19

Cofactor Engineering Enhances the Physiological Function of an Industrial Strain

Liming Liu and Jian Chen
State Key Laboratory of Food Science and Technology
Jiangnan University
China

1. Introduction

Microorganisms are able to produce a wide range of valuable chemicals and materials, and microbial fermentation is widely used as an alternative route for the production of chemicals in industry[1]. The key elements that determine the efficiency of a fermentation process are high titer, high yield, high productivity and process robustness[2]. These parameters are highly dependent on the host microorganism. In order to enhance the metabolic capabilities of the host microorganism, early research focused on screening appropriate microorganisms that naturally overproduce target products and improving their performance by random mutagenesis and by optimizing the fermentation processes. With the advent of metabolic engineering, many different genetic or metabolic engineering strategies have been adopted to improve the metabolic capabilities of the host strains, including relief of feedback inhibition, deletion of competing pathways, up-regulation of primary biosynthetic pathways, re-direction of central metabolism towards the target pathway, over-expression of export processes and insertion of new metabolic pathways. More recently, the emergence of systems biology integrated with metabolic engineering has provided a comprehensive understanding of microbial physiology, followed by a more global-wide identification of the target genes to be manipulated[3]. Those approaches have been proven to be powerful in developing microbial strains for the commercial production of organic acids[4], amino acids, biofuels and pharmaceuticals[5,6,7]. Nevertheless, problems such as the accumulation of toxic intermediates or metabolic stress resulting in decreased cellular fitness are still far from being solved. Over-expression, deletion or introduction of heterologous genes in target metabolic pathways does not always result in the desired phenotype. A good example is the attempts to increase the glycolytic flux, which cannot be increased by individual or combinational over-expression of genes encoding the key enzymes in either a eukaryotic or prokaryotic microorganism[8]. The essence of the problems listed above lies in the fact that, in addition to the modification of key genes by metabolic engineering, the researcher needs to study the effects of the internal environment (e.g. the intracellular energy charge and the interior redox potential and intracellular pH) on the phenotype, based on an accurate analysis of the metabolic network structure. If such an approach is adopted, manipulation of the form and level of intracellular cofactors can potentially be an efficient strategy for obtaining a desired phenotype.

In 1998, Hugenholtz from Delft University of Technology introduced the *nox-2* gene, which encodes the H_2O-forming NADH oxidase, into *Lactococcus lactis* resulting in a shift from homolactic to mixed-acid fermentation during aerobic glucose catabolism. The magnitude of the shift was directly dependent on the level of NADH oxidase overproduced[9]. This result is different from that of metabolic engineering. Most current metabolic engineering strategies have focused on manipulating enzyme levels through the amplification, interruption or addition of a metabolic pathway. The cofactors involved in microbial physiology include ATP / ADP / AMP, NADH / NAD^+, NADPH / $NADP^+$, acetyl coenzyme A and its derivatives, vitamins and trace elements. As illustrated in KEGG (www.kegg.com)[10], cofactors are essential to a large number of biochemical reactions; for example, NADH is involved in 740 biochemical reactions with 433 enzymes and NADPH is involved in 887 biochemical reactions with 462 enzymes (Table 1, updated in Mar 30, 2011). Their manipulation is expected to have substantial effects on metabolic networks. Cofactor engineering, therefore, has potential as a tool for metabolic manipulation.

	ATP	ADP	NADH	NAD+	NADPH	NADP+	CoA	Acetyl-CoA
Number of reactions	496	347	740	750	887	889	480	169
Number of enzymes	454	350	433	455	462	462	250	119

Table 1. The reactions and enzymes involved with nucleotide cofactors, as listed in KEGG

2. Strategies and applications of ATP manipulation

ATP, a kind of nucleotide, widely serves as substrate, product, activator or/and inhibitor in metabolic networks. Based on these four basic functions, the demand and supply of ATP could affect active transportation, peptide folding, subunit assembly, protein relocation and phosphorylation, cell morphology, signal transduction, and stress response. Through these complicated physiology process, ATP is involved in many metabolic pathways and production of almost all of the metabolites by industrial strains. Therefore, the manipulation of ATP supply and demand could be a powerful tool to increase the metabolic performance of industrial strains. Substrate-level phosphorylation (anaerobic conditions) and oxidative phosphorylation (aerobic conditions) were two different ATP regeneration pathways. It seems that manipulation of oxidative phosphorylation was a more efficient way to regulate the intracellular ATP concentration, because under aerobic conditions, most ATP production origin from oxidative phosphorylation pathway. It is conceivable that NADH availability, electron transfer chain (ETC), proton gradient, F_0F_1-ATPase and oxygen supply could all be regulatory candidates for manipulating the intracellular ATP availability.

2.1 Strategies for manipulation ATP availability

Intracellular NADH, produced from glycolysis, fatty acid oxidation, and the citric acid cycle, can be converted to NAD in three separate ways. Under aerobic growth, NADH oxidation occurs through ETC, in which oxygen is used as the final electron acceptor, and a large

amount of ATP is produced. Under anaerobic growth and in the absence of an alternate oxidizing agent, the oxidation of NADH can occur by fermentative pathways, such as aldehyde dehydrogenase[11], or lactate dehydrogenase[12]. In this case, energy production is mainly from substrate-level phosphorylation. NADH can also be directly oxidized into water and NAD through NADH oxidase[13]. Therefore, manipulating the availability and oxidation pathway of NADH may be an efficient strategy to manipulate the intracellular ATP level.

There are three different strategies to manipulate the NADH availability to adjust the intracellular ATP content, based on NADH-related metabolic pathways. Firstly, manipulating NADH availability through over-expression or deletion of the key NADH related enzymes, such as *ackA* (acetate kinase)[14], *aldA* (aldehyde dehydrogenase)[11], *ldh* (lactate dehydrogenase), and *pfl* (pyruvate formate-lyase). Secondly, supplement the culture medium with specific substrates for NAD-dependent dehydrogenase, such as formate, citrate and oxalate. Finally, overexpression of NADH oxidase genes, such as *noxE* from *Lactococcus lactis* or *nox* from *Streptococcus pneumoniae*, that oxidize NADH into NAD and water without ATP regeneration[13].

Complex I, II, III and IV are the key components of ETC and play the vital role in ATP production. Focusing on those four different complexes, three separate strategies have been used to disrupt the ETC's capacity to reduce energy production. To decrease ATP content by disrupting ETC, specific inhibitors of ETC components were added to the culture broth and a reduced ATP level was observed[15]. For yeast *T. glabrata*, when 10 mg·L^{-1} rotenone or antimycin A was added to the culture broth at the mid-exponential growth phase, the intracellular ATP level decreased 43% and 27.7%, respectively[8]. The second strategy was deficiency of key components by mutagenesis or genetic operation. The deficiency of cytochrome aa_3 and b in yeast led to an energy level decrease of 25%. The third method was disruption of ETC by ectopic expression of some enzymes, such as alternative oxidase (*AOX1*) from *Histoplasma capsulatum*[16], which disrupted ETC through oxidation of electrons and decreased ATP supply.

In aerobic growth, when oxygen is used as the final electron acceptor of the ETC, the abundance of oxygen in culture broth is the decisive environmental factor of ATP production, especially for some fermentation processes, which are high-density, high-viscous and high-energy requiring. Many studies have demonstrated that an increased ATP supply can be achieved by increasing oxygen supply. In the past decades, the strategies of process control and genetic modification have been applied to enhance the ATP production efficiency through increasing oxygen supply. The first strategy can be further divided into two different approaches. One is controlling the aeration rate through the agitation speed in bioreactors, or aeration with pure oxygen[17,18,19]. Many complicated oxygen-supply control strategies were developed based on these simple methods. Another approach is adding oxygen vectors to the culture broth, such as *n*-hexane, *n*-heptane and *n*-dodecane, result in high oxygen solubility in culture broth. In an example of a genetic strategy, hemoglobin from *Vitreoscilla* (*vgb*) was ectopically expressed in different industrial strain to improve oxygen transfer by binding oxygen at low extracellular oxygen content[20].

F_0F_1-ATPase, the final component of oxidative phosphorylation, plays the central role in ATP production. Three different methods have been performed to reduce the intracellular ATP by decreasing F_0F_1-ATPase activity. The first was supplementing the culture medium with an external and specific inhibitor of F_0F_1-ATPase, such as oligomycin, neomycin and

N′,N′-dicyclohexylcarbodiimide[21]. The second was genetic manipulation and traditional mutation of F_0F_1-ATPase[22]. In prokaryotic microorganisms, *Bacillus subtilis* and *Corynebacterium glutamicum* mutants defective in the activity of F_0F_1-ATPase have a decreased ATP supply and intracellular ATP level, thus increasing ATP demand through the glycolytic pathway[23]. For yeast *Torulopsis glabrata*, mutagenesis to decrease F_0F_1-ATPase activity by about 65% decreased the intracellular ATP level by 24%[24]. In *Saccharomyces cerevisiae*, F_0F_1-ATPase may be under strict regulation by autologous ATPase inhibitor peptides, such as IF1, which could affect the oligomerization of F_0F_1-ATPase and thus affect its activity[25]. All three methods described above used down-regulation of F_0F_1-ATPase. Over-expression of F_0F_1-ATPase was believed to be a most direct method for up-regulation of activity of the enzyme complex, which is not achieved for a long period. A most recent study has shown that over-expression of a mitochondrial *ATP6* gene from *Arabidopsis thaliana* in *S. cerevisiae* and *A. thaliana* could effective enhance the activity of F_0F_1-ATPase and ATP regeneration, thus enhance the tolerance to several kind of common stress[26].

2.2 Applications of ATP manipulation

The strategies to enhance the concentration, the yield, and the productivity of the target metabolites with ATP-based manipulation could be divided into three groups: (1) decreasing ATP supply; (2) increasing ATP supply; (3) multi-phase ATP-supply regulation strategies. The ultimate objective of ATP manipulation is to achieve the highest product concentration, the highest yield and the highest productivity, singly or in combination. In the past decades, ATP-oriented bioprocess optimization has developed expeditiously, and has successfully extended the boundaries of metabolic engineering. Here we present some representative works to further illustrate the concept of bioprocess optimization based on the regulation of ATP availability.

A higher target metabolite concentration in the fermentation broth increases the bioreactor utility and reduces the expense for the subsequent extraction process. Regulation of the ATP availability in industrial strain could further increase the target metabolite concentration. Three examples are presented to illustrate the feasibility of further increasing target metabolite concentration by increasing ATP supply.

Studies had demonstrated that a continuous and abundant supply of ATP was essential for glutathione (GSH) synthesis and secretion. A direct, efficient, but costly method to further increase GSH production is to supplement the culture broth with pure ATP, although this is too expensive to use on an industrial scale. Since 1978, researchers have been attempting to establish a coupled system for GSH production using genetically engineered *Escherichia coli* with *gshI*/*gshII* for GSH synthesis and permeabilized *S. cerevisiae* for ATP regeneration. Recently, using an improved coupling system, a high GSH concentration of 8.92 mM was achieved without supplement of ATP[27]. Another example is the enhancement of xanthan gum concentration by increasing ATP supply through two different methods. The first concentrated on the improvement of oxygen supply to increase ATP production. The second was to feed cells with an extra energy source, such as citric acid. It was interesting that the average molecular weight of xanthan gum was also improved by increasing ATP supply. With pulse-feeding of citric acid, similar results for the synthesis of poly-γ-glutamate (PGA) from glutamate by *Bacillus subtilis* (35 $g \cdot L^{-1}$ PGA concentration and 1 $g \cdot L^{-1} \cdot h^{-1}$ PGA productivity) was also achieved[28].

Increasing the fermentation productivity is an efficient way to increase the economy of bioprocess, because a high productivity decreases the fermentation period, the cost of

equipment and energy expenditure. For this aim, it is extremely important to increase the rate of carbon flux through central metabolic pathways, *e.g.* the glycolytic pathway and the citric acid cycle. It has been demonstrated that the flux through the glycolytic pathway towards energy production is enhanced by decreasing the energy level of prokaryotic and eukaryotic cells. It has been shown that mutants with a deficiency in ATP synthase have a decreased intracellular ATP level, resulting in an accelerated glycolytic flux and enhanced productivity[19,23,24].

On the other hand, an elevated intracellular ATP level can also improve the productivity of some metabolites. For hyaluronic acid (HA) production by *Streptococcus zooepidemicus*, it has been shown that cell growth and HA production are closely associated with ATP level in fermentation processes by metabolic flux analysis. The continuous production of HA by increasing the supply of ATP through glucose limitation and increased yeast extract supply, could reduce the time spent on repeated bioreactor cleaning processes, thus improving the total productivity.

During fine or bulk chemicals production by industrial strain, many byproducts, such as acetic acid, lactic acid and glycerol, are secreted into the culture broth. The accumulation of byproducts results in a decrease in the yield of product on substrate and an increase in the bioprocess cost, and the environmental burden. The following examples illustrate how to decrease the byproduct formation by manipulating ATP-related metabolic pathways[29,30].

For production of penicillin and its derivatives by *Penicillium chrysogenum*, a higher ATP supply is required for the fast growth of *P. chrysogenum*. Moreover, both the synthesis (73 mol of ATP per mol of penicillin-G) and secretion of penicillin are high ATP-requiring processes. The ATP supply during these processes is very important and should be under strict regulation. Otherwise, organic acids and other intermediate metabolites accumulate in the culture broth due to the deficiency of ATP. Previous research showed that a low oxygen supply can result in a decrease in the yield of penicillin on glucose. In order to further increase the penicillin G yield on glucose, 200 mM of formate was co-fed to penicillin G cultures as an energy source. As a result, the yield of biomass or penicillin G on glucose increased from 49% to 62%[31,32,33]. Lactic acid production is an anaerobic process, in which all of ATP is generated from glycolysis. A high yield of lactic acid on glucose was achieved by increasing ATP demand and accelerating the glycolytic flux through deficiency of pyruvate-formate-lyase (*pfl*) and oxygen limitation. Increasing ATP demand promoted the rate of glycolysis and inhibited the synthesis of byproducts under the oxygen limitation condition. Moreover, the deficiency of *pfl* efficiently eliminated the accumulation of formate. By this strategy, the yield of lactic acid on glucose was improved by 72.5%[12,34].

During the bioprocess, industrial strain may encounter a series of environmental stresses, such as acid, cold, oxidative and osmotic changes. As a consequence, the survival, growth, and metabolic function of industrial strain are affected by those stresses. A number of environmental stress resistance mechanisms have been identified and characterized. It was hypothesized that the supply of ATP plays significant roles in facilitating the stress response of industrial strain, through active transport and signaling pathways[35,36]. The primary mechanism by which industrial strain survive high stress is to control the intracellular environment by membrane-bound ATPases, which translocate specific ions to the environment at the expense of ATP hydrolysis. A deficiency in those ATPases greatly weakens the cells' resistance to environmental challenges, resulting in the cessation of growth and target metabolite accumulation. For instance, a mutant of *S. cerevisiae* deficient

in the activity of vacuolar proton-translocating ATPase has chronic oxidative stress. Both the aluminum and NaCl tolerance were sharply decreased in an *S. cerevisiae* strain deficient in the H^+-ATPase. In contrast, it is well documented that the tolerance of *S. cerevisiae* to high ethanol content is improved with increased ATP level. An enhanced ATPase system could well facilitate the cells in dealing with harsher conditions[37,38,39].

The ATP-based stress-induced signaling pathways have been widely studied in industrial strain. ATP was an essential substrate for signal pathways. Several signal transduction nodes in the high osmotic glycerol (HOG) pathway were shown to use ATP as an energy source in protecting against high osmotic stress. Similarly, ATP also facilitated signaling in other stress response networks, such as the signal of cold stress, heat stress and oxidative stress. In turn, some signaling pathways could also affect ATP synthesis under stress. In *Streptococcus*, mutants deficient in the minimally conserved bacterial signal recognition particle (SRP) elements remain viable but are more sensitive to environmental stress because the SRP deficiency decreases ATPase activity and limits the ATP supply[40,41]. It was found that industrial strain grown on high environmental stress have a high ATP demand[42] and increase ATP supply[43] could well facilitate the resistance to stress. However, most researches are focused on ATP-related mechanisms or phenomenon description during resistance to different stresses. Few reports are available on the deliberately up-regulating ATP levels to enhance the ability of industrial strain to deal with stress challenges.

3. Strategies and applications of NADH manipulation

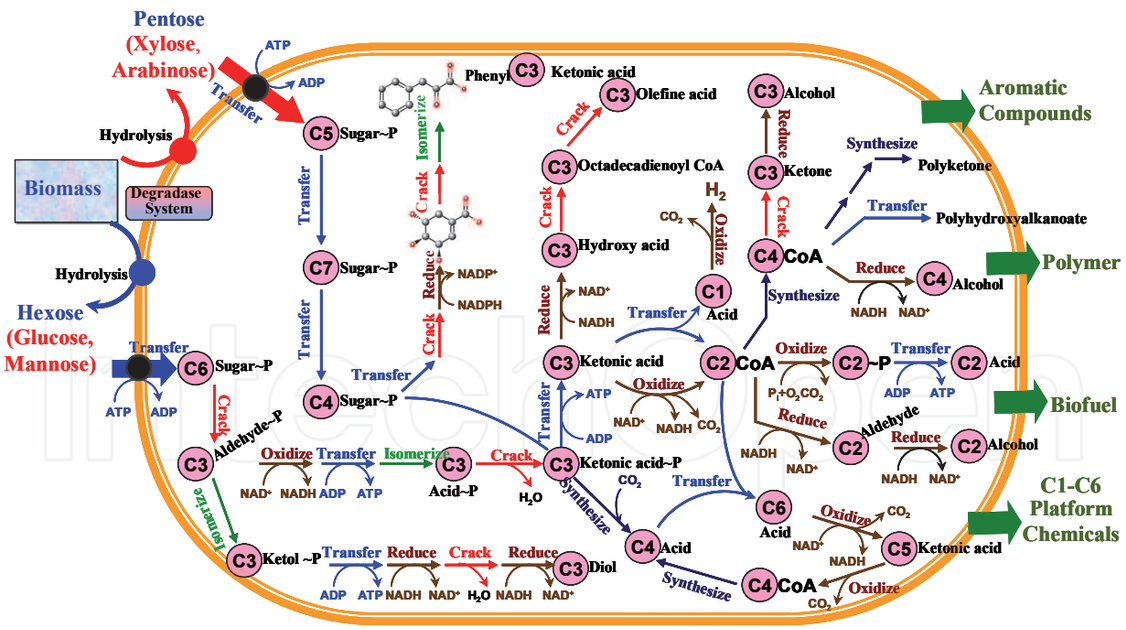


Fig. 1. An illustration of the broad use of the cofactor couple NADH/NAD+ in different metabolic reactions involved in the production of key metabolic products identified as platform chemicals

As the predominant redox product of catabolism, NADH has been found to be involved in more than 700 biochemical reactions in the microbial metabolic network (Table 1). Its

Manipulation strategy	Results/conclusion	Ref.
Biochemical engineering		
Feeding external electron acceptors		
Acetaldehyde	Decreased NADH/NAD^+ ratio	[47]
Fumarate or nitrate	Decreased NADH/NAD^+ ratio	[48]
Acetoin	Decreased NADH level	[49,50]
Pyruvate, citrate, O_2 or fructose	Decreased NAD(P)H level	[51]
Furfural	Decreased NADH level	[52]
Adding carbon sources with different oxidation states		
Sorbitol	Increased NADH availability	[44]
Gluconate	Decreased NADH/NAD^+ ratio	[53]
Adding a NAD^+ precursor		
Nicotinic acid	Increased NAD^+ level	[47]
Altering culture conditions		
Lower temperature	Increased NADH/NAD^+ ratio	
Increased dissolved oxygen level	Increased NADH availability	[54]
Extracellular oxidoreduction potential	Decreased NAD^+/NADH ratio in a relatively oxidative environment	[55]
Metabolic engineering		
Over-expressing enzymes association with NADH metabolism		
Nicotinic acid phosphoribosyltransferase	Increased NAD^+ levels and decreased NADH/NAD^+ ratio	[56]
Eliminating NADH competition pathways		
Inactivating aldehyde dehydrogenase	Increased NADH availability	[57]
Deactivating *adh*E, *ldh*A and *ack-pta*, simultaneously	Increased NADH availability	[58]
Introducing heterogeneous NADH metabolic pathways		
H_2O-NADH oxidase	Decreased NADH level and NADH/NAD^+ ratio	[59]
Alternative oxidase	Decreased NADH/NAD^+ ratio	[60]
NAD^+-dependent formate dehydrogenase	Increased NADH availability	[53,61]

Table 2. Manipulation strategies of NADH

physiological roles can be divided into five aspects (Fig. 1): (1) regulation of energy metabolism – NADH uses oxygen as the final electron acceptor to produce a large quantity of ATP through the electron transport chain in mitochondria; (2) adjustment of the microbial intracellular redox state – NADH/NAD+ is the main index of redox potential; (3) manipulation of carbon flux – NADH can redistribute carbon flux by activating or inhibiting

key enzymes in the target metabolic pathway; (4) modification of mitochondrial function – NADH can modify mitochondrial function by affecting mitochondrial permeability, controlling the mitochondrial membrane anion channel and increasing the mitochondrial membrane potential; (5) regulation of cell life cycle. Based on the above, it is conceivable that NADH/NAD+ could potentially act as an efficient tool to manipulate microbial growth and phenotype. In general, there are two different manipulation strategies for NAD(H/$^+$) availability (Table 2): (1) biochemical engineering approaches that include feeding external electron acceptors, adding carbon sources with different oxidation potentials or NAD synthesis precursors to the fermentation broth and controlling the culture conditions, such as the dissolved oxygen content, temperature and extracellular oxidoreduction potential[44,45]; (2) metabolic engineering methods such as over-expressing enzymes associated with NAD^+(NADH) metabolism, eliminating NAD^+(NADH) competition pathways and introducing an NAD^+(NADH) regeneration system[46]. These strategies have been proven to provide efficient control of the intracellular NAD^+(NADH) content.

3.1 Manipulation of NADH availability through biochemical engineering approaches

Many reports have demonstrated that aldehydes, ketones, organic acids, molecular nitrogen or nitrate can be used as internal electron acceptors to enhance NADH oxidation to maintain an optimum oxidoreduction level (using the NADH/NAD^+ ratio as the index) in industrial microorganisms. For example, during the heterofermentative lactic acid fermentation by *Lactobacillus* strains with hexose as the substrate, fructose, pyruvate, citrate, and O_2 were separately fed to the fermentation broth as external electron acceptors to accelerate the oxidation rate of NADH, to increase the growth rate of the lactic acid bacteria and to decrease the production of the byproducts erythritol and glycerol. When the external electron receptor acetoin was supplemented to the process of ethanol fermentation by *Saccharomyces cerevisiae* TMB3001 and *Fusarium oxysporum* with xylose as the substrate, the intracellular NAD^+ content and the yield of ethanol were efficiently increased. Another example was 4 mM acetyldehyde fed to the culture broth of *T. glabrata*, which led to a decrease in the NADH/NAD^+ ratio to 0.22 and improvement in the glucose consumption rate and the pyruvate titer of 26.3% and 22.5%, respectively.

When glucose, sorbitol and gluconate were compared as carbon sources in microbial glycolysis, sorbitol produced more NADH than glucose while gluconate was transformed directly to pyruvate with no NADH production. Therefore, the oxidation states of these three different carbon sources were -1 (sorbitol), 0 (glucose) and +1 (gluconate). It is thus conceivable that these carbon sources will have a pronounced effect on the intracellular NADH/NAD^+ ratio and subsequently on the carbon flux distribution. In a series of chemostat experiments under anaerobic conditions, San et al used three different carbon sources as a simple way of manipulating the cellular NADH/NAD^+ ratio from 0.51 (gluconate) to 0.75 (glucose) to 0.94 (sorbitol). The changes in the NADH/NAD^+ ratio increased the ethanol to acetate ratio from 1.00 with glucose to 3.62 with sorbitol and decreased it to 0.29 with gluconate. This result provided a simple method for manipulating the distribution of metabolic flux to the desired metabolites. In the case of succinate production by an engineered strain of *E. coli*, supplementation of sorbitol made the intracellular NADH content increase to 0.33 mmol/DCW and, as a result, the titer and yield of succinate increased by 96% and 81%, respectively.

In microbial cells, there are two different NAD synthesis pathways for maintaining the total NADH/NAD^+ intracellular pool: the de novo pathway and the pyridine nucleotide salvage

pathway. For the de novo pathway, NAD is synthesized from aspartate and dihydroxyacetone phosphate. The pyridine nucleotide salvage pathway recycles intracellular NADH breakdown products, such as nicotinamide mononucleotide (NMN), as well as other preformed pyridine compounds from the environment, such as nicotinamide and nicotinic acid (NA). As the NA concentration (8 mg/L) increased in the fermentation medium of *T. glabrata*, the glucose consumption rate and the pyruvate concentration increased by 48.4% and 29%, respectively.

External environmental conditions, such as the dissolved oxygen concentration and temperature, also modulate the intracellular NADH, NAD^+ and ATP levels, thus shifting the metabolic pattern. Under oxygen limited conditions, oxidation of NADH in *Aspergillus niger* mainly depends on mannitol-phosphate dehydrogenase. When the dissolved oxygen concentration increased from 1% to 10%, the intracellular NADH content in *T. glabrata* IFO 0005 increased by 50%; however, the intracellular NADH content did not continue to increase with further increases in the dissolved oxygen concentration. Under conditions of high dissolved oxygen, the specific enzyme activities and gene expression levels of NAD^+-related glucose-6-phosphate dehydrogenase, malate dehydrogenase and isocitrate dehydrogenase were significantly increased, but the activities of NAD^+-related enzymes in the TCA cycle were significantly inhibited. Singh et al demonstrated that *Pseudomonas fluorescens* could adjust NAD^+ kinase and $NADP^+$ phosphatase activity to supply enough NADPH while limiting NADH synthesis. According to the fact that microbial cells demand different NADH availabilities at different dissolved oxygen concentrations, Liu et al have shown that the NADH/NAD^+ ratio was efficiently reduced by adding acetaldehyde to the *T. glabrata* fermentation broth at 20% dissolved oxygen content. The reduction in NADH/NAD^+ led to increases in the pyruvate titer, yield and productivity of 68%, 44% and 45%, respectively. For the case of temperature effects on NADH availability, Cuihua Wang et al demonstrated that lower temperatures are beneficial to higher NADH/NAD^+ ratios in *T. glabrata*.

The extracellular oxidoreduction potential (ORP) of the fermentation medium is a comprehensive index of environmental conditions, essentially depending on the chemical composition, pH, temperature and dissolved oxygen (DO) concentration of the culture medium. Many reports have revealed that ORP plays a major role in the distribution of carbon flux through changes in the activities of key enzymes that have a metal cofactor in the active site. Du suggested that ORP manipulates the NADH level by affecting the activities of some NADH- or NAD^+-related enzymes that participate in electron transcription. Another example was presented by Qin et al, when potassium ferricyanide was added to the *T. glabrata* culture broth at 20% DO concentration, leading to the NADH content, NADH/NAD^+ ratio and ATP level decreasing by 45.3%, 60.3% and 15.2%, respectively. As a consequence, the specific glucose consumption rate increased by 45.5%.

3.2 Manipulation of NADH availability through metabolic engineering strategies

The second strategy that can be used to manipulate NADH availability is the metabolic engineering approach. The application of genetic and metabolic engineering has the potential to considerably affect NADH availability through the amplification, addition or deletion of NAD-related metabolic pathways. Two distinct genetic engineering methods can be used: the first approach aims at increasing the total NAD(H/$^+$) pool while the second approach focuses on changing the NADH/NAD^+ ratio. It is also conceivable that a

combination of these two approaches may lead to both an increased NAD(H/$^+$) pool and an increased ratio. As previously mentioned, nicotinic acid can be used to directly synthesize nicotinate mononucleotide, a direct precursor of NAD, catalyzed by nicotinic acid phosphoribosyltransferase (NAPRTase; EC2.4.2.11). This enzyme is encoded by the *pncB* gene. San et al has found that the total level of NAD(H/$^+$) in *E. coli* was increased by 26% but the NADH/NAD$^+$ ratio did not exhibit a consistent trend when the *pncB* gene was over-expressed. Similarly, Heuser et al over-produced the *pncB* and *nadE* genes, encoding nicotinic acid phosphoribosyltransferase and NAD synthetase in *E. coli*, which led to increases in the total NAD(H) and NADP(H) pools of 7-fold and 2-fold, respectively. For the NAD$^+$-dependent dehydrogenase, Cordier et al manipulated NADH regeneration and decreased NADH consumption through deletion of ADH1 and over-expression of ALD3, encoding, respectively, the major NAD$^+$-dependent alcohol dehydrogenase and a cytosolic NAD$^+$-dependent aldehyde dehydrogenase. As a metabolic response to the changing levels of intracellular NADH/NAD$^+$, the engineered *S. cerevisiae* secreted 0.46 g glycerol/g glucose at a rate of 3.1 mmol/g dry mass/h in aerated batch cultures. Another example in *S. cerevisiae* is over-expression of malic enzyme in the mitochondria or in the cytosol, which has a pronounced effect on the intracellular NAD(P)(H) pool. It was found that the total levels of NAD(H/$^+$) remained constant when the mitochondrial malic enzyme was over-expressed but decreased by 34% with over-expression of the cytosolic malic enzyme; both mitochondrial and cytosolic malic enzymes efficiently decreased the NADH/NAD$^+$ ratio. For NADP(H), the over-expression of mitochondrial malic enzyme decreased the total levels of NADP(H/$^+$) (by 17%) and the ratio of NADPH/NADP (by 6%).

The second approach to manipulating NADH is the deletion or weakening of the NADH competition pathways (Fig.2), to redirect NADH to the target metabolic pathway to enhance the production of the desired metabolites. By inactivating aldehyde dehydrogenase (ALDH) in *Klebsiella pneumonia*, an enzyme that competes with 1,3-PD oxidoreductase for NADH, the final titer, the productivity of 1,3-PD and the yield of 1,3-PD relative to glycerol reached 927.6 mmol L^{-1}, 14.05 mmol L^{-1} h^{-1} and 0.699 mol mol^{-1}, respectively. During the production of glycerol from glucose with *S. cerevisiae*, Geertman adopted a series of metabolic engineering strategies, which included: (1) maintaining flexibility at fructose-1,6-bisphosphatase and triosephosphate isomerase; (2) deleting pyruvate decarboxylases, NADH dehydrogenases and glycerol-3-phosphate dehydrogenase; (3) feeding formate, to make the glycerol yield from glucose reach 1.08 mol mol^{-1}. Similarly, inactivation of competing NADH pathways (alcohol dehydrogenase and lactate dehydrogenase) and heterologous production of pyruvate carboxylase (PYC) in *E. coli*, to provide maximum quantities of NADH for succinate synthesis, led to achievement of a succinate yield from glucose of 1.31 mol/mol. Based on the above result, Sanchez et al reconstructed a recombinant *E. coli* strain with deactivated adhE, ldhA and ack-pta and, by activating the glyoxylate pathway through the inactivation of iclR, reduced the NADH demand for production of a mole of succinate from 2 moles to 1.25 moles.

The third approach for manipulating NADH is heterologous production of oxido-reduction related enzymes, to change the ways NADH is regenerated or oxidized, thus changing the ratio NADH/NAD$^+$. In microbial cells, cytosolic NADH needs to shuttle to the mitochondria and be oxidized. It is conceivable that an increase in the efficiency and rate of NADH oxidation may be achieved through over-expression of water-forming NADH oxidase, which directly oxidizes NADH to NAD$^+$ in the cytoplasm. In *L. lactis*, the

heterologous expression of water-forming NADH oxidase led to a significantly decreased NADH/NAD^+ ratio and to a shift from homolactic to mixed-acid fermentation. Over-expression of the *noxE* gene, encoding the water-forming NADH oxidase in *S. cerevisiae*, resulted in decreases in the level of NADH and the NADH/NAD^+ ratio of 5- and 6-fold, respectively. As a consequence, the glucose consumption rate increased by 10%. In mitochondria, the over-expression of NADH alternative oxidase redirects the NADH oxidation pathway from the oxidative phosphorylation pathway to alternative oxidation and could effectively decrease the NADH/NAD^+ ratio and ATP content. In addition to accelerated NADH oxidation, some specific metabolite syntheses require a large quantity of NADH. Therefore, increasing the NADH availability is the limiting step for improving the production efficiency of the target metabolites. To this aim, Zhao et al significantly increased NADH availability through over-expression of phosphite dehydrogenase (PTDH), which catalyzes phosphate to phosphite and reduces NAD+ to NADH, from *Pseudomonas stutzeri*. Similarly, Berríos-Rivera et al reconstructed an efficient NADH regeneration pathway in *E. coli* through over-expression of NAD^+-dependent formate dehydrogenase from *Candida boidinii*, replacing the corresponding enzyme, which does not depend on any cofactor. The mutant exhibited high NADH synthesis ability, from 1 mole to 4 moles (under aerobic conditions) and 3 moles (under anaerobic conditions) per mole glucose.

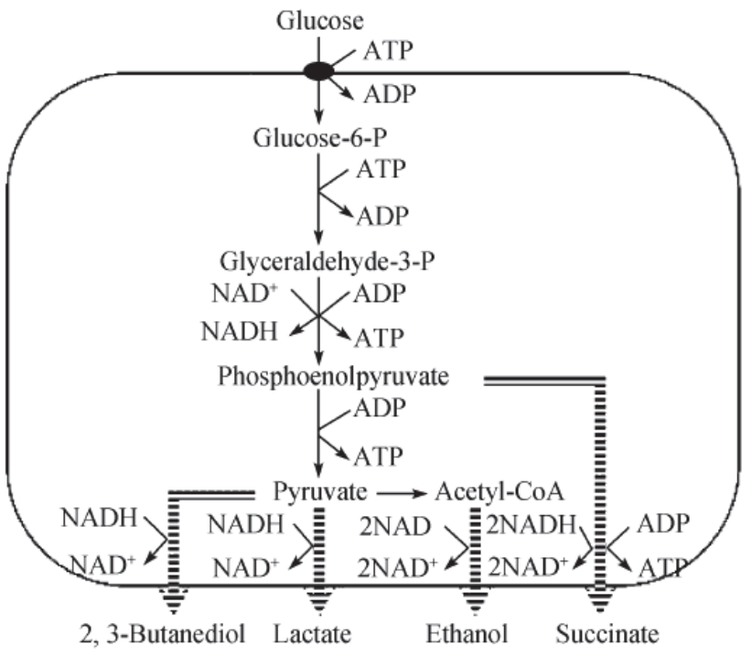


Fig. 2. The NADH competing pathway at the pyruvate node

4. Strategies and applications of CoA manipulation

As acetyl carriers, coenzyme A (CoA) (Table 2) and its derivatives, acetyl-CoA, succinyl coenzyme A (succinyl-CoA) and malonyl coenzyme A (malonyl-CoA), are involved in more than 600 biochemical reactions in microbial cell metabolism. Acetyl-CoA is an essential intermediate in many energy-yielding metabolic pathways and is a substrate in enzymatic

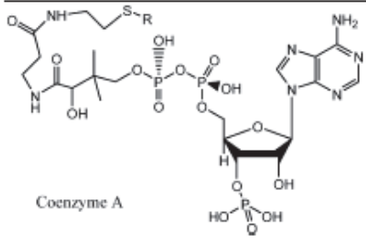
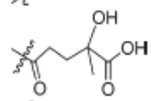
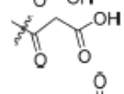
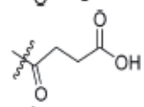
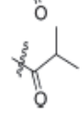
	Compound	R	Formula	Exact mass
 Coenzyme A	CoASH	H	$C_{21}H_{36}N_7O_{16}P_3S$	767.1152
	Acetyl-CoA		$C_{23}H_{38}N_7O_{17}P_3S$	809.1258
	3-Hydroxy-3-methyl-glutaryl-CoA		$C_{27}H_{44}N_7O_{20}P_3S$	911.1575
	β-Hydroxybutyryl-CoA		$C_{25}H_{42}N_7O_{18}P_3S$	853.1520
	Malonyl-CoA		$C_{24}H_{38}N_7O_{19}P_3S$	853.1156
	Succinyl-CoA		$C_{25}H_{40}N_7O_{19}P_3S$	867.1313
	Propionyl-CoA		$C_{24}H_{40}N_7O_{17}P_3S$	823.1414
	Isobutyryl-CoA		$C_{25}H_{42}N_7O_{17}P_3S$	837.1571

Table 3. CoA and its thioester derivatives

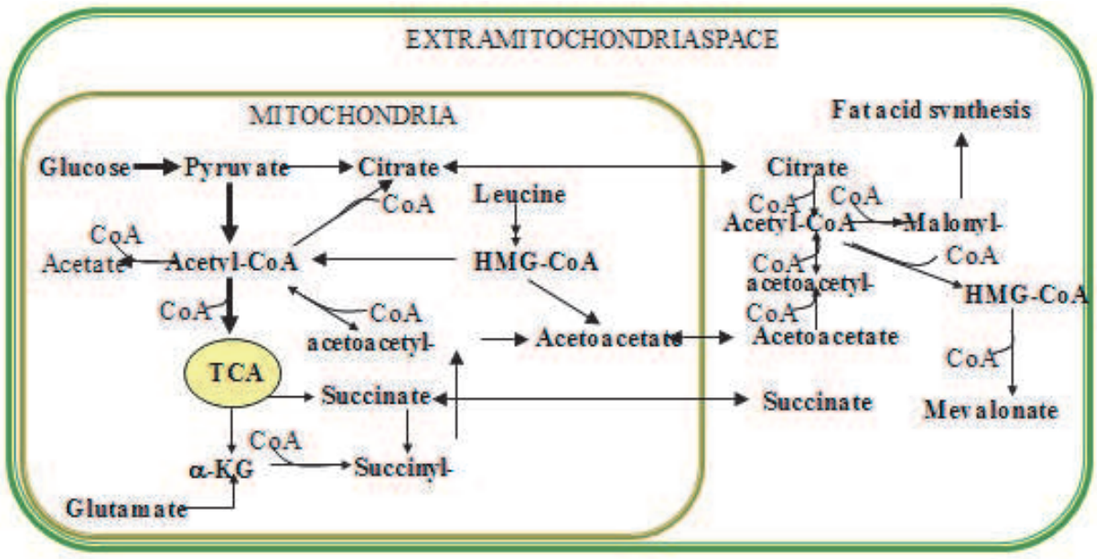


Fig. 3. Involvement of CoA and its derivates in metabolic networks

production of industrially useful compounds such as esters and lipid molecules. As illustrated in Fig. 3, CoA and its derivatives take part in a variety of metabolic functions,

such as the citric acid cycle, fatty acid synthesis and decomposition, macromolecule fat synthesis, amino acid metabolism, ketogenesis, sterol synthesis and as regulators to control some key enzymes in specific metabolic pathway[62,63].

4.1 Strategies of CoA manipulation

The total levels of CoA and its derivatives are dependent on the kinds and amounts of carbon sources in the fermentation medium. When *E. coli* were grown on a medium with glucose as the sole carbon source, the content of CoA reached a maximum value (400 umol/L) with acetyl-CoA as a derivative. However, the content of CoA decreased to 100 umol/L when the *E. coli* culture medium contained the amino acid mixture from casein hydrolysis. For pantothenic acid auxotrophic strains, the intracellular CoA content is determined by the concentration of acetate (or carboxylic acid salt). Due to the fact that the auxotrophic strain does not synthesize pantothenic acid when grown on a limited medium with glucose as the sole carbon source, this leads to a deficiency of succinyl-CoA for the synthesis of amino acids and proteins; as a consequence, the growth of the microbial cells is very slow. For the acetic acid auxotrophic *E. coli* strain, the intracellular acetyl-CoA level is low, and the total CoA level decreases with the hydrolysis and further metabolism of CoA. In contrast, the intracellular CoA level significantly increases with over-expression of CoA synthase in *E. coli*, and supplementation of pantothenic acid in the medium results in an increase in acetate production. Furthermore, it was found that the addition of acetate does not increase the acetyl-CoA content, but decreases the acetate concentration in the medium quickly reduce the level of intracellular acetyl-CoA. The kinds of carbon sources also have a pronounced effect on the CoA/acetyl-CoA ratio. Many studies have shown that the level of intracellular acetyl-CoA rapidly increases when microbial cells use D-glucose, D-fructose, D-mannose, glycerol or sorbitol as the sole carbon source, but remains constant with L-glucose, sucrose, maltose, succinate or acetate as the carbon source. Compared with that of glucose as a carbon source, acetate as the carbon source has a high CoA/acetyl-CoA ratio[64]. When microbial cells switched from acetate to glucose, the intracellular CoA level increased 50%. Consistent with this result, an increase in the CoA/acetyl-CoA ratio was observed. Furthermore, it was found that the specific activity of CoA synthase decreased with a shift from glucose to acetate[65]. This result demonstrated that CoA synthase is not sensitive to acetyl-CoA. Based on these findings, Lee et al enhanced PHB production by increasing intracellular acetyl-CoA and acetoacetyl-CoA levels in *E. coli* by adding a synthetic nitrogen-based amino acid and oleic acid[66].

4.2 Applications of CoA manipulation

From the above discussion, it can be concluded that the ratio of acetyl-CoA/CoA is a key index that reflects the metabolic state of carbon and energy metabolism during the fermentation process. San et al over-expressed pantothenate kinase in *E. coli*, by a combination of pantothenic acid addition, to significantly increase the intracellular CoA level. As a metabolic response to the change in CoA level, the carbon flux redistributed to isoamyl acetate, resulting in a significantly increased titer and yield of isoamyl acetate[67,68]. Similarly, the over-expression of pantothenate kinase gene also increased the CoA level (10-fold), acetyl-CoA level (5-fold) and the acetyl-CoA/CoA ratio, which channeled more carbon flux into acetate formation and to excessive accumulation of acetate[69].

Apart from metabolic engineering strategies, the biochemical engineering strategy has been proven to be an effective way to manipulate CoA and acetyl-CoA levels and the acetyl-

CoA/CoA ratio. An example is the manipulation of thiamine, biotin and Ca^{2+} levels as a tool for redistributing carbon flux from pyruvate to α-ketoglutarate in *T. glabrata*: (1) the carbon flux was blocked at the pyruvate node (69 g/L) with the sub-optimization of vitamins in the fermentation medium; (2) the titer of α-ketoglutarate reached 10.3 g/L by selectively opening the valve of carbon flux from pyruvate to the pyruvate dehydrogenase complex in the pyruvate carboxylase pathway by increasing the concentrations of thiamine and biotin; (3) the concentration of α-ketoglutarate (43.7 g/L) was further increased through increasing the pyruvate carboxylase level with Ca^{2+} present in the fermentation medium[70].

5. Concluding remarks and future directions

Recently, many studies have demonstrated that the fermentation process from sugar to the target product is not just a simple biochemical reaction but rather a comprehensive network, including the gene regulatory network, protein-protein interaction network, signal transduction network and metabolic network, dependent on the physical and chemical interactions of genes, proteins and metabolites. Therefore, interest in cofactor engineering in the future should be concerned with: (1) identification of the active site of the cofactor in the biochemical reaction, metabolic pathway and metabolic network; (2) evaluation of the effect of the cofactor on the metabolic reaction, pathway and network; (3) finding the threshold values at which the metabolic networks and regulatory networks respond to cofactor changes; (4) development of directed, precise strategies for cofactor manipulation. The increasing availability of genome sequences and accumulation of high-throughput biological data allow us to understand the physiological functions of cofactors and to propose precise strategies for cofactor manipulation of microbial physiology.

6. References

[1] Nielsen J, Otero JM (2010) Industrial Systems Biology. Biotechnology and Bioengineering 105: 439-460.

[2] Nielsen J, Liu LM, Agren R, Bordel S (2010) Use of genome-scale metabolic models for understanding microbial physiology. FEBS Letters 584: 2556-2564.

[3] Lee SY, Park JH (2008) Towards systems metabolic engineering of microorganisms for amino acid production. Current Opinion in Biotechnology 19: 454-460.

[4] Lin H, Bennett GN, San KY (2005) Fed-batch culture of a metabolically engineered Escherichia coli strain designed for high-level succinate production and yield under aerobic conditions. Biotechnology and Bioengineering 90: 775-779.

[5] Liao JC, Atsumi S (2008) Metabolic engineering for advanced biofuels production from Escherichia coli. Current Opinion in Biotechnology 19: 414-419.

[6] Wendisch VF, Bott M, Eikmanns BJ (2006) Metabolic engineering of Escherichia coli and Corynebacterium glutamicum for biotechnological production of organic acids and amino acids. Current Opinion in Microbiology 9: 268-274.

[7] Xian M, Yu C, Cao YJ, Zou HB (2011) Metabolic engineering of Escherichia coli for biotechnological production of high-value organic acids and alcohols. Applied Microbiology and Biotechnology 89: 573-583.

[8] Chen J, Liu LM, Li Y, Li HZ (2006) Significant increase of glycolytic flux in Torulopsis glabrata by inhibition of oxidative phosphorylation. FEMS Yeast Research 6: 1117-1129.

[9] Lopez de Felipe F, Kleerebezem M, de Vos WM, Hugenholtz J (1998) Cofactor engineering: a novel approach to metabolic engineering in Lactococcus lactis by controlled expression of NADH oxidase. Journal of Bacteriology 180: 3804-3808.
[10] Aoki-Kinoshita KF (2006) Overview of KEGG applications to omics-related research. Journal of Pesticide Science 31: 296-299.
[11] Cao Z, Zhang YP, Li Y, Du CY, Liu M (2006) Inactivation of aldehyde dehydrogenase: A key factor for engineering 1,3-propanediol production by Klebsiella pneumoniae. Metabolic Engineering 8: 578-586.
[12] Zhu J, Shimizu K (2004) The effect of pfl gene knockout on the metabolism for optically pure D-lactate production by Escherichia coli. Applied Microbiology and Biotechnology 64: 367-375.
[13] Nielsen J, Vemuri GN, Eiteman MA, McEwen JE, Olsson L (2007) Increasing NADH oxidation reduces overflow metabolism in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America 104: 2402-2407.
[14] Underwood SA, Zhou S, Causey TB, Yomano LP, Shanmugam KT, et al. (2002) Genetic changes to optimize carbon partitioning between ethanol and biosynthesis in ethanologenic Escherichia coli. Applied and Environmental Microbiology 68: 6263-6272.
[15] Miyoshi H, Abe M, Kubo A, Yamamoto S, Hatoh Y, et al. (2008) Dynamic function of the spacer region of acetogenins in the inhibition of bovine mitochondrial NADH-ubiquinone oxidoreductase (complex I). Biochemistry 47: 6260-6266.
[16] Johnson CH, Prigge JT, Warren AD, McEwen JE (2003) Characterization of an alternative oxidase activity of Histoplasma capsulatum. Yeast 20: 381-388.
[17] Huang H, Qu L, Ji XJ, Ren LJ, Nie ZK, et al. (2011) Enhancement of docosahexaenoic acid production by Schizochytrium sp. using a two-stage oxygen supply control strategy based on oxygen transfer coefficient. Letters in Applied Microbiology 52: 22-27.
[18] Huang H, Peng C, Ji XJ, Liu X, Ren LJ, et al. (2010) Effects of n-Hexadecane Concentration and a Two-Stage Oxygen Supply Control Strategy on Arachidonic Acid Production by Mortierella Alpina ME-1. Chemical Engineering & Technology 33: 692-697.
[19] Li Y, Hugenholtz J, Chen J, Lun SY (2002) Enhancement of pyruvate production by Torulopsis glabrata using a two-stage oxygen supply control strategy. Applied Microbiology and Biotechnology 60: 101-106.
[20] Tang KX, Zhang L, Li YJ, Wang ZN, Xia Y, et al. (2007) Recent developments and future prospects of Vitreoscilla hemoglobin application in metabolic engineering. Biotechnology Advances 25: 123-136.
[21] Johnson KM, Cleary J, Fierke CA, Opipari AW, Jr., Glick GD (2006) Mechanistic basis for therapeutic targeting of the mitochondrial F1F0-ATPase. ACS Chem Biol 1: 304-308.
[22] Yokota A, Henmi M, Takaoka N, Hayashi C, Takezawa Y, et al. (1997) Enhancement of glucose metabolism in a pyruvic acid-hyperproducing Escherichia coli mutant defective in F-1-ATPase activity. Journal of Fermentation and Bioengineering 83: 132-138.
[23] Sekine H, Shimada T, Hayashi C, Ishiguro A, Tomita F, et al. (2001) H+-ATPase defect in Corynebacterium glutamicum abolishes glutamic acid production with enhancement of glucose consumption rate. Appl Microbiol Biotechnol 57: 534-540.

[24] Liu LM, Li Y, Du GC, Chen J (2006) Increasing glycolytic flux in Torulopsis glabrata by redirecting ATP production from oxidative phosphorylation to substrate-level phosphorylation. Journal of Applied Microbiology 100: 1043-1053.

[25] Garcia JJ, Morales-Rios E, Cortes-Hernandez P, Rodriguez-Zavala JS (2006) The inhibitor protein (IF1) promotes dimerization of the mitochondrial F1F0-ATP synthase. Biochemistry 45: 12695-12703.

[26] Zhang X, Liu S, Takano T (2008) Overexpression of a mitochondrial ATP synthase small subunit gene (AtMtATP6) confers tolerance to several abiotic stresses in Saccharomyces cerevisiae and Arabidopsis thaliana. Biotechnol Letter 30: 1289-1294.

[27] Liao X, Deng T, Zhu Y, Du G, Chen J (2008) Enhancement of glutathione production by altering adenosine metabolism of Escherichia coli in a coupled ATP regeneration system with Saccharomyces cerevisiae. Journal of Applied Microbiology 104: 345-352.

[28] Ahn WS, Park SJ, Lee SY (2000) Production of Poly(3-hydroxybutyrate) by fed-batch culture of recombinant Escherichia coli with a highly concentrated whey solution. Applied Environmental Microbiology 66: 3624-3627.

[29] Karakashev D, Thomsen AB, Angelidaki I (2007) Anaerobic biotechnological approaches for production of liquid energy carriers from biomass. Biotechnology Letter 29: 1005-1012.

[30] Suwannakham S, Huang Y, Yang ST (2006) Construction and characterization of ack knock-out mutants of Propionibacterium acidipropionici for enhanced propionic acid fermentation. Biotechnology and Bioengineering 94: 383-395.

[31] Harris DM, van der Krogt ZA, van Gulik WM, van Dijken JP, Pronk JT (2007) Formate as an auxiliary substrate for glucose-limited cultivation of Penicillium chrysogenum: impact on penicillin G production and biomass yield. Applied Environmental Microbiology 73: 5020-5025.

[32] Garrido JM, van Benthum WA, van Loosdrecht MC, Heijnen JJ (1997) Influence of dissolved oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. Biotechnology and Bioengineering 53: 168-178.

[33] van Gulik WM, de Laat WT, Vinke JL, Heijnen JJ (2000) Application of metabolic flux analysis for the identification of metabolic bottlenecks in the biosynthesis of penicillin-G. Biotechnology and Bioengineering 68: 602-618.

[34] Neves AR, Pool WA, Kok J, Kuipers OP, Santos H (2005) Overview on sugar metabolism and its control in Lactococcus lactis - the input from in vivo NMR. FEMS Microbiol Review 29: 531-554.

[35] Lokanath NK, Matsuura Y, Kuroishi C, Takahashi N, Kunishima N (2007) Dimeric core structure of modular stator subunit E of archaeal H+ -ATPase. J Mol Biol 366: 933-944.

[36] Salinas P, Ruiz D, Cantos R, Lopez-Redondo ML, Marina A, et al. (2007) The regulatory factor SipA provides a link between NblS and NblR signal transduction pathways in the cyanobacterium Synechococcus sp. PCC 7942. Mol Microbiol 66: 1607-1619.

[37] Shima J, Ando A, Takagi H (2008) Possible roles of vacuolar H+-ATPase and mitochondrial function in tolerance to air-drying stress revealed by genome-wide screening of Saccharomyces cerevisiae deletion strains. Yeast 25: 179-190.

[38] Milgrom E, Diab H, Middleton F, Kane PM (2007) Loss of vacuolar proton-translocating ATPase activity in yeast results in chronic oxidative stress. Journal of Biological Chemistry 282: 7125-7136.

[39] Stewart CM, Cole MB, Legan JD, Slade L, Schaffner DW (2005) Solute-specific effects of osmotic stress on Staphylococcus aureus. Journal of Applied Microbiology 98: 193-202.

[40] Hasona A, Crowley PJ, Levesque CM, Mair RW, Cvitkovitch DG, et al. (2005) Streptococcal viability and diminished stress tolerance in mutants lacking the signal recognition particle pathway or YidC2. Proc Natl Acad Sci U S A 102: 17466-17471.

[41] Hasona A, Zuobi-Hasona K, Crowley PJ, Abranches J, Ruelf MA, et al. (2007) Membrane composition changes and physiological adaptation by Streptococcus mutans signal recognition particle pathway mutants. Journal of Bacteriology 189: 1219-1230.

[42] Canovas M, Bernal V, Sevilla A, Iborra JL (2007) Salt stress effects on the central and carnitine metabolisms of Escherichia coli. Biotechnology and Bioengineering 96: 722-737.

[43] Sanchez C, Neves AR, Cavalheiro J, dos Santos MM, Garcia-Quintans N, et al. (2008) Contribution of citrate metabolism to the growth of Lactococcus lactis CRL264 at low pH. Appl Environ Microbiol 74: 1136-1144.

[44] Lin H, Bennett GN, San KY (2005) Effect of carbon sources differing in oxidation state and transport route on succinate production in metabolically engineered Escherichia coli. J Ind Microbiol Biotechnol 32: 87-93.

[45] Ma B, Pan SJ, Zupancic ML, Cormack BP (2007) Assimilation of NAD(+) precursors in Candida glabrata. Mol Microbiol 66: 14-25.

[46] Cordier H, Mendes F, Vasconcelos I, Francois JM (2007) A metabolic and genomic study of engineered Saccharomyces cerevisiae strains for high glycerol production. Metabolic Engineering 9: 364-378.

[47] Liu LM, Li Y, Shi ZP, Du GC, Chen J (2006) Enhancement of pyruvate productivity in Torulopsis glabrata: Increase of NAD(+) availability. J Biotechnol 126: 173-185.

[48] de Graef MR, Alexeeva S, Snoep JL, Teixeira de Mattos MJ (1999) The steady-state internal redox state (NADH/NAD) reflects the external redox state and is correlated with catabolic adaptation in Escherichia coli. J Bacteriol 181: 2351-2357.

[49] Panagiotou G, Christakopoulos P (2004) NADPH-dependent D-aldose reductases and xylose fermentation in Fusarium oxysporum. J Biosci Bioeng 97: 299-304.

[50] Panagiotou G, Christakopoulos P, Villas-Boas SG, Olsson L (2005) Fermentation performance and intracellular metabolite profiling of Fusarium oxysporum cultivated on a glucose-xylose mixture. Enzyme Microb Technol 36: 100-106.

[51] Zaunmuller T, Eichert M, Richter H, Unden G (2006) Variations in the energy metabolism of biotechnologically relevant heterofermentative lactic acid bacteria during growth on sugars and organic acids. Applied Microbiology Biotechnology 72: 421-429.

[52] Wahlbom CF, Hahn-Hagerdal B (2002) Furfural, 5-hydroxymethyl furfural, and acetoin act as external electron acceptors during anaerobic fermentation of xylose in recombinant Saccharomyces cerevisiae. BiotechnologyBioengineering 78: 172-178.

[53] San KY, Bennett GN, Berrios-Rivera SJ, Vadali RV, Yang YT, et al. (2002) Metabolic engineering through cofactor manipulation and its effects on metabolic flux redistribution in Escherichia coli. Metabolic Engineering 4: 182-192.

[54] Qiang H, Shimuzu K (1999) Effect of dissolved oxygen concentration on the intracellular flux distribution for pyruvate fermentation. Journal of Biotechnology 68: 135-147.

[55] Du CY, Yan H, Zhang YP, Li Y, Cao ZA (2006) Use of oxidoreduction potential as an indicator to regulate 1,3-propanediol fermentation by Klebsiella pneumoniae. Applied Microbiology Biotechnology 69: 554-563.

[56] Berrios- Rivera SJ, San KY, Bennett GN (2002) The effect of NAPRTase overexpression on the total levels of NAD, the NADH/NAD(+) ratio, and the distribution of metabolites in Escherichia coli. Metabolic Engineering 4: 238-247.

[57] Zhang YP, Li Y, Du CY, Liu M, Cao Z (2006) Inactivation of aldehyde dehydrogenase: A key factor for engineering 1,3-propanediol production by Klebsiella pneumoniae. Metabolic Engineering 8: 578-586.

[58] Sanchez AM, Bennett GN, San KY (2005) Novel pathway engineering design of the anaerobic central metabolic pathway in Escherichia coli to increase succinate yield and productivity. Metabolic Engineering 7: 229-239.

[59] Heux S, Cachon R, Dequin S (2006) Cofactor engineering in Saccharomyces cerevisiae: Expression of a H2O-forming NADH oxidase and impact on redox metabolism. Metabolic Engineering 8: 303-314.

[60] Vemuri GN, Eiteman MA, McEwen JE, Olsson L, Nielsen J (2007) Increasing NADH oxidation reduces overflow metabolism in Saccharomyces cerevisiae. PNAS 104: 2402-2407.

[61] Saanchez AM, Bennett GN, San KY (2005) Effect of different levels of NADH availability on metabolic fluxes of Escherichia coli chemostat cultures in defined medium. Journal Biotechnologyl 117: 395-405.

[62] Jackowski S, Rock CO (1986) Consequences of reduced intracellular coenzyme A content in Escherichia coli. Journal of Bacteriology 166: 866-871.

[63] Chohnan S, Furukawa H, Fujio T, Nishihara H, Takamura Y (1997) Changes in the size and composition of intracellular pools of nonesterified coenzyme A and coenzyme A thioesters in aerobic and facultatively anaerobic bacteria. Applied Environmental Microbiology 63: 553-560.

[64] Vallari DS, Jackowski S, Rock CO (1987) Regulation of pantothenate kinase by coenzyme A and its thioesters. Journal of Biological Chemistry 262: 2468-2471.

[65] Chohnan S, Izawa H, Nishihara H, Takamura Y (1998) Changes in size of intracellular pools of coenzyme A and its thioesters in Escherichia coli K-12 cells to various carbon sources and stresses. Bioscience Biotechnolology and Biochemical 62: 1122-1128.

[66] Lee SY, Chang HN (1995) Production of poly(3-hydroxybutyric acid) by recombinant Escherichia coli strains: genetic and fermentation studies. Candia Journal Microbiology 41 Suppl 1: 207-215.

[67] Vadali RV, Bennett GN, San KY (2004) Enhanced isoamyl acetate production upon manipulation of the acetyl-CoA node in Escherichia coli. Biotechnol Prog 20: 692-697.

[68] Vadali RV, Bennett GN, San KY (2004) Applicability of CoA/acetyl-CoA manipulation system to enhance isoamyl acetate production in Escherichia coli. Metabolic Engineering 6: 294-299.

[69] Vadali RV, Bennett GN, San KY (2004) Cofactor engineering of intracellular CoA/acetyl-CoA and its effect on metabolic flux redistribution in Escherichia coli. Metabolic Engineering 6: 133-139.

[70] Liu L, Li Y, Zhu Y, Du G, Chen J (2007) Redistribution of carbon flux in Torulopsis glabrata by altering vitamin and calcium level. Metabolic Engineering 9: 21-29.

Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications

Edited by Prof. Angelo Carpi

ISBN 978-953-307-268-5
Hard cover, 646 pages
Publisher InTech
Published online 01, August, 2011
Published in print edition August, 2011

This book provides an example of the successful and rapid expansion of bioengineering within the world of the science. It includes a core of studies on bioengineering technology applications so important that their progress is expected to improve both human health and ecosystem. These studies provide an important update on technology and achievements in molecular and cellular engineering as well as in the relatively new field of environmental bioengineering. The book will hopefully attract the interest of not only the bioengineers, researchers or professionals, but also of everyone who appreciates life and environmental sciences.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Liming Liu and Jian Chen (2011). Cofactor Engineering Enhances the Physiological Function of an Industrial Strain, Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications, Prof. Angelo Carpi (Ed.), ISBN: 978-953-307-268-5, InTech, Available from: http://www.intechopen.com/books/progress-in-molecular-and-environmental-bioengineering-from-analysis-and-modeling-to-technology-applications/cofactor-engineering-enhances-the-physiological-function-of-an-industrial-strain

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen