Methanol fermentation increases the production of NAD(P)H-dependent chemicals in synthetic methylotrophic Escherichia coli

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Abstract

Background: Methanol has attracted increased attention as a non-food alternative carbon source to sugar for biological production of chemicals and fuels. Moreover, the high degree of reduction of methanol offers some advantages in increasing the production yields of NAD(P)H-dependent metabolites. Here, we demonstrate an example of methanol bioconversion with the aim of improving production of NAD(P)H-dependent chemicals in synthetic methylotrophic Escherichia coli.

Results: A synthetic methylotrophic E. coli was engineered with a nicotinamide adenine dinucleotide (NAD+)-dependent methanol dehydrogenase (MDH) and ribulose monophosphate (RuMP) pathway. Regarding the limited MDH activity, the role of activator proteins in vivo was investigated, and the NudF protein was identified capable of improving MDH activity and triggering increased methanol metabolism. Using 13C-methanol-labeling experiments, we confirmed methanol assimilation in the methylotrophic E. coli. A cycling RuMP pathway for methanol assimilation was also demonstrated by detecting multiple labeled carbons for several compounds. Finally, using the NAD(P)H-dependent metabolite lysine as a test, the potential of methanol bioconversion to generate value-added metabolites was determined. To further characterize the benefit of methanol as the carbon source, extra NADH from methanol oxidation was engineered to generate NADPH to improve lysine biosynthesis by expression of the POS5 gene from Saccharomyces cerevisiae, which resulted in a twofold improvement of lysine production. Moreover, this new sink further pulled upstream methanol utilization.

Conclusion: Through engineering methanol metabolism, lysine biosynthesis, and NADPH regeneration pathway from NADH, the bioconversion of methanol to improve chemical synthesis was successfully achieved in methylotrophic E. coli.

Keywords: Methanol, Synthetic methylotrophic E. coli, Cofactor regeneration, NAD(P)H-dependent chemicals

Background

Microbial production of chemicals and biofuels from feedstock that are both inexpensive and abundant, such as natural gas, offers sustainable and economically attractive alternatives to traditional fermentation processes [1–3]. Given advances in methods of converting natural gas to methanol, methanol has attracted increased attention as a non-food alternative carbon source to sugar in microbial production processes [4–6]. Meanwhile, as the higher reduction degree of methanol than most lignocellulosic sugars, it might be used to enhance the production of some reductive products, such as alcohols, carboxylic acids, and fatty acids when used alone or as a co-substrate.

Methylotrophs represent a group of organisms that use methane or methanol as carbon and energy sources to
produce metabolites, including biofuels and chemicals. Efforts to engineer native methylotrophs (e.g., *Bacillus methanolicus*, and *Methylobacterium extorquens*) [7–9] have been hampered due to the inefficient genetic tools. Developing synthetic methylotrophy using platform organisms, such as *Corynebacterium glutamicum* and *E. coli*, has become an increasingly attractive possibility for methanol bioconversion [10–12].

In aerobic methylotrophs, methanol is initially oxidized to formaldehyde by methanol dehydrogenase (MDH). Formaldehyde is subsequently assimilated for energy generation via the serine pathway, the ribulose monophosphate (RuMP) pathway, or the ribulose bisphosphate (RuBP) pathway [5]. The MDHs can be divided into three classes based on their electron acceptor: pyrroloquinoline quinone (PQQ) dependent in Gram-negative bacteria, NAD dependent in Gram-positive bacteria, and oxygen dependent in methylotrophic yeasts, respectively [5, 13, 14]. As a favorable option for synthetic methylotrophy [5], electrons, derived from methanol oxidation that was catalyzed by NAD+-dependent MDHs, are stored in NADH, which can be used to improve production of target metabolites without sacrificing additional carbons. For the formaldehyde assimilation process, the RuMP pathway, which fixes formaldehyde to the pentose phosphate pathway (PPP) intermediate ribulose-5-phosphate via the two core enzymes, including 3-hexulose-6-phosphate synthase (HPS) and 6-phospho-3-hexuloseisomerase (PHI) [8], has been shown to be more bioenergetically favorable in terms of ATP generation.

*Escherichia coli* is an important platform organism that has been extensively engineered for superior industrial production of an enormous range of useful metabolites. In the past few years, several efforts to engineer improved methanol utilization ability in *E. coli* have been made. For example, in 13C-methanol-labeling experiments, 13C-labeled glycolytic intermediates were detected in synthetic methylotrophic *E. coli* [12]. Using MDH from *B. steaorhotorophilus* and the RuMP pathway from *B. methanolicus*, 30% improvement in biomass was observed in a methylotrophic *E. coli* strain and methanol-derived naringenin production [15]. Bennett et al. [16] further improved methanol assimilation by expressing the nonoxidative pentose phosphate pathway (PPP) from *B. methanolicus*. However, the effect of methanol consumption on intracellular cofactor levels, such as NADH and NADPH, was rarely characterized in synthetic methylotrophy, as well as the use of excess electrons from MeOH consumption to improve the yields of desirable metabolites.

In the present study, a synthetic methylotrophic *E. coli* was engineered by employing the NAD+-dependent MDH and RuMP pathways. Two MDH activator proteins were then coexpressed to analyze their role in regulating methanol metabolism in vivo, and the protein NudF was found capable of improving methanol metabolism. After optimizing the culture condition, 13C-methanol-labeling experiments were carried out to confirm the methanol assimilation in methylotrophic *E. coli*. Finally, with lysine as an example, the possibility that bioconversion of methanol to generate value-added metabolites was determined. The extra NADH from methanol oxidation was also engineered as an alternative way to generate NADPH for improving biosynthesis of the desired metabolites.

**Results**

**The construction of synthetic methylotrophy *E. coli* for methanol utilization**

Due to the higher generations of ATP and NAD(P)H, we considered the NAD-dependent MDH and RuMP pathways being most favorable for engineering synthetic methylotrophy [5, 12, 15]. Enzymes with high catalytic activity are prerequisites for efficient engineering of the desired phenotype, and *Mdhl, Hps* and *Phi* from *B. methanolicus* had previously been determined to be the most effective [12]. Based on these previous results, the methanol metabolic pathway was assembled in *E. coli* BL21(DE3) (Fig. 1).

We then analyzed the activities of MDH and HPS-PHI to identify whether the enzymes for methanol metabolism were functionally produced and activated in *E. coli* BL21(DE3). The MDH activity in *E. coli* BL21(DE3) was evaluated by the measurement of formaldehyde accumulated. After 120 min, the formaldehyde accumulation was successfully detected (Fig. 2a), followed by a decrease in formaldehyde concentration, suggesting the activity of endogenous formaldehyde degradation pathway.

To identify the activity of HPS-PHI, formaldehyde degradation of the whole-cells was tested. Formaldehyde was degraded in both the wild-type strain and HPS-PHI-expressing strain, although the HPS-PHI-expressing *E. coli* strain exhibited a higher degree of degradation (Fig. 2b), confirming the activity of HPS-PHI in *E. coli* BL21(DE3). These data indicate that the methylotrophic enzymes required for methanol utilization are functionally expressed in the engineered *E. coli* BL21(DE3) strain. However, the endogenous formaldehyde degradation pathway in wild-type *E. coli* was found to be highly activated. To avoid their effect on methanol fixation efficiency, an *E. coli* mutant strain, ΔfrmA, with a deletion in formaldehyde dehydrogenase, was constructed to prevent the endogenous degradation of formaldehyde and used as the host cell in the following experiment.
The modification of MDH activity by the coexpression of activator protein

The NAD\(^+\)-dependent MDHs from Bacillus spp. have lower affinity toward MeOH compared to higher alcohols. It has been reported that in vitro activity of B. methanolicus MDH could be increased by the endogenous activator protein ACT [17]. To test whether the ACT from B. methanolicus could increase the MDH activity in E. coli in vivo, the ACT protein was coexpressed in E. coli BL21(DE3). The presence of ACT had a positive effect on the in vivo activity of MDH in E. coli, which was increased to 29.1 mU/mg from 22.1 mU/mg (Table 1).

ACT belongs to the enzyme family of Nudix hydrolases and uses NAD\(^+\) as a substrate [18]. Ochsner previously showed that in vitro activity of B. methanolicus MDH could also be improved by other ACT-like Nudix hydrolases [18]. As a member of the Nudix hydrolase family, NudF from E. coli [17] was overexpressed to test its effect on in vivo activity of MDH in E. coli BL21(DE3), which significantly increased MDH activity by 2.1 times compared with that by ACT (Table 1).

To further determine the effect of activator proteins on methanol utilization, the engineered strains BL21/ΔfrmA-Mdh2-Hps-Phi, BL21/ΔfrmA-ACT-Mdh2-Hps-Phi, and BL21/ΔfrmA-NudF-Mdh2-Hps-Phi were cultured in M9 medium supplemented with 55 mM glucose and 100 mM methanol. Little difference in growth was observed in the strains BL21/ΔfrmA-pETDuet-1 and BL21/ΔfrmA-ACT-Mdh2-Hps-Phi, and a slight growth increase was observed in the strain BL21/ΔfrmA-NudF-Mdh2-Hps-Phi (Fig. 2c). After fermenting for 50 h, the strain BL21/ΔfrmA-NudF-Mdh2-Hps-Phi consumed 11.9 mM, while 8.0 mM methanol was consumed by the stain BL21/ΔfrmA-ACT-Mdh2-Hps-Phi (Fig. 2d). These
results further indicated the positive role of protein NudF in regulating methanol utilization.

The optimization of methanol utilization in recombinant strain BL21/ΔfrmA-NudF-Mdh2-Hps-Phi

The addition of yeast extract could improve methanol utilization [15]. Given the important role of the nitrogen source, different nitrogen sources were added into the medium to investigate their influence on methanol metabolism of the strain BL21/ΔfrmA-NudF-Mdh2-Hps-Phi (Fig. 3a). Consistent with the previous study [15], the addition of yeast extract moderately improved the methanol utilization from 10.2 mM to 11.3 mM. With regard to the other nitrogen sources, the presence of peptone and steepwater had little effect on methanol metabolism. Notably, the methanol metabolism was largely improved by the addition of malt extract, where 18.4 mM methanol was consumed after fermenting for 50 h. Subsequently, the methanol concentration in the medium was optimized. Under the methanol concentration ranging from 25 to 100 mM, its inhibition on growth was not observed (Additional file 1: Figure S1). A maximum methanol consumption rate was observed when 50 mM of methanol was supplemented (Fig. 3b). After fermenting for 50 h, 21.2 mM of methanol was utilized totally, which was the
highest methanol consumption level in synthetic methylotrophic *E. coli* reported to date [12, 15]. Meanwhile, a higher biomass was obtained when methanol was used as a co-substrate (Additional file 1: Figure S1).

**13C-methanol-labeling experiments to identify incorporation of methanol-derived carbon into intracellular metabolites**

To confirm methanol assimilation by the recombinant *E. coli* BL21/ΔfrmA-NudF-Mdh2-Hps-Phi, 13C-methanol-labeling experiments were carried out in M9 medium supplemented with 55 mM glucose and 50 mM 13C-methanol. The cells were sampled after about 25 h when the maximum methanol consumption rate was observed. Detected metabolites mainly included the intermediates associated with glycolysis, the pentose phosphate pathway (PPP), and the tricarboxylic acid (TCA) cycle (Fig. 4).

After cultivation for 25 h, 58.7% of Glu-6-p, 3.6% of DHAP, 22.4% of Fru-1,6-p, 8.0% of Gly-3-p, and 22.5% of pyruvate contained labeled carbon that originated from methanol (Fig. 4a). The labeling of TCA intermediates included 6.8% of oxaloacetate, 11.3% of aconitate, 6.4% of α-KG, 12.9% of malate, and 4.8% of succinate from methanol (Fig. 4b). In addition, we also measured 5.8% labeling ribose-5-p and 25.4% labeling sedoheptulose-7-p in the PPP pathway (Fig. 4a). These results showed that carbon from methanol could successfully pass through the glycolysis, TCA cycle, and PPP pathway.

We also detected 19 amino acids, including Ala, Arg, Asp, Glu, Asn, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val, and 13 amino acids were labeled. Among the labeled amino acids, Val, Ala, Leu, Trp, Phe, and Met were derived from the glycolysis intermediates. Thr, Ile, Arg, Glu, Gln, and Pro were the amino acids derived from TCA cycle intermediates. Although the labeling pool of these amino acids was lower than 10%, except for Thr (11.7%) (Fig. 4c, d), their detection indicated that carbon from methanol could be assimilated into metabolic pathways that branched off of central carbon metabolism, and then used for synthesizing important cell components.

In addition, among the detected metabolites, many of them were labeled by multiple carbons. For example, approximately 52.9% of Glu-6-p, 5.0% of Gly-3-p, and 13.6% of pyruvate involved in the glycolysis pathway exhibited M+2 labeling (Fig. 4b). For the compound α-ketoglutarate (α-KG), 0.4% contained M+4 labeling and 0.5% was fully labeled. 2.1% of the malate contained M+2 labeling, 1.4% contained M+3 labeling, and 4.1% was fully labeled (Fig. 4b). Similarly, 0.6% of succinate contained M+2 labeling (Fig. 4b). We also found that approximately 8.6% of Thr contained M+2 labeling (Fig. 4d). The detection of multiple carbon-labeled metabolites indicated that the RuMP cycle successfully worked in the recombinant *E. coli* BL21/NudF-Mdh2-Hps-Phi.

The bioconversion of methanol to enhance l-lysine production

Whitaker et al. [15] previously demonstrated in vivo conversion of methanol to naringenin in synthetic *E. coli*. Leifmeier also confirmed that the nonnatural carbon substrate methanol could be converted at least partially to cadaverine as a nonnative product by a recombinant *C. glutamicum* strain [10]. These works show the potential of methanol to act as a carbon source for the production of value-added chemicals in synthetic methylotrophy. As methanol possesses a high degree of reduction, its consumption can improve the intracellular NADH availability used for facilitating the synthesis of some reductive products. Here, we attempted to use the NADH from methanol consumption to generate NADPH to expand
the bioconversion of methanol for improving the synthesis of reductive products in synthetic methylotrophic *E. coli*.

Amino acids represent one of the largest classes of fermentation products whose syntheses are closely correlated with the availability of NAD(P)H. The synthesis of 1 mol lysine, for example, requires 4 mol NADPH. Several efforts have been made to enhance the supply of NADPH for improved lysine production [19]. Here, we selected lysine as a test case, and engineered the lysine synthetic pathway and NADPH generation pathway from NADH in the recombinant *E. coli* BL21/ΔfrmA-NudF-Mdh2-Hps-Phi (Fig. 5a). As no lysine was labeled from the above 13C-methanol-labeling experiment in *E. coli* BL21/ΔfrmA-NudF-Mdh2-Hps-Phi (Fig. 5a), we also focused on whether the carbon flux from methanol could be directed to generate lysine after the reconstruction of the metabolic pathway.

With the recombinant *E. coli* BL21/ΔfrmA-NudF-Mdh2-Hps-Phi, no lysine was detected in the medium. To improve lysine production, the key genes involved in lysine biosynthesis, including *dapA*, *dapB*, *PPC*, and *lysC* (a lysine-insensitive aspartokinase) [20, 21], were co-overexpressed in the recombinant *E. coli* BL21/ΔfrmA-NudF-Mdh2-Hps-Phi to generate *E. coli* BL21/ΔfrmA-ML. Thereafter, 0.06 g/L lysine was produced after fermenting for 24 h, with glucose as the sole carbon source (Fig. 5b). Then, methanol was added into the medium to determine its effect on lysine production. After fermenting for 24 h, *E. coli* BL21/ΔfrmA-ML consumed 9.5 mM of methanol (Fig. 5c), while the methanol metabolism significantly increased the intracellular NADH pool (Fig. 5d). However, production of lysine moderately increased in *E. coli* BL21/ΔfrmA-ML when methanol was used as a co-substrate (Fig. 5b).

To explore the potential of methanol metabolism in improving lysine production, we constructed a NADPH-regenerating system from NADH by expressing NADH kinase (Pos5p) from *S. cerevisiae* [22] in *E. coli* BL21/ΔfrmA-ML (Fig. 5a). The intracellular NADH pool was largely decreased when POS5 was expressed in *E. coli* BL21/ΔfrmA-ML (Fig. 5d). This engineering strategy successfully enhanced lysine production by twofold when methanol was present, while lysine production was only
moderately affected in the absence of methanol (Fig. 5b). At the same time, the methanol utilization ability was also improved by the overexpression of POS5. With the synthesis of lysine, the NADPH level decreased (Fig. 5e).

Finally, a $^{13}$C-methanol labeled experiment was carried out to measure $^{13}$C-labeling patterns of metabolic lysine in recombinant *E. coli* BL21/ΔfrmA-ML, and *E. coli* BL21/ΔfrmA-ML-POS5 (Fig. 5f). No labeled lysine had been detected in *E. coli* BL21/ΔfrmA-Mdh-Hps-Phi. Through engineering the lysine biosynthetic pathway, 4.7% of lysine exhibited M + 1 labeling in the strain BL21/ΔfrmA-ML. With the overexpression of POS5, 9.0% of
lysine contained M + 1 labeling. Lysine labeled by multiple carbons was not detected in the recombinant strains.

Discussion
The bioconversion of methanol has received considerable attention given methanol’s abundance, low price, and high electron and energy content. In recent years, several efforts have been made to engineer *E. coli* as a synthetic methylotroph [15, 16, 23, 24]. However, little methanol utilization has been demonstrated to date [12, 15]. MDH activity is one of the key factors limiting methanol metabolism efficiency. To address this problem, several strategies—including a scaffoldless strategy to organize MDH, HPS, and PHI into an supramolecular enzyme strategies—to improve methanol utilization has been demonstrated to date [12, 15].

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amplified from plasmid pTrc99a-dapA E. coli the and Target-frmA-R and followed by self-ligation. A 1000-obtained by inverse PCR with the primer Target-frmA-F the plasmid pTarget as a template, the pTarget-frmA was BL21(DE3) with plasmid pCas9 and donor DNA. With sequence, was constructed and cotransformed to gene NudF-Mdh2-Hps-Phi. The fragment of ACT generate plasmid pETDuet-NudF-Mdh2 and pETDuet-sites of pETDuet-Mdh2 and pETDuet-Mdh2-Hps-Phi to pETDuet-ACT-Mdh-Hps-Phi.

I/Sac Sal with primer 1 and primer 2 was inserted into the gene E. coli amplified from MG1655 genome NudF and pETDuet-1 to generate plasmid pETDuet-Mdh2-was inserted into NdeI/SacI and primer 8 [26], which was then inserted into I sites of pCWJ-PPC. For deleting frmA gene, the plasmid pTarget-frmA, dapA-dapB-LysC<sup>Thr</sup> constructed in our previous study with primer 7 and primer 8 [26], which was then inserted into SpeI/NcoI sites of pCWJ-PPC to yield the plasmids pCWJ-dapA-dapB-LysC<sup>Thr</sup>-PPC.

For deleting frmA gene, the plasmid pTarget-dapA, used in frmA modification with a targeting N20 sequence, was constructed and cotransformed to E. coli BL21(DE3) with plasmid pCas9 and donor DNA. With the plasmid pTarget as a template, the pTarget-dapA was obtained by inverse PCR with the primer Target-dapA-F and Target-dapA-R and followed by self-ligation. A 1000-bp donor sequence was designed by overlap PCR with the E. coli genome as a template.

Media and growth condition

The E. coli strains were cultured in Luri-Bertani (LB) medium which contained 10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl. The M9 medium supplemented with 10 g/L glucose, 0.5 g/L NaCl, 10 g/L NH₄Cl, 3 g/L KH₂PO₄, and 17.1 g/L NaHPO₄·12H₂O was used for the analysis of methanol consumption and enzyme assays. Gene expression was induced by 0.05 mM of isopropyl-1-thiogalactopyranoside (IPTG). The strain was cultured at 200 rpm and 37 °C. Appropriate antibiotics were added at the following concentrations: ampicillin, 100 µg/mL, chloramphenicol, 34 µg/mL.

For the fermentation, a single colony was inoculated into the LB medium supplemented with 100 µg/mL. Amp or 34 µg/mL Cm, and cultivated for 10 h approximately. Then, the cultures were centrifuged, and resuspended in 50 mL M9 medium. The initial OD<sub>600</sub> was 0.3 and incubated at 37 °C and 200 rpm. After about 3 h, 0.05 mM IPTG and certain concentration of methanol were added. To optimize the methanol utilization, the experiment was carried out at the different methanol concentrations ranging from 50 mM to 200 mM. The effects of nitrogen sources, including peptone, yeast extract, steepwater, and malt extract were also investigated. The concentrations of the nitrogen source was 1 g/L.

In vivo enzyme assays

The in vivo activity of MDH was assayed by the production of the formaldehyde. Cells were grown over night in LB medium. Subsequently, amount of cells corresponding to an OD<sub>600</sub> of 1 in a volume of 10 mL was pelleted by centrifugation at 5000 × g for 5 min and resuspended in 9 mL M9 medium. The experiment was started by the addition of 1 mL 500 mM methanol, and the cultures were kept at 37 °C. Samples were taken at the specific intervals, and 100 µL of the supernatant was mixed with the same volume of Nash reagent to determine the formaldehyde concentration [27]. One unit (U) was defined as the amount of enzyme that is required to produce 1 µmol formaldehyde produced per minute.

The activity of Hps-Phi operon was assayed by the consumption of the formaldehyde. Cells were prepared as described above. The reaction was started by adding 0.35 mM formaldehyde. One unit (U) was defined as the amount of enzyme that is required to consume 1 µmol formaldehyde per minute.

Determination of 13C-labeled experiments

For the determination of 13C-labeled intracellular metabolites, the assimilation of methanol in recombinant E. coli strains was monitored by a 13C-methanol-labeling experiment. The strain E. coli BL21/ΔfrmA-NudF-Mdh-Hps-Phi was cultivated in LB medium supplemented with 100 µg/mL Amp and 0.5 mM IPTG for 10–12 h. Cells were then transferred to M9 medium with 50 mM
13C-methanol (Sigma-Aldrich). After the fermentation of 25 h, cells were collected and pretreated as the following steps. Quenching and metabolite extraction were performed according to the methods described previously with minor modifications [27]. The supernatant (800 μL) of metabolite extracts was transferred into a 1.5 mL EP tube, and lyophilized under low temperature. The dry extracts were then reconstituted in 300 μL of acetonitrile:H2O (1:1, v/v), sonicated for 10 min and centrifuged 15 min at 10,000×g and 4 °C to remove insoluble debris. The supernatants were transferred to HPLC vials and stored at −80 °C prior to LC–MS/MS analysis. For the determination of 13C-labeled lysine, E. coli BL21/ML-P0S5 was cultivated, sampled, and treated as the above steps. For each sample, three replicates were carried out.

Analytical methods

Cell growth was determined by measuring the OD600 using a Beckman Coulter DU370 spectrophotometer. Methanol concentrations were measured using an Agilent 1290 Infinity System (Santa Clara, CA, USA) equipped with an Aminex HPX-87H column. l-Lysine was determined using SBA-40E immobilized enzyme biosensor (Shandong, China) [21]. NADH and NADPH were assayed by means of the NAD/NADH quantitation Kit and the NADP/NADPH quantitation Kit (Sigma-Aldrich).

LC–MS/MS analyses were performed using an UHPLC system (1290, Agilent Technologies) equipped with a UPLC BEH Amide column (1.7 μm, 1.0×100 mm, Waters) coupled to Triple TOF 6600 (Q-TOF, AB Sciex). The mobile phase consisted of 25 mM NH4OAc and 25 mM NH4OH in water (pH = 9.75) (A), and acetonitrile (B) was carried with elution gradient as follows: 0 min-95% B, 0.5 min-95% B, 0.5 min-65% B, 7 min-40% B, 9 min-40% B, 9.1 min-95%, and B, 12 min-95% B, which was delivered at 0.5 mL/min. The injection volume was 1 μL. The Triple TOF mass spectrometer was used to assess its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during a LC/MS experiment. ESI source conditions were set as follows: Ion source gas 1 as 60, Ion source gas 2 as 60, Curtain gas as 35, source temperature 550 °C, Ion Spray Voltage Floating (ISVF) 5500 V or −5500 V in positive or negative modes, respectively.

Abbreviations

DHAP: dihydroxyacetone phosphate; α-KG: α-ketoglutarate; His: histidine; Met: methionine; Val: valine; Ala: alanine; Leu: leucine; Phe: phenylalanine; Tyr: tyrosine; Gly: glycine; Ser: serine; Trp: tryptophane; Thr: threonine; Ile: isoleucine; Asn: asparagine; Asp: aspartic acid; Lys: lysine; Arg: arginine; Gl: glutamic acid; Gln: glutamine; Pro: proline.

Authors’ contributions

WX, LX, WXL, CKq, and OYPK designed the experiments; WX conducted the experiments; and WX and LX, wrote the paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of supporting data

Data will be made available from the corresponding author on reasonable request.

Consent for publication

All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Additional file

Additional file 1: Table S1. Strains and plasmids used in this work. Table S2. Primers used in this study. Figure S1. The growth of BL21/ΔΔmVA-NudF-Mdh2-Hps-Phi under the different concentration of methanol.
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