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Original Research Article

Metabolic engineering of *Bacillus amyloliquefaciens* for efficient production of α-glucosidase inhibitor1-deoxynojirimycin

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ABSTRACT

Owing to the feature of strong α-glucosidase inhibitory activity, 1-deoxynojirimycin (1-DNJ) has broad application prospects in areas of functional food, biomedicine, etc., and this research wants to construct an efficient strain for 1-DNJ production, basing on *Bacillus amyloliquefaciens* HZ-12. Firstly, using the temperature-sensitive shuttle plasmid T2 (2)-Ori, gene *ptsG* in phosphotransferase system (PTS) was weakened by homologous recombination, and non-PTS pathway was strengthened by deleting its repressor gene *iolR*, and 1-DNJ yield of resultant strain HZ-S2 was increased by 4.27-fold, reached 110.72 mg/L. Then, to increase precursor fructose-6-phosphate (F-6-P) supply, phosphofructokinase was weaken, fructose phosphatase GlpX and 6-phosphate glucose isomerase Pgi were strengthened by promoter replacement, moreover, regulator gene *nanR* was deleted, 1-DNJ yield was further increased to 267.37 mg/L by 2.41-fold. Subsequently, promoter of 1-DNJ synthetase cluster was optimized, as well as 5′-UTRs of downstream genes in synthetase cluster, and 1-DNJ produced by the final strain reached 478.62 mg/L. Last but not the least, 1-DNJ yield of 1632.50 mg/L was attained in 3 L fermenter, which was the highest yield of 1-DNJ reported to date. Taken together, our results demonstrated that metabolic engineering was an effective strategy for 1-DNJ synthesis, this research laid a foundation for industrialization of functional food and drugs based on 1-DNJ.

1. Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases in the world, and is also a major public health issue [1]. Basing on the latest data reported by International Diabetes Foundation, the number of global diabetes patients reached 537 million in 2021, which was expected to rise to 643 million in 2030 (IDF Diabetes Atlas 2021). Acting as an antidiabetic drug, α-glucosidase inhibitory (α-GI) has showed the good efficiency compared with other oral hypoglycemic drugs, and are the most commonly used oral diabetes drugs in Asian countries [2]. At present, commercial α-GI inhibitors include miglitol, acarbose, voglibose, etc, however, these drugs may cause adverse reactions, such as diarrhea or abdominal distension, and prolonged use causes liver toxicity [3]. Therefore, people need to find more effective and safer α-GI.

1-DNJ is an important α-GI that separated from mulberry leaves, and it could beacted as the bulk drug for the treatmentof non-insulin-dependent (type II) diabetes [4]. In addition, 1-DNJ possesses various biological activities, such as anti-obesity, antiviral, etc [5,6]. Compared with acarbose, 1-DNJ showed better anti-hyperglycemia properties in the treatment of hyperglycemia rats induced by streptozotocin and high-fat diet, and the hyperglycemic state was returned more slowly in 1-DNJ-treated group, compared to acarbose-treated group [5].

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Moreover, 1-DNJ can reduce liver damage and improve hepatic glucose metabolism in mouse [7]. *Bacillus* and *Streptomyces* are the promising strains for 1-DNJ synthesis. Previously, 1-DNJ-producing strain *Streptomyces* SID9135 was isolated from soil, which yield was increased to 640 mg/L by optimizing fermentation process [8]. *Bacillus amyloliquefaciens* AS385 was screened from 750 microorganisms, and 1-DNJ yield was increased to 460 mg/L in the medium with sorbitol addition [9]. The biosynthetic pathway of microbial 1-DNJ begins from fructose-6-phosphate [10], an intermediate product of glycolysis, and genes *gabT1*, *yktC1*, and *gutB1* were proven to be involved in 1-DNJ biosynthesis pathway [9,11]. Meantime, metabolic engineering approach was also applied for the efficient synthesis of 1-DNJ. Through over-expressing fructokinase gene *yjf* and fructose phosphatase gene *glpX* in *E. coli*, 1-DNJ yield was reached 273 mg/L, combining with medium optimization [12]. *B. amyloliquefaciens* HZ-12 is a 1-DNJ producing strain that isolated in our previous research [13], and over-expression of 1-DNJ synthetase gene cluster and glucose transporter GlcP led to a 32.52% increase of 1-DNJ yield [14]. However, the synthesis level of 1-DNJ by microorganisms is still too low, which limits its application and promotion.

With the continuous progress of bio-breeding technology driven by synthetic biology and metabolic engineering, biomanufacturing has achieved various achievements in the areas of energy, chemical, food, agriculture, etc. Based on the promising effect of artemisinin in malaria treatment, Jay et al. have established the *de novo* synthesis pathway of artemisinin in *Saccharomyces cerevisiae*, which overturned the traditional method of artemisinin production [15]. Meanwhile, to increase N-acetylglucosamine (GlcNAc) production in *Bacillus subtilis*, Liu et al. have systematically modified glucose transport, central carbon metabolism, and GlcNAc synthesis pathway, etc., which significantly enhanced GlcNAc production [16]. In addition, in order to achieve the fine regulation of genes and metabolic pathways in *Bacillus*, a variety of gradient promoter libraries were constructed by our group [17], for efficient production of proteins [18], poly γ-glutamic acid [19], 2-phenylethanol [20], etc. Based on the guidance of synthetic biology concept and preliminary basis of our group in *Bacillus* biotechnology, the synthesis capability of 1-DNJ was expected to be improved through the approach of metabolic engineering breeding.

In the previous work of our group, a 1-DNJ synthesis strain *B. amyloliquefaciens* HZ-12 was isolated, and the solid-state fermentation process for 1-DNJ production was also established [13]. Furthermore, the 1-DNJ yield was significantly increased by over-expression of glucose transporter GlcP [14]. Here, to increase 1-DNJ production in *B. amyloliquefaciens* HZ-12, the modules of glucose transportation, central carbon metabolism, and 1-DNJ synthetase cluster were systematically engineered, and fermentation process was also optimized in 3 L fermenter. Our results demonstrated that metabolic engineering was an effective strategy for enhanced production of 1-DNJ, and this research laid a foundation for the industrialization of functional food and drugs based on 1-DNJ.

2. Material and methods

2.1. Bacteria, plasmids andcultivation conditions

The strains and plasmids used in this study were listed in Table S1. *B. amyloliquefaciens* HZ-12 was used as the parental strain for recombinant strain construction, and *E. coli* DH5α was served as the host for plasmid establishment. Plasmid pHY300PLK was used for constructing gene expression vector, and plasmid T2(2)-Ori was applied for gene integration, gene deletion and promoter replacement. The primers used for strain construction and gene transcriptional level assays were provided in Table S2. LB medium (10 g/L Tryptone, 5 g/L Yeast, 10 g/L NaCl) was served as the basic medium for strain cultivation, and corresponding antibiotic (20 μg/mL tetracycline or 20 μg/mL kanamycin) was added, when necessary. For 1-DNJ production in flask, the seeds were cultivated in a 250 mL shake flask containing 50 mL LB medium for 8–12 h, then transferred into 250 mL shake flask containing 30 mL fermentation medium (40 g/L glucose, 20 g/L peptone, 15 g/L yeast extract, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.2 g/L $FeSO_4{\cdot}7H_2O$, 0.02 g/L $MnCl_2$), and then cultivated for 60 h. Each fermentation experiment was repeated at least three times.

2.2. Construction of gene over-expression strain

The gene expression plasmid was established basing on plasmid pHY300PLK, according to our previously reported method [21], and gene *nanR* over-expression strain was served as an example. Briefly, P43 promoter from *B. subtilis* 168, *nanR* from *B. amyloliquefaciens* HZ-12, *amyL* terminator from *Bacillus licheniformis* WX-02 were amplified by corresponding primers, and fused by spliced overlap extension PCR (SOE-PCR). Then, the fused fragment was inserted into pHY300PLK at the restriction sites *Eco*RI/*Xba*I, diagnostic PCR and DNA sequence was applied to verify the successful construction of recombinant vector, named as pHY-NanR. Then, pHY-NanR was electro-transferred into *B. amyloliquefaciens* HZ-12 to attain *nanR* overexpression strain HZ/pHY-NanR.

2.3. Gene deletion in B. amyloliquefaciens

Gene deletion in *B. amyloliquefaciens* was referred to our previously reported method [22], basing on vector T2(2)-Ori, and the construction procedure of gene *nanR* deletion strain was served as an example. Briefly, the upstream and downstream homologous arms of gene *nanR* were amplified, and fused by SOE-PCR. The fused fragment was inserted into T2(2)-Ori at the restriction sites *Sac*I/*Xba*I, resulting in gene deletion vector T2-nanR. Then, T2-nanR was electro-transformed into *B. amyloliquefaciens,* and the positive transformants were verified by kanamycin resistance and diagnostic PCR. The positive transformants were cultivated in LB medium (containing 20 mg/L kanamycin) at 45 °C for three generations, and then transferred into LB medium without kanamycin for six generations at 37 °C. Colonies without kanamycin resistance were screened, and *nanR*-deficient strain was verified by diagnostic PCR and DNA sequencing. Similarly, other gene deletion strains were attained by the same method.

2.4. Construction of promoter replacement strain in B. amyloliquefaciens

Promoter replacement is an effective strategy to regulate the intensities of gene expression and metabolic pathway. Here, the construction procedure of gene *ptsG* promoter replacement strain was served as an example. Briefly, upstream and downstream homologous arms of original promoter of gene *ptsG*, promoter P_{undh} attained in our previous research [17], were amplified and fused. Then, the fused fragment was inserted into T2(2)-Ori to attain the promoter replacement vector, named as T2-Pundh-ptsG. T2-P_{undh}-ptsG was electro-transformed into *B. amyloliquefaciens*, and the promoter replacement strain was obtained by homologous double-exchanges, the same as that of gene deletion.

2.5. 1-DNJ production in 3 L fermenter

For 1-DNJ production in 3 L fermentor, *B. amyloliquefaciens* was cultivated in 1.8 L 1-DNJ production medium. The inoculation ratio was 6%, and aeration rate was 1.0 vvm throughout the fermentation process. The agitation rate was 300 rpm at lag phase (0–4 h), and the dissolved oxygen was associated with stirring, to maintain the dissolved oxygen percentage between 15 and 25% (6–60 h). Glucose was feed to maintain the glucose concentration between 5 and 10 g/L, and samples were taken every 6 h to measure the cell biomass and 1-DNJ concentration.

2.6. Analysis methods

To determine the concentration of 1-DNJ, the volume of 1 mL fermentation supernatant was mixed 20 mL distilled water. The 1-DNJ concentration was determined by Agilent HPLC 1260 (Agilent Technologies, USA), equipped with Agilent Lichrospher C18 column (4.6 mm × 250 mm, 5 μm), the detection wavelength was 254 nm, and mobile phase was acetonitrile: 0.1% acetic acid (11:16, V/V). The injection volume was 10 μL, and flow rate was 1.0 mL/min. In addition, cell biomass was measured by dilution method. Glucose concentration was detected using a biosensor analyzer. Gene transcriptional levels were measured by RT-qPCR, according to our previous research [23].

2.7. Statistical analysis

Each experiment was performed at least three replicates, and software SPSS was used for data analysis. All data were subjected to analysis of variance at $P < 0.05$ (*) and $P < 0.01$ (**), and *t*-test was performed with statistical software STATISTICA 6.0.

3. Results

3.1. Optimization of glucose transport pathway for 1-DNJ synthesis

Acting as the most common carbon source for cell growth and metabolite synthesis, glucose efficient utilization is essential for the synthesis of target product. In *Bacillus*, glucose is mainly transported into cell by phosphotransferase system (PTS), accompanying with the conversion of phosphoenolpyruvate (PEP) to pyruvate (PYR). This phenomenon leads to the accumulation of excess pyruvate, coupled with the generally weak TCA cycle, resulted in the vigorous overflow metabolic and overproduction of acetoin, 2,3-butanediol and acetate in *Bacillus*. Thus, *Bacillus* was proven as the wonderful strain for acetoin and 2,3-butanediol production [24]. In addition, there is another glucose transport pathway (Non-PTS pathway) in *Bacillus*, and glucose transports by which was not accompanied with pyruvate generation. Served as the critical gene in PTS pathway, *ptsG* expression determines the metabolic flux of PTS pathway, glucose transporter GlcP and glucokinase GlcK served as the vital genes in non-PTS pathway, and both of them were negatively regulated by regulator IolR [25]. Here, we want to optimize the glucose transport pathway to increase the synthesis capabilityof 1-DNJ (see Fig. 1).

Basing on the original strain *B. amyloliquefaciens* HZ-12 (HZ-S), the promoter of gene *ptsG* was replaced by promoter P_{undh}, a weak promoter attained in our previous research [17], resulting in strain HZ-S1. Based on our results of Fig. 2, the maximum cell biomass and glucose consumption rates were significantly reduced by 33.74% and 25.00% in HZ-S1, respectively. However, 1-DNJ produced by HZ-S1 was increased to 93.04 mg/L by 3.42-fold, compared with that of original strain HZ-S (21.03 mg/L). Furthermore, to increase the glucose transportation and cell growth, non-PTS pathway was strengthened by knocking out of regulator gene *iolR*, and transcriptional level of genes *glcP* and *glcK* were respectively increased by 2.62-fold and 4.21-fold in the resultant strain HZ-S2, and 1-DNJ produced by HZ-S2 reached 110.72 mg/L, increased by 19.00% and 4.26-fold compared to HZ-S1 and HZ-S, respectively. Meanwhile, cell growth of HZ-S2 was dramatically improved, and the maximum cell biomass was increased by 23.75% compared to HZ-S1. Glucose consumption rate of HZ-S2 was reduced by 16.67%, and by-products, acetoin and 2, 3-butediol, were respectively reduced by 34.73% and 32.39%, compared to HZ-S. Thus, these results indicated that rewiring glucose transportation pathway weakened the overflow metabolism and promoted 1-DNJ synthesis.

3.2. Strengthening precursor fructose 6-phosphate supply for 1-DNJ synthesis

Sufficient precursor supply is an important basis for high-level production of target product. Fructose 6-phosphate (F-6-P) is an important intermediate in glycolytic pathway, and is also the key precursor for DNJ synthesis. Here, genes around F-6-P metabolite node were manipulated to improve its supply for 1-DNJ synthesis.

Phosphofructokinase, encoded by two iso-enzyme genes *pfkA* and *pfkB*, is responsible for catalyzing the reactionfrom F-6-P to fructose 1,6-bisphosphate (F-1,6–2P) [26], additionally, fructose 1,6-bisphosphatase GlpX catalyzes the reverse reaction in gluconeogenic pathway. Firstly, genes *pfkA* and *pfkB* were deleted to improve F-6-P accumulation,

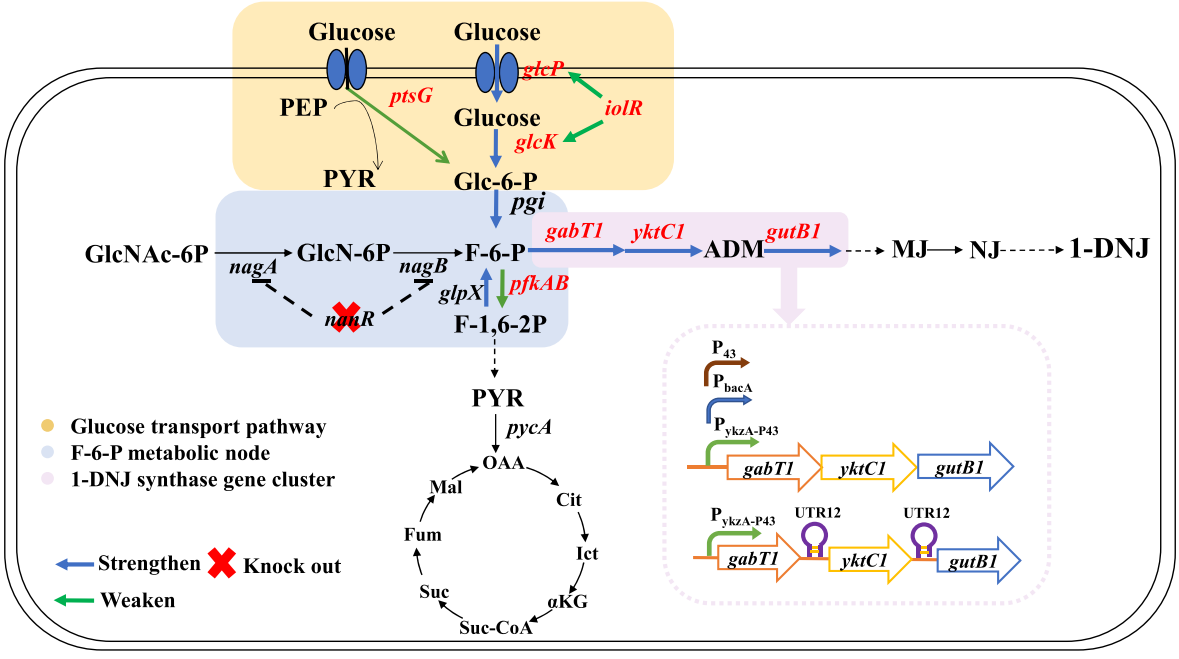

Fig. 1. Metabolic engineering of *B. amyloliquefaciens* for efficient production of α-glucosidase inhibitor 1-deoxynojirimycin, including the modules of glucose transport pathway, F-6-P metabolic node and 1-DNJ synthetase gene cluster.

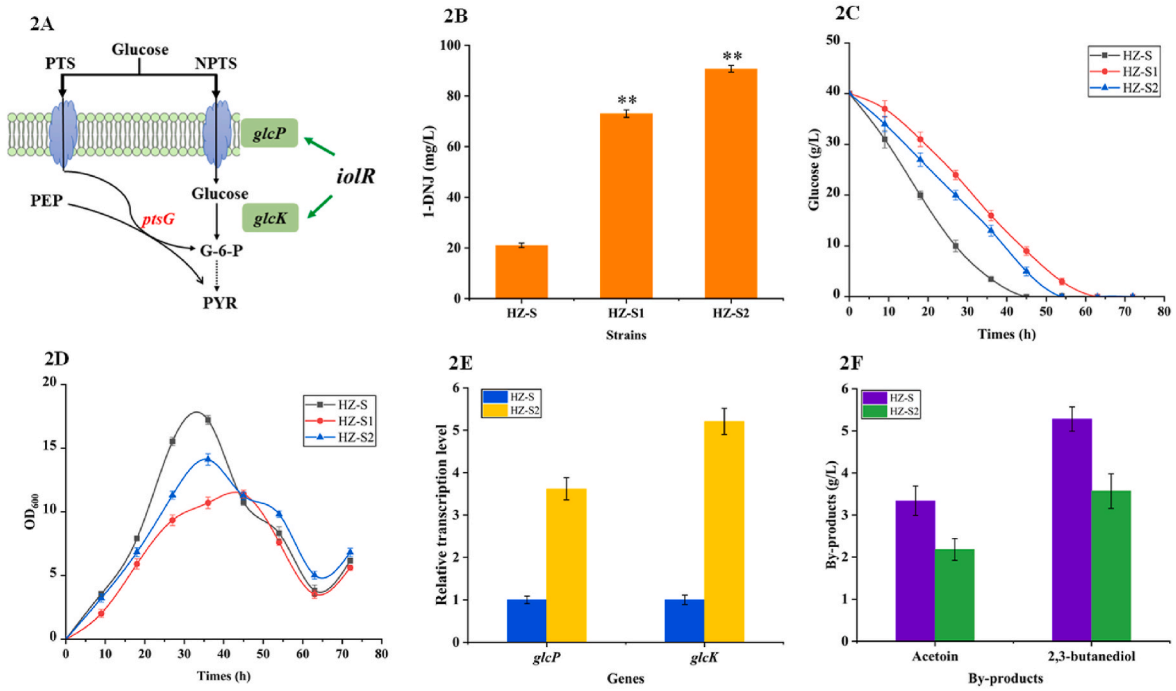

Fig. 2. Optimization of glucose transport pathways for 1-DNJ synthesis. **A**: Schematic diagram of glucose transport in *B. amyloliquefaciens* HZ-12, **B**: 1-DNJ yields, **C**: Glucose concentrations, **D**: Cell biomass, **E**: Transcriptional levels of genes *glcP* and *glcK*, **F**: The yields of by-products (acetoin and 2,3-butandieol).

resulting in strain HZ-S3 and HZ-S4, respectively. Based on our results of Fig. 3A, knocking out of *pfkB* benefited 1-DNJ synthesis, and 1-DNJ yield was increased to 118.82 mg/L by 7.31%. However, the growth status of *pfkA*-null strain (HZ-S3) was very poor, resulting in the significant reduction of 1-DNJ yield, and which may be due to that *pfkA* plays as the major function of phosphofructokinase, and metabolic disorder was occurred in *pfkA* deletion strain. Then, promoter P_{undh} was applied to weaken *pfkA* expression in HZ-S4, and transcriptional level of *pfkA* was reduced by 62.00% in resultant strain HZ-S4-P_{undh}-PfkA (HZ-S5), 1-DNJ yield was 137.29 mg/L, increased by 15.54% compared to HZ-S4. In addition, cell biomass showed no significant difference between HZ-S5 and HZ-S4. Furthermore, the original promoter of *glpX* was replaced by strong promoter P_{bacA}, to strengthen the transformation from F-1, 6–2P to F-6-P in gluconeogenic pathway, and 1-DNJ yield produced by the attained strain HZ-S5-PbacA-GlpX (HZ-S6) was 169.41 mg/L, increased by 23.40% compared to HZ-S5.

Generally, F-6-Pcan also be generated by GlcNAc-6P, under the catalysis of N-acetylglucosamine-6-phosphate deacetylase NagA and

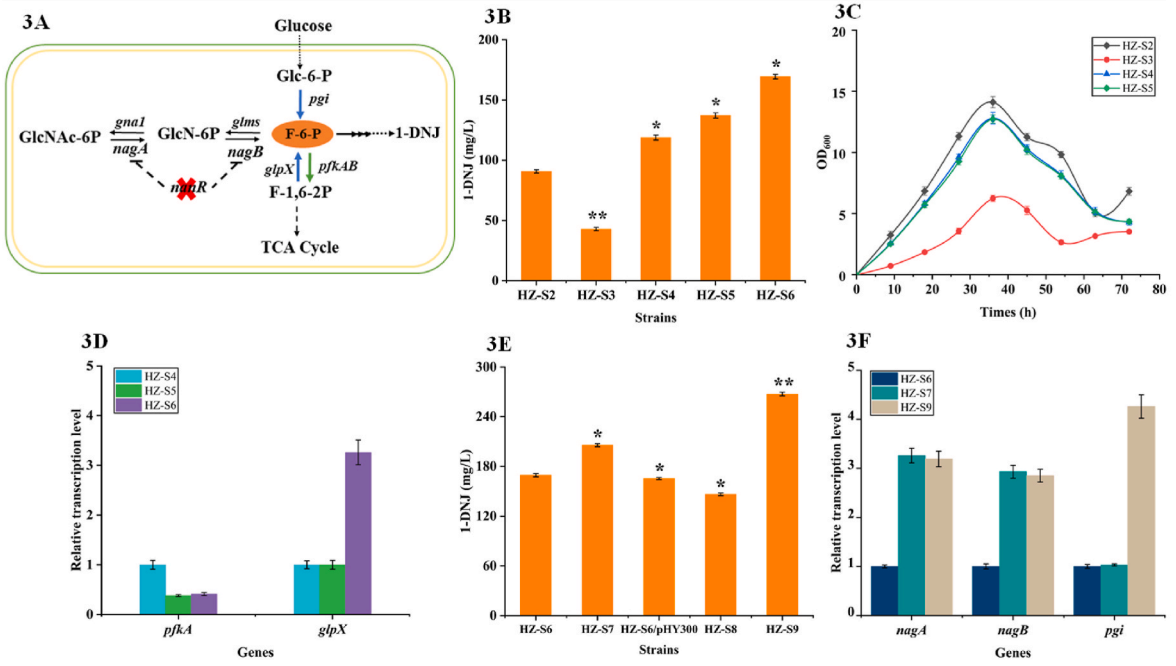

Fig. 3. Strengthening precursor F-6-P supply for 1-DNJ synthesis. **A**: Schematic diagram of F-6-P metabolic node in *B. amyloliquefaciens* HZ-12, **B**: 1-DNJ yields, **C**: Cell biomass, **D**: The transcriptional levels of genes *pfkA* and *glpX*. **E**: 1-DNJ yields, **F**: The transcriptional levels of genes *nagA*, *nagB* and *pgi*.

glucosamine-6-phosphate deaminase NagB, both of them are regulated by transcription factor NanR. Here, to strengthen the catalysis reaction from GlcNAc-6P to F-6-P, gene *nanR* was respectively deleted and overexpressed in HZ-S6, resulting in recombinant strains HZ-S6△nanR(HZ-S7) and HZ-S6/pHY-NanR(HZ-S8), respectively. The transcriptional levels of genes *nagA* and *nagB* were respectively increased by 2.26-fold and 1.93-fold in *nanR* deletion strain, which led to a 21.40% increase of 1-DNJ yield, compared to HZ-S6, meanwhile, 1-DNJ yieldwas decreased by 11.54% in NanR overexpression strain HZ-S8 (Fig. 3E).

Glucose 6-phosphate isomerase Pgi catalyzes the conversion of G-6-P to F-6-P, which is an important source of F-6-P. Here, the original promoter was replaced by promoter P_{bacA}, a strong promoter confirmed in our previous research [27], attaining recombinant strain HZ-S9 (HZ-S7-P_{bacA}-Pgi). Based on our results of Fig. 3F, the transcriptional level of gene *pgi* was increased by 3.26-fold, and 1-DNJ yield of strain HZ-S9 reached 267.37 mg/L, increased by 30.00% compared to HZ-S7.

3.3. Enhancing the expression intensity of 1-DNJ synthesis gene cluster for 1-DNJ production

Genes *gabT1*, *yktC1* and *gutB1*, encoding for 4-aminobutyrate aminotransferase, myo-inositol-1(or 4)-monophosphatase and zinc-binding dehydrogenase [10], were proven as the critical genes in 1-DNJ synthesis pathway in *Bacillus* [9,11], all of these genes were driven by promotrt P_{gabT1}. To improve the gene cluster expression for 1-DNJ synthesis, the original promoter was replaced by promoters P_{43}, P_{bacA}, and dual promoter $P_{ykzA\text{-}P43}$, attaining recombinant strains HZ-S10(HZ-S9-P_{43}-TYB), HZ-S11(HZ-S9-P_{bacA}-TYB) and HZ-S12 (HZ-S9-$P_{ykzA\text{-}P43}$-TYB), respectively. Based on the results of Fig. 4, promoter replacement benefited 1-DNJ synthesis, and 1-DNJ yields produced by the relative strains were respectively increased by 25.49%, 41.80% and 47.47%, which positively consisted with the results of gene transcriptional levels in Fig. 4C.

In general, the expression level of downstream gene in the gene clusters is limited, due to lower transcription and translation efficiency, and gene *gabT1* transcriptional level was higher than those of *yktC1* and *gutB1* in HZ-S11.5′-UTR was confirmed to play the critical role in mRNA translation initiation and stability, thereby affecting the gene expression level. In the previous research of our group, a 5′-UTR library has been established for gene expression regulation in *Bacillus* [28]. Here, to improve gene *gutB1* expression, the original 5′-UTR of *gutB1* was replaced by UTR12 (GTATATTAGAAAGGAGGAATATATA), attained recombinant strain HZ-S13, and 1-DNJ yield produced by HZ-S13 reached 426.71 mg/L, increased by 8.22% compared to HZ-S12 (Fig. 5A). Additionally, 5′-UTR replacement decreased the △G of mRNA secondary structure (Fig. 5B and **C**), which benefited mRNA translation initiation and secondary stability. Meanwhile, similar strategy was applied for *yktC1* expression enhancement in HZ-S13, and 1-DNJ produced by the resultant strain HZ-S14 was 478.6 mg/L, increased by 12.17% compared to HZ-S13.

3.4. 1-DNJ production of B. amyloliquefaciens HZ-S14 in 3 L fermenter

To further increase 1-DNJ yield, fed-batch fermentation of strain HZ-S14 was performed in a 3-L fermenter (Fig. 6), with a fermentation period of 96 h. The pH was controlled between 6.5 and 7.4 throughout the fermentation process, and glucose was supplemented to maintain its concentration at 5–10 g/L. In the first 50 h of fermentation, cells grew rapidly, and the maximum cell biomass (OD_{600}) reached 57.81 at 72 h. 1-DNJ was synthesized from 48 h, and the synthesis rate was significantly accelerated after 60 h, and the maximum1-DNJ yield was 1632.5 mg/L at 96 h, whichwas the highest yield of 1-DNJ reported so far.

4. Discussions

Synthetic biology and metabolic engineering promoted the development of food biotechnology, and boosted the basic research and application of future food science. Post et al., have made the first artificial meat burger in the world [29], and researchers have successfully constructed the cell factory for the production of hemoglobin, the colorant of artificial meat in *Yarrowia lipolytica* [30]. Furthermore, co-expression of molecular chaperone led to the 2-fold increases of thaumatin production in *Pichia pastoris* [31], and microbial production of lactoprotein has also become a research hotspot in recent years, which laid the foundation for nutritional protein production by microorganism. Meanwhile, synthetic biology has also been widely used in the production of metabolites. Through metabolic engineering breeding, the yield of citric acid reached 200 g/L in *Y. lipolytica AWG7* INU 8, with a yield of 0.51 g/L·h [32]. Meanwhile, one-step synthesis process of Vitamin C was established by introducing the different combinations of L-sorbose dehydrogenases and L-sorbosone dehydrogenases in *Gluconobacter oxydans* WSH-003, which overturned the traditional manufacturing process of vitamin C [33]. In this research, efficient synthesis of α-glucosidase inhibitor1-DNJ was achieved basing on the rational-design and optimization of metabolic pathways, which might change the traditional bio-manufacturing process of 1-DNJ production that mainly exacted from mulberry leaves, and is expected to be applied in the production of hypoglycemic functional foods. Taken together, developments in synthetic biology and metabolic engineering are transforming the food industry [34], which could improve the traditional food production and manufacturing, as well as enhance food nutrition and functions, in addition, the traditional fermented food production can also be altered by the genetically engineered microbial [35].

The optimal regulation of central carbon metabolism plays the vital role in cell growth and target metabolite synthesis, which could improve the utilization ratio of carbon sources for target product production. For example, in order to improve PEP accumulation for tryptophan synthesis

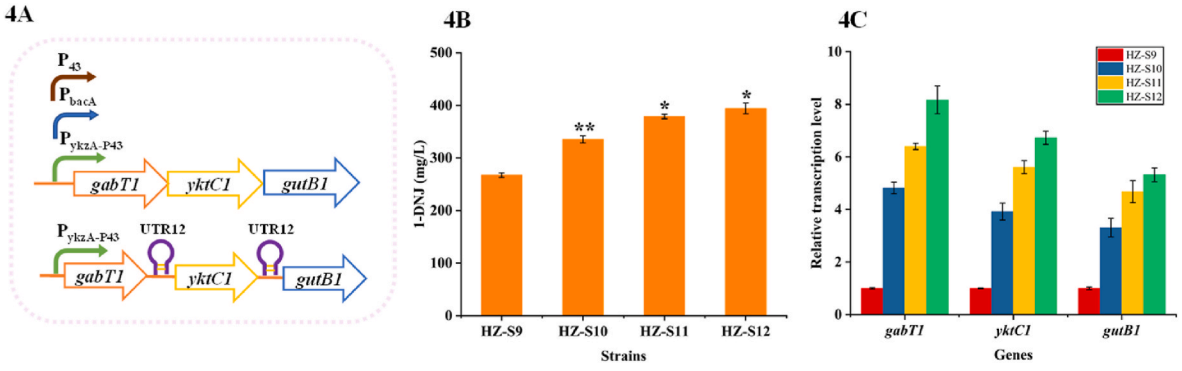

Fig. 4. Effects of promoter replacement of 1-DNJ synthetase gene cluster on 1-DNJ production. **A**: Schematic diagram of promoter and 5′-UTR replacement of 1-DNJ synthetase gene cluster, **B**: 1-DNJ yields, **C**: Genes transcription level.

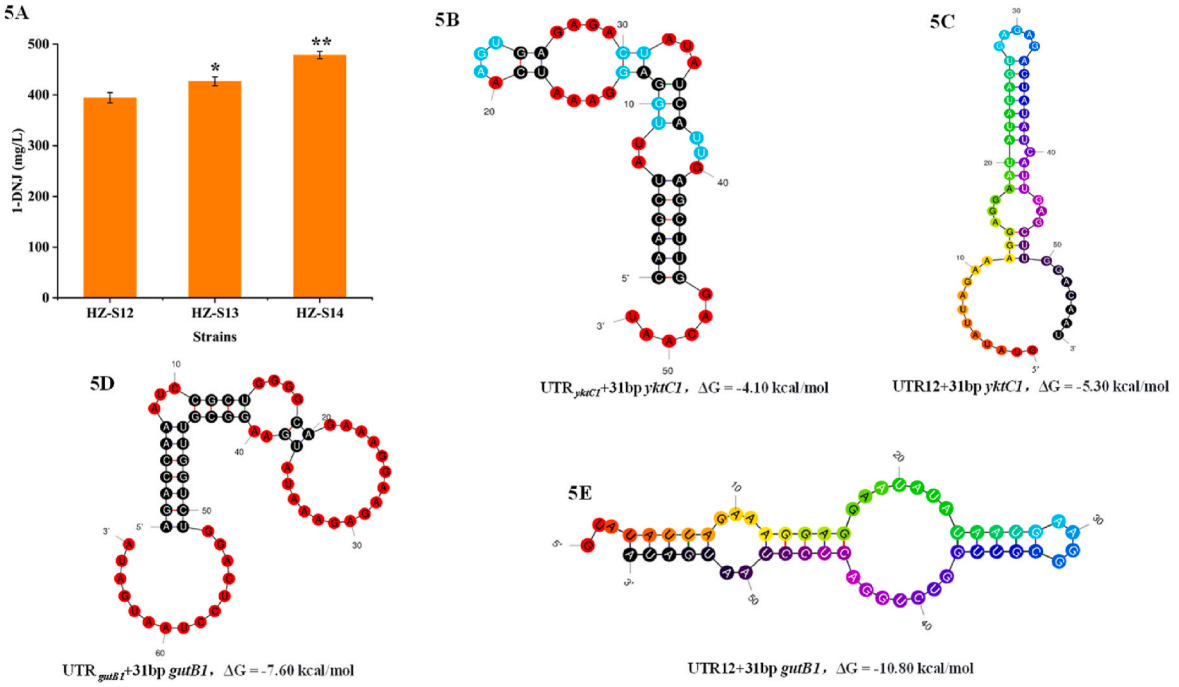

Fig. 5. Effects of 5′-UTR replacement of genes *yktC1* and *gutB1* on 1-DNJ production. **A**: 1-DNJ yields, **B**: The mRNA secondary structure of *yktC1* with its original 5′-UTR, **C**: The mRNA secondary structure of *yktC1* with UTR12, **D**: The mRNA secondary structure of *gutB1* with its original 5′-UTR, **E**: The mRNA secondary structure of *gutB1* with UTR12.

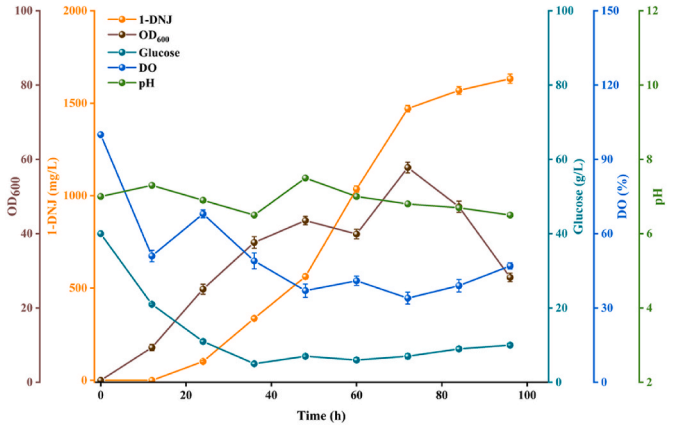

Fig. 6. The fermentation process curve of strain HZ-S14 in 3 L fermenter.

in *E. coli*, genes encoding for pyruvate oxidase B (*poxB*), pyruvate formate lyase (*pflB*), lactate dehydrogenase (*ldhA*), alcohol dehydrogenase (*adhE*), acetate kinase A (*ackA*) and phosphoacetyltransferase (*pta*) were knocked out in *E. coli*, and tryptophan yield was increased by 12% [36]. In *B. subtilis*, through repressing genes *pfkA* and *zwf* by CRISPRi, the carbon flux was partially diverted from pentose phosphate pathway (PPP) and glycolysis to hyaluronic acid biosynthesis, which led to the 108% increase of hyaluronic acid titer [37]. Meanwhile, a "push-pull--promote" strategy was established to rewire carbon metabolism for GlcNAc synthesis, and GlcNAc yield was increased to 24.5 g/L by 3.71 folds, and this research provided new methods and ideas for reconstructing metabolic networks to improve carbon atom economy [38]. Also, various approaches were conducted to optimize the glucose metabolism and shikimic acid synthesis pathway in *E. coli*, and shikimic acid yield was increased to 78.4 g/L in final strain [39]. In this study, in order to improve the intracellular accumulation of F-6-P, the expression levels of genes in glucose transport, glycolysis, peptidoglycan synthesis, and gluconeogenesis pathways were manipulated, which led to a 86.74%increase of 1-DNJ yield, and byproducts (acetoin and 2,3-butandieol) were also reduced simultaneously. In addition, our research found that NanR acted as a negative regulator for the repression of *nagA* and *nagB* expression in *B. amyloliquefaciens* HZ-12, which positively correlated with the previous result [40]. Moreover, the intracellular 1-DNJ might affect cell metabolism, and no intracellular 1-DNJ was detected by our results, suggested that 1-DNJ could be rapidly transported to extracellular. Unfortunately, there was no report about 1-DNJ transporter, we tried to excavate the suspected transporters surround 1-DNJ synthetase gene cluster in our previous work, but failed, and we will continue to carry out the relevant research on this aspect of work in future research. Taken together, all these researches demonstrated that precise regulation of central carbon metabolism is an effective strategy to achieve efficient synthesis of target products.

Gene cluster is widespread in the metabolitesynthesis, and the existence of which is in favor of genome simplification and genetic manipulation, however, it might also affect gene expression level, especially genes that are supposed to maintain the metabolic balance. Thus, it is necessary to modify the relevant elements to optimize its expression. 5′-UTR affects gene expression via regulating the mRNA translation initiation and secondary stability. Previously, a 5′-UTR library was established in our group [28], which significantly increased the yields of red fluorescence protein, green fluorescence protein, keratinase, bacitracin, etc, however, all these modifications mainly focused on the single gene or gene cluster. Here, our results implied that lower transcriptional levels of downstream genes (*yktC1* and *gutB1*) in 1-DNJ synthetase gene cluster might be the bottleneck for 1-DNJ synthesis, and 5′-UTR replacement significantly improved mRNA secondary stability, which benefited the improvement of target gene expression and 1-DNJ synthesis. This study expands the application field of 5′-UTR, and provided a new strategy for efficient synthesis of metabolites based on streamlined genomes.

5. Conclusions

1-DNJ, an efficient α-glucosidase inhibitory, has broad application prospects in the areas of functional food, biomedicine, etc. 1-DNJ is mostly extracted from mulberry leaves with high cost and low purity, which limited its extension application. Synthetic biology has made the great achievements in the fields of food, agriculture, chemical industry, etc. Here, to improve 1-DNJ production in *B. amyloliquefaciens*, the modules of glucosetransportation, F-6-P supply, 1-DNJ synthetase cluster were rewired, combining with feed-fermentation in 3 L fermenter, 1-DNJ yield was reached 1632.5 mg/L, increased by 77.62-fold compared to the original strain HZ-12, and which was the highest yield of 1-DNJ synthesis until now. Our results demonstrated that metabolic engineering was an efficient approach for enhanced production of 1-DNJ, and this research provided a promising strain for 1-DNJ industrial production.

Data availability statement

The data used to support the findings of this study are available from the corresponding authors upon request.

CRediT authorship contribution statement

Xujie Li: Methodology, Investigation, Data curation, Software, Writing – original draft. **Meng Zhang:** Investigation, Data curation, Software. **Yu Lu:** Methodology, Investigation, Data curation. **Ningyang Wu:** Investigation. **Jian'gang Chen:** Methodology, Investigation, Data curation. **Zhixia Ji:** Writing – review & editing. **Yangyang Zhan:** Writing – review & editing. **Xin Ma:** Data curation, Writing – review & editing. **Junyong Chen:** Data curation, Writing – review & editing. **Dongbo Cai:** Supervision, Data curation, Writing – review & editing. **Shouwen Chen:** Supervision, Writing – review & editing.

Declaration of competing interest

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2023.05.002.

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