had a negative impact on ammonium removal of bacterium (Yang et al., 2012; Zhang et al., 2015, 2022). A noteworthy observation is that NO$_2^-$ had no impact on the ammonium efficiency of the $\Delta$nirBD strain, which reached 97.5% (Figure 5D), possibly due to nirBDs knockout leading to increased NH$_4^+$ assimilation and strain growth, thereby enhancing the tolerance of the strain to the toxic NO$_2^-$.

Conclusion

nirBDs, which conventionally encode an NADH nitrite reductase, also influence the ammonium transformation of P. putida Y-9. Knocking out nirBDs accelerated the ammonium assimilation and inhibited the emission of the greenhouse gas N$_2$O, thus alleviating the toxicity of nitrite in an ammonium and nitrite system.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: GenBank, KP410740.

Author contributions

XH: visualization, investigation, data curation, writing—original draft preparation, reviewing, and funding acquisition. YL: investigation, data curation, and formal analysis. LL: investigation and data curation. DX: project administration and supervision. ZL: conceptualization, methodology, and writing—reviewing and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

XH was employed by Guangxi Bossco Environmental Protection Technology Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.982674/full#supplementary-material

SUPPLEMENTARY FIGURE 1
nirBD deletion mutations detection (Lanes 1–6: deletion mutant; Lane 8: wild-type strain Y-9; Lane 9: DL5000 DNA Marker).
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