REVIEW ARTICLE

Contemplating 3-Hydroxypropionic Acid Biosynthesis in *Klebsiella pneumoniae*

Ying Li · Pingfang Tian

Abstract 3-Hydroxypropionic acid (3-HP) is a commercially valuable platform chemical from which an array of C_3 compounds can be generated. *Klebsiella pneumoniae* has been considered a promising species for biological production of 3-HP. Despite a wealth of reports related to 3-HP biosynthesis in *K. pneumoniae*, its commercialization is still in infancy. The major hurdle hindering 3-HP overproduction lies in the poor understanding of glycerol dissimilation in *K. pneumoniae*. To surmount this problem, this review aims to portray a picture of 3-HP biosynthesis, involving 3-HP-synthesizing strains, biochemical attributes, metabolic pathways and key enzymes. Inspired by the state-of-the-art advances in metabolic engineering and synthetic biology, here we advocate protocols for overproducing 3-HP in *K. pneumoniae*. These protocols range from cofactor regeneration, alleviation of metabolite toxicity, genome editing, remodeling of transport system, to carbon flux partition via logic gate. The feasibility for these protocols was also discussed.

Keywords 3-Hydroxypropionic acid · *Klebsiella pneumoniae* · Cofactor regeneration · Genome editing · Logic gate · Synthetic biology

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Introduction

Biosynthesis has emerged as an alternative to conventional chemical synthesis owing to fast consumption of petroleum resources. 3-Hydroxypropionic acid (3-HP) is one of the 12 top-added platform compounds suggested by US DOE [1]. As a versatile precursor, 3-HP can be readily converted into a series of economically important chemicals, including 1,3-propanediol (1,3-PDO), 3-hydroxypropionaldehyde (3-HPA), acrylic acid, and malonic acid [2]. Additionally, the hydroxyl and carboxyl in 3-HP molecule can be polymerized to form poly-3-hydroxypropionate [3], which is a novel polymer displaying higher mechanical strength and elongation at break compared with poly lactic acid (2-hydroxypropionic acid). Because of its broad applications, 3-HP has been fueled in recent years.

So far myriads of 3-HP biosynthesis pathways have been identified [4]. Based on carbon sources, 3-HP pathways can be divided into two types: glucose- and glycerol-based routes. In the glucose-based pathway, glucose is converted to acetyl-CoA which is catalyzed to propionyl-CoA by Acetyl-CoA carboxylase. Next, propionyl-CoA is catalyzed to 3-HP by malonyl-CoA reductase [5]. Except this, there exist other biosynthetic pathways where glucose is converted to glycerol and next to 3-HP [6]. In the glycerol pathway, glycerol dehydratase catalyzes glycerol into 3-hydroxypropionaldehyde (3-HPA), next, 3-HPA is converted to 3-HP by aldehyde dehydrogenase (AldH) [7], and to 1,3-propanediol by 1,3-propanediol oxidoreductase [8] (see Fig. 1).

As a main byproduct of biodiesel production, glycerol is a competitive substrate for 3-HP biosynthesis due to its decreasing price [9, 10]. In contrast to glucose pathway, glycerol pathway seems to be more applicable owing to its simple catalytic reactions and tractable manipulation. Apart
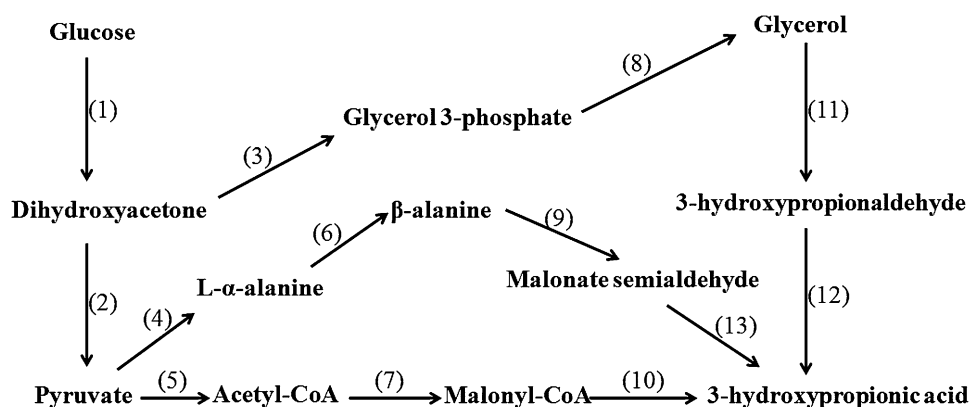
Fig. 1 Representative pathways of 3-HP production from glucose or glycerol. (*1*) and (*2*) indicate the enzymes in glycolytic pathway, (*3*) glycerol-3-phosphate dehydrogenase EC:1.1.1.8, (*4*) beta-alanine/pyruvate aminotransferase EC:2.6.1.18, (*5*) pyruvate oxidase EC:1.2.3.6, (*6*) alanine 2,3-aminomutase EC:5.4.3.8, (*7*) acetyl-CoA carboxylase EC:6.4.1.2 or biotin carboxylase EC:6.3.4.14, (*8*) glycerol 3-phosphatase EC:3.1.3.21, (*9*) beta -alanine/pyruvate aminotransferase EC:2.6.1.18, (*10*) malonyl-CoA reductase EC:1.2.1.75, (*11*) large subunit of glycerol dehydratase EC:4.2.1.30, (*12*) aldehyde dehydrogenase EC:1.2.1.4, (*13*) 3-hydroxypropionate dehydrogenase (NADP$^+$) EC:1.1.1.298

from above mentioned two synthetic pathways, 3-HP can be derived from other carbon sources like sucrose and maltose [11]. Most of the carbon sources are converted to 3-HP via intermediate pyruvate or glycerol in vivo. In addition, recent research demonstrates that 3-HP can also be generated from CO_2 through 3-HP cycle in *Pyrococcus furiosus* [12], which was previously reported as carbon dioxide fixation pathway [13].

Klebsiella pneumoniae and Glycerol Dissimilation

Among 3-HP-synthesizing strains, *Klebsiella pneumoniae* is of great attractiveness due to its biochemical attributes, including remarkable capacity to metabolize glycerol, active cell proliferation, and native capability to synthesize vitamin B_{12} [14]. Since vitamin B_{12} is the cofactor of glycerol dehydratase, *K. pneumoniae* has obvious advantage over *E. coli* which needs the addition of expensive vitamin B_{12} to fermentation medium [15]. Although *Lactobacillus reuteri* also naturally synthesizes 3-HPA and is benign to environment, its growth is significantly slow compared with *K. pneumoniae*, thus increasing production cost. Under anaerobic or microaerobic conditions, *K. pneumoniae* grow actively with glycerol as sole carbon source. An operon named *dha* governs the glycerol dissimilation which relates to glycerol oxidation and reduction pathways [16]. In glycerol oxidation pathway, glycerol dehydrogenase catalyzes glycerol into dihydroxyacetone (DHA), next, DHA is mutated into dihydroxyacetone phosphate (DHAP) by dihydroxyacetone kinase. In glycerol reduction pathway, glycerol is changed into 3-hydroxypropionaldehyde (3-HPA) by B_{12}-dependent glycerol dehydratase (GDHt). Subsequently, 3-HPA is converted to

3-HP by aldehyde dehydrogenase (AldH), and to 1,3-propanediol (1,3-PDO) by 1,3-propanediol oxidoreductase [7, 17] (Fig. 1). It seems that *dha* regulon was tailored for the adaption to anaerobic or microaerobic environment. Of diverse 3-HP-synthesizing bacteria reported thus far, *Klebsiella* and *Clostridium* species harbor intact *dha* regulon [14]. These bacteria have developed a suite of mechanisms to adapt the changing milieus. For example, under anaerobic conditions glycerol dissimilation in *K. pneumoniae* is mediated by *dha* regulon. However, under aerobic conditions, *glp* instead of *dha* works because *glp* regulon is more efficient for glycerol consumption and cell respiration [16].

Despite *K. pneumoniae* can naturally synthesize 3-HP due to its innate aldehyde dehydrogenases (e.g., KPN_01468,KPN_01919) [15], the yield is far from commercialization. Namely, 3-HP pathway needs significant modification. Among all identified 3-HP pathways, the pathway native to *K. pneumoniae* has attracted most attention. Previous reviews have documented the research advances [11, 18], here we rethink the glycerol dissimilation in *K. pneumoniae* in the hope of addressing the problems arising from molecular breeding.

3-HP Production Relies Largely on the Activity of Aldehyde Dehydrogenase

As mentioned above, glycerol dehydratase catalyzes glycerol into 3-HPA, which is subsequently converted into 1,3-PDO by 1,3-propanediol oxidoreductase, or, in parallel, into 3-HP by aldehyde dehydrogenase [19]. Wild type *K. pneumoniae* strain produces more 1,3-PDO yet less 3-HP because 1,3-propanediol oxidoreductase shows higher activity than aldehyde dehydrogenase. The reasons behind

include but are not limited to the following: (1) Compared with 3-HP, 1,3-PDO has lower polarity and does not cause significant pH fluctuation. Moreover, 1,3-PDO is an anti-freeze that may enable *K. pneumoniae* to survive at low temperature. Thus, from the perspective of evolution, we hypothesize that *K. pneumoniae* prefers to synthesize 1,3-PDO rather than 3-HP. (2) High activity of aldehyde dehydrogenase resulted in the accumulation of 3-HP, which slowed down the cell growth [20]. In other words, low activity ALDH restricts the formation of 3-HP and benefits cell growth. (3) Biosynthesis of 3-HPA represents a self-protection mechanism for *K. pneumoniae*. This viewpoint is evidenced by the fact that some viruses are sensitive to aldehyde [21, 22], and intracellular 3-HPA may diffuse out of the cell to prevent virus spreading. Hence, low activity of ALDH enables the existence of 3-HPA, which is ready to cope with viral invasion. (4) 3-HPA acts as the core metabolite in glycerol dissimilation pathways, and its biosynthesis contributes to both rigidity and flexibility of *dha* operon. Presumably, when glycerol is ample, 3-HPA is converted to 3-HP or 1,3-PDO. Conversely, when glycerol is exhausted, 3-HPA as an intermediate blocks the conversion of glycerol to 3-HP and 1,3-PDO.

Aldehyde dehydrogenases are common in nearly all living organisms. The ubiquitous aldehyde dehydrogenases catalyze aldehydes to corresponding carboxylic acids [23]. In plants and bacteria, aldehyde dehydrogenases are expressed under extreme conditions to cope with hostile stimuli such as drought and high concentration of aldehyde [23]. Upon long term evolution, aldehyde dehydrogenase currently exhibits low activity because its overexpression collides with other functional aldehyde compounds. To overproduce 3-HP, high activity ALDH is imperative and the below are approaches toward this goal. One is screening strains from extreme environment, given that the adaptation to harsh conditions is usually correlated with high activity enzyme. One such example is the screened enzyme "KGSADH" that exhibited a high activity toward 3-HPA [15]. Another approach towards high ALDH activity is "directed evolution". Through error-prone PCR or DNA shuffling and subsequent high throughput screening, high activity ALDH could be acquired. The third strategy is chemical synthesis of the enzyme gene based upon molecular simulation with emphasis on substrate specificity.

Insufficient Cofactor NAD^+ Limits 3-HP Production

Cofactor availability is crucial for aldehyde dehydrogenase (ALDH) activity and 3-HP biosynthesis [24]. In glycerol reductive pathway, ALDH together with NAD^+ catalyzes 3-HPA to 3-HP [25]. Since ALDH activity is lower than GDHt, plasmid-dependent ALDH overexpression is a common strategy for promoting 3-HPA to 3-HP. One

drawback of this strategy is massive NAD^+ consumption by the overexpression of ALDH, which leads to NAD^+ exhaustion and the cessation of 3-HP formation. Another factor affecting ALDH activity is the formation of byproducts. Accompanied by 3-HP biosynthesis, lactate dehydrogenase and phosphotransacetylase catalyze the formation of lactic acid and acetic acid, respectively. Since phosphotransacetylase also utilizes NAD^+ as a cofactor, there exists a competition for the limited amount of NAD^+ between this enzyme and ALDH. Evidently, both ALDH activity and 3-HP biosynthesis are associated with the formation of lactic acid and acetic acid.

To boost 3-HP production, NAD^+ regeneration system could be engineered in *K. pneumoniae*. Previously, NAD^+ was regenerated mainly by overexpression of the enzymes using NADH as a cofactor. For example, both lactate dehydrogenase and glutamine dehydrogenase are NADH-dependent enzymes, and were expressed in host cell to regenerate NAD^+ [26, 27]. However, plasmid-dependent cofactor regeneration overburdens the host cell and therefore hinders 3-HP productivity. To coordinate cofactor regeneration and cell growth, coproduction of two or more desired compounds was implemented. For example, 3-HP and 1,3-PDO were successfully coproduced, achieving titer up to 24.4 g/L of and 49.3 g/L, respectively [28]. The overall yield on glycerol is around 50–80 % [15]. So far although coenzyme regeneration systems have been engineered in *K. pneumoniae* for the production of 3-HP and 1,3-PDO, there existed an imbalance between oxidation and reduction pathways, because emphasis was preferentially placed on glycerol reduction pathway. Namely, the structural rigidity of *dha* regulon was ignored. Based on available conclusions, coproduction of 3-HP and 1,3-PDO seems to be feasible.

Excessive Lipopolysaccharide Attenuates the Carbon Flux Toward 3-HP

The third hurdle retarding 3-HP biosynthesis is lipopolysaccharide secreted from the cell wall of Gram-negative bacteria. As an endotoxin and the major ingredient of cell wall, lipopolysaccharide is generated when bacteria are subjected to lysozyme degradation or virus infection. Considering *K. pneumoniae* is a pathogenic bacterium, we reckon that it may produce more lipopolysaccharide than that of nonpathogenic bacteria. Since the biosynthesis of lipopolysaccharide consumes a large amount of carbon source, the conversion of glycerol to 3-HP or 1,3-PD is attenuated. Aside from this drawback, excess accumulation of lipopolysaccharide entangles the downstream separation and thereby increases the cost of waste disposal, particularly the quenching of pathogenicity or sterilization [29]. Considering lipopolysaccharide makes *K.*

pneumoniae robust to survive under hostile environment, it is postulated that moderate rather than excessive lipopolysaccharide may benefits the formation of 3-HP and 1,3-PD. Park and his colleagues screened a new *K. pneumoniae* strain J2b, which produced less lipopolysaccharide yet more 1,3-PDO [30]. To reduce the formation of lipopolysaccharide, deletion or down-regulation of its synthetic genes is imperative.

3-HP Formation is Related to Cell Tolerance

3-HP cannot be overproduced because it is toxic to the host. Previously 3-HP was shown to have nematicidal activity. For example, some endophytic fungi secrete 3-HP to protect host plant from the invasion of nematode [31]. Also, some bacteria are sensitive to 3-HP [32, 33] because low concentration 3-HP is sufficient to inhibit bacteria. Beyond cytotoxicity, 3-HP overburdens the host strain *K. pneumoniae*, which in turn restricts its biosynthesis. One strategy to address this dilemma is the addition of a chemical into fermentation medium, which can specifically bind to 3-HP and therefore attenuate its toxicity. This strategy had been applied to the industrial production of 3-HPA, whereby achieving titer up to 108 g/L [34]. Another strategy is expression of a channel protein that timely pumps 3-HP out of cell once it is generated. The unknown protein may be discovered via computer stimulation. Although it seems difficult to uncover or engineer such a channel protein, this idea is a fascinating solution to this problem.

Attenuating the Metabolic Burden Caused by Plasmid

In microbial metabolic engineering, plasmid vectors are widely used for overexpression of the enzymes closely related to desired metabolite. Previous 3-HP production was achieved mainly by plasmid-dependent overexpression of glycerol dehydratase and aldehyde dehydrogenase in *K. pneumoniae* or *E. coli* [28, 35]. However, plasmid-dependent metabolic engineering shows the following drawbacks. First, plasmid replication overburdens the host *K. pneumoniae*, which retards cell growth and blocks 3-HP biosynthesis. Second, the addition of antibiotics and IPTG into fermentation medium increases the production cost. Third, the engineered strains show genetic instability mainly due to the plasmid loss. In fact, it is difficult to simultaneously express multiple enzymes in a single vector. Moreover, co-transformation of two or more vectors into host cell leads to metabolic imbalance which in turn hampers 3-HP production. Although 3-HP concentration was partially enhanced by plasmid-dependent method, the engineered strains are often fragile and unstable when they are fermented in a larger bioreactor, and, moreover, the productivity cannot maintain as high as that in 5 L

bioreactor. Considering most industrialized products such as ethanol and 2,3-butanediol are achieved by using plasmid-free strategy [36, 37], plasmid-independent metabolic engineering is suggested. One such strategy is incorporation of 3-HP synthesis module into chromosome. Not only alleviating the burden to host cell, genome engineering also endows strain with genetic stability. Along this line, the engineered *K. pneumoniae* strain may divert ample carbon flux towards 3-HP.

Exploiting Synthetic Biology Approaches to 3-HP Production

Since glycerol dissimilation in *K. pneumoniae* involves parallel oxidation and reduction pathways, it is unlikely to preferentially intensify any branched pathway. Given the topological rigidity of *dha* operon, novel strategies are required to remodel 3-HP pathways. Recent years have been a watershed for microbial breeding due to the emergence of synthetic biology. The repertoire of synthetic biology is beyond imagination. For instance, a series of genome editing approaches have tapped into horizon, which are powerful in sculpting genome.

Rewiring 3-HP Pathway Via Genome Editing

Early endeavors have revealed the involvement of multiple enzymes in 3-HP biosynthesis, indicating that 3-HP production depends on quantitative trait loci (QTL). In fact, overexpression or disruption of one or two specific genes has proven inefficient to significantly accumulate 3-HP. Hence, large-scale genome editing seems imperative. Recent years have seen great strides in the area of genome editing. Among the approaches, a creative technique named multiplex automated genomic engineering (MAGE) is eye-catching [38]. In light of Red recombination mechanism, artificially synthetic DNA fragments specific to target genes are continuously transferred into bacteria (see Fig. 2). Subsequently, high-yield strains are screened and analyzed for the mutation sites that affect 3-HP yield. The advantage of this method is twofold. First, the chromosome is globally modified and the resulting clones are subjected to high throughput screening. Second, the strains are genetically stable because the genes are modified at the DNA level. For genome editing of *K. pneumoniae* via MAGE, the candidate genes may include those governing the formation of lipopolysaccharide, porin, lactic acid, and acetic acid. Also, the regulatory genes closely related to 3-HP synthesis are incorporated. By means of MAGE, the above mentioned genes could be deleted or mutated, and the biosynthesis pathways of byproducts could be determined. Apart from modification of multiple genes, one or two specific genes also deserve to be overexpressed or
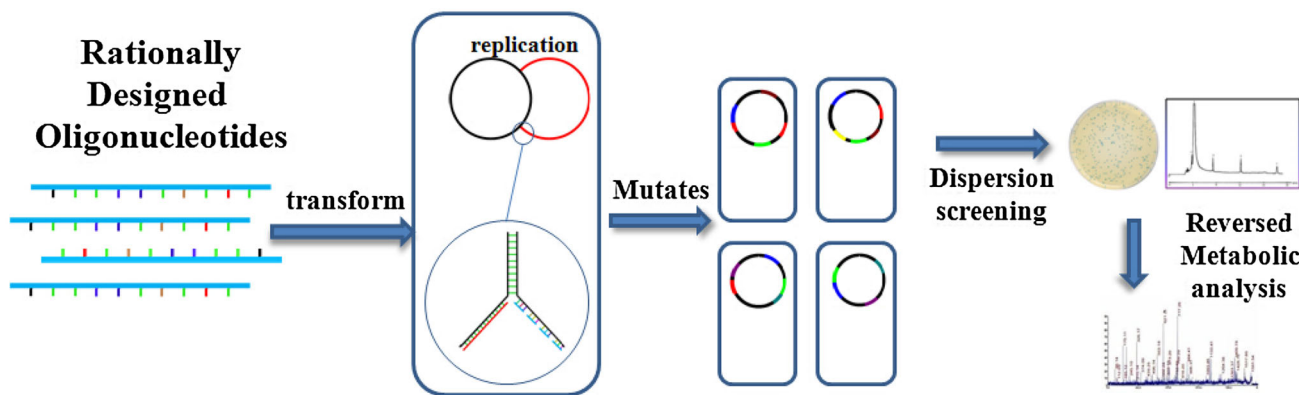
Fig. 2 Protocols for enhancing 3-HP production via MAGE. Different oligonucleotides are chemically synthesized and transformed into *K. pneumoniae*, leading to insertion, mutation or deletion in 3-HP synthetic pathways. Metabolic flux is preferentially diverted into 3-HP pathway and high yield strains are acquired. DNA sequencing of the mutated nucleotides may reveal the limitations on 3-HP production, which facilitates further modification of the *K. pneumoniae* genome
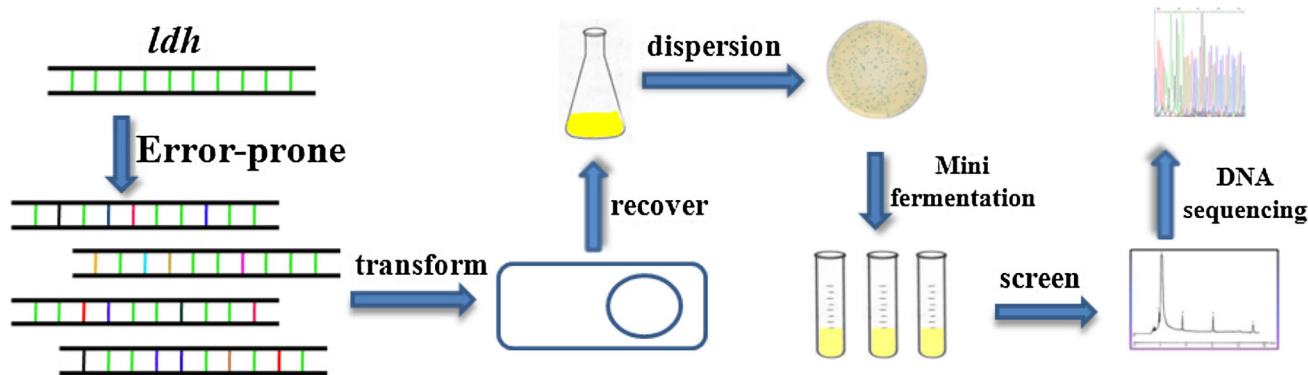

Fig. 3 Chromosome engineering of *K. pneumoniae* using double-strand DNA fragments. Primer design is based on reported sequence. Error-prone PCR is repeatedly performed. The mutated DNA fragments are transformed into host cell and subjected to homologous recombination. Positive clones are fermented for screening high-yielding strain. This method is free from selection marker and thus suitable for strain improvement

mutated. Briefly, the primers containing mutated gene sequence could be artificially designed, chemically synthesized, and electro-transformed into the host. The resulting positive clones display phenotype of the target genes (see Fig. 3). Aside from MAGE, other novel genome editing technologies also come to light, including CAGE [39], TALEN [40] and CRISPR-Cas9 System [41]. With the aid of these techniques, the *K. pneumoniae* genome can be thoroughly reshaped and 3-HP production may be significantly enhanced.

Switching Metabolic States Via On/Off Logic Gate

In *K. pneumoniae*, 3-HP production is positively correlated with biomass accumulation during log-phase. Interestingly, 3-HP in turn retards cell growth. To resolve this dilemma, two-phase fermentation strategy came into being. That is,

biomass accumulation and 3-HP biosynthesis were implemented separately. Previously, biomass accumulation was achieved by using glucose as carbon source, and 3-HP biosynthesis was initiated by addition of glycerol into medium. To date, the burgeoning synthetic biology offers novel approach to two-phase fermentation. Based on bi-stability in bacteria [42, 43], an "on/off" logic gate could be engineered in *K. pneumoniae*, which executes the switch between two metabolic states: cell growth and 3-HP formation (see Fig. 4).

Although glucose is the most efficient carbon source to bacterial growth, it impedes the consumption of other carbohydrates, which is so-called Crabtree effect [44]. Fortunately, *K. pneumoniae* grows actively using glycerol as the sole carbon. Hence, for glycerol-based 3-HP biosynthesis in *K. pneumoniae*, biomass accumulation is accomplished by the addition of glycerol into medium at the initial stage of fermentation. Once cell concentration
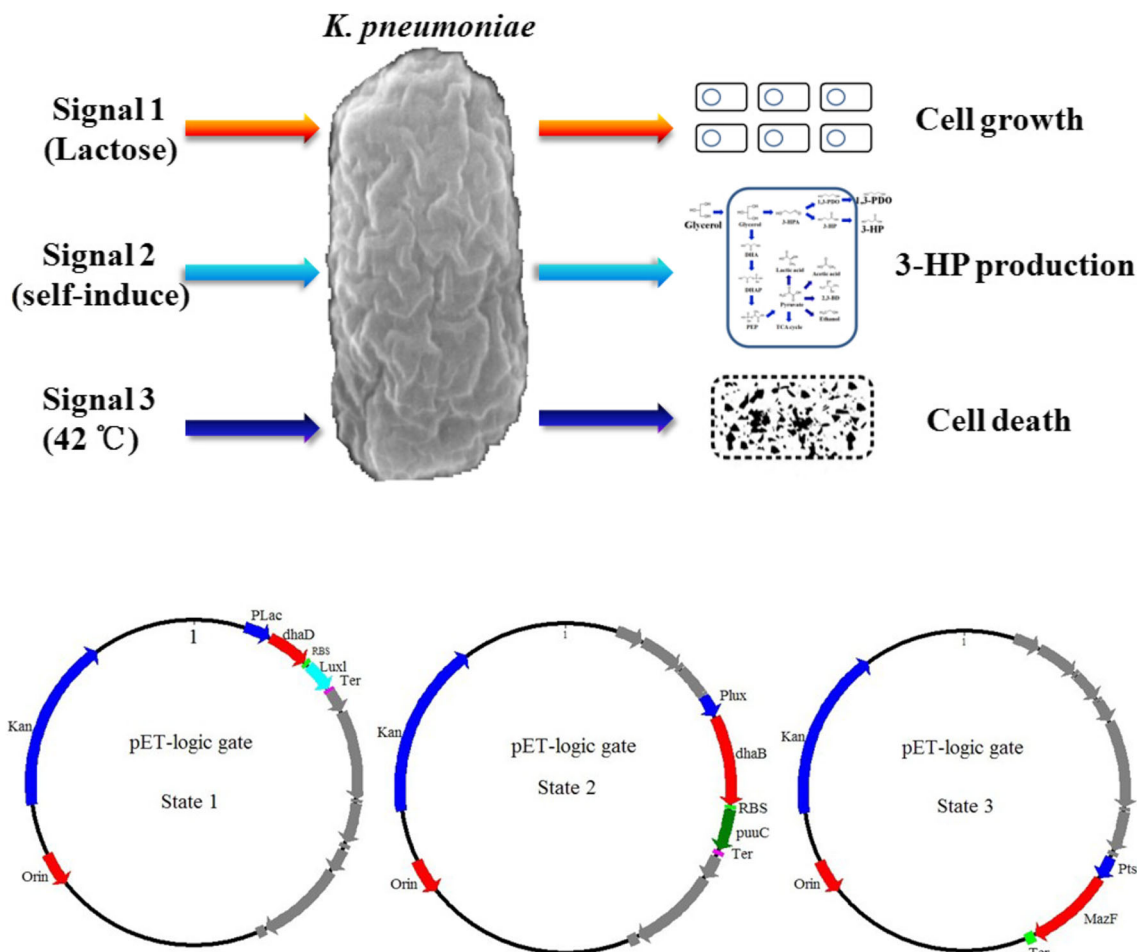
Fig. 4 The entire 3-HP production is divided into three phases by logic gate. First, cell growth is facilitated by overexpression of *dhaD* to divert carbon flux into oxidative pathway. Meanwhile, Lux I is expressed to accumulate AHL, which is the inducer of next step. Second, when AHL concentration reaches a set threshold, *dhaB* and *puuC* are expressed for biosynthesis of 3-HP. This step is controlled by promoter *Plux*. Third, the gene *MazF* from *E. coli* is expressed for cell suicide and simplifying downstream separation process. This *Pts*-driven process is silenced at 37 °C but reactivated at 42 °C

reaches a set threshold, 3-HP biosynthesis is triggered by switching to another state (see Fig. 5). At this state, glycerol oxidation pathway is simultaneously running to coordinate the reduction and oxidation pathways. This proof-of-concept "on/off" strategy can alleviate feedback inhibition and attenuate 3-HP toxicity. The entire fermentation process is exactly controlled and the balance between oxidation and reduction is maintained.

Enhancing Enzymatic Activities Via Surface Display

3-HP biosynthesis depends largely on the activities of GDHt and ALDH. Previous metabolic engineering of *K. pneumoniae* centered upon in vivo biosynthesis but ignored in vitro catalysis. Surface display can enhance enzymatic activity due to enlarged specific surface area. In principle,

scaffold proteins were expressed in vivo and assembled in vitro on cell surface. For 3-HP biosynthesis, the key enzymes GDHt and ALDH as ligands are transferred to cell surface with the aid of signal peptide and then specifically bind to the scaffold protein which acts as receptor. GDHt and ALDH could even be fused to form a "superbug" that continuously catalyzes glycerol to 3-HP. Due to enlarged specific surface area and spatial proximity, glycerol conversion rate may be enhanced (see Fig. 6). Although this strategy may encounter obstacles, it represents a novel strategy to implement sequential reactions. In fact, surface display has been applied to ethanol production from renewable carbon sources and CO_2 fixation [45, 46]. Surface display is an important addition to the toolbox for improving enzymatic activity, attenuating toxicity, and alleviating the feedback inhibition arising from metabolite accumulation.

Logic gate for switching glucose and glycerol-utilizing states

Glucose utilizing state
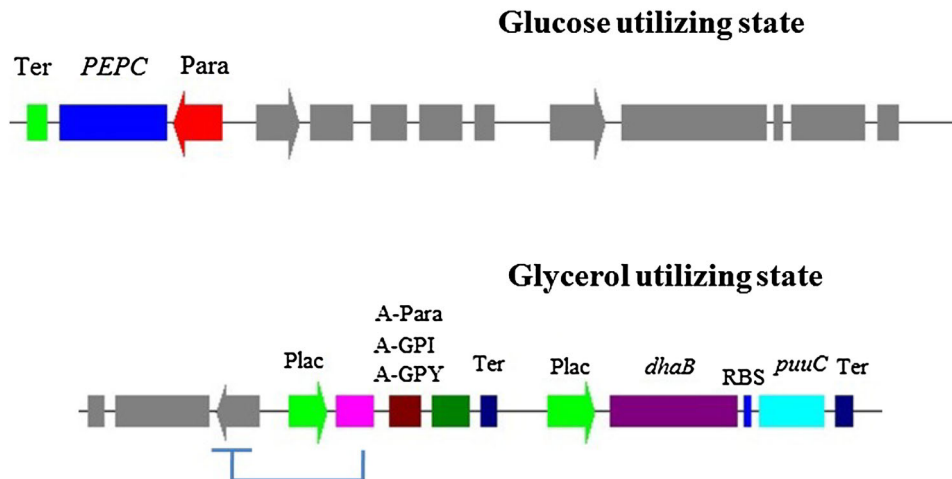

Glycerol utilizing state

Fig. 5 Logic gate for switching between glucose- and glycerol-utilizing states in *K. pneumoniae*. At the initial stage, cells grow using glucose instead of glycerol as carbon source, meanwhile, phospho-enolpyruvate carboxylase (PEPC) is expressed to benefit anaerobic respiration and cell proliferation. When glucose is exhausted, lactose is used to induce glycerol-utilizing state. Three antisense mRNA modules (A-Para, A-GPI, and A-GPY) are employed to turn off glucose metabolism, and P$_{lac}$ promoter drives the expression of *dhaB* and *puuC* for production of 3-HP. Bacteria prefer to metabolize glucose when it coexists with glycerol, therefore, when glycerol-utilizing state is turn on, the glucose-utilizing state must be turn off

Fig. 6 Diagram of scaffold protein for loading enzymes on the cell surface of *K. pneumoniae*. GDHt and ALDH are synthesized in vivo and tethered via a linker. A supramolecular enzyme catalyzing glycerol into 3-HP is generated and shipped to cell surface with the aid of signal peptide. The supermolecule is adhered to scaffold protein. The catalytic activity is enhanced due to enlarged surface area
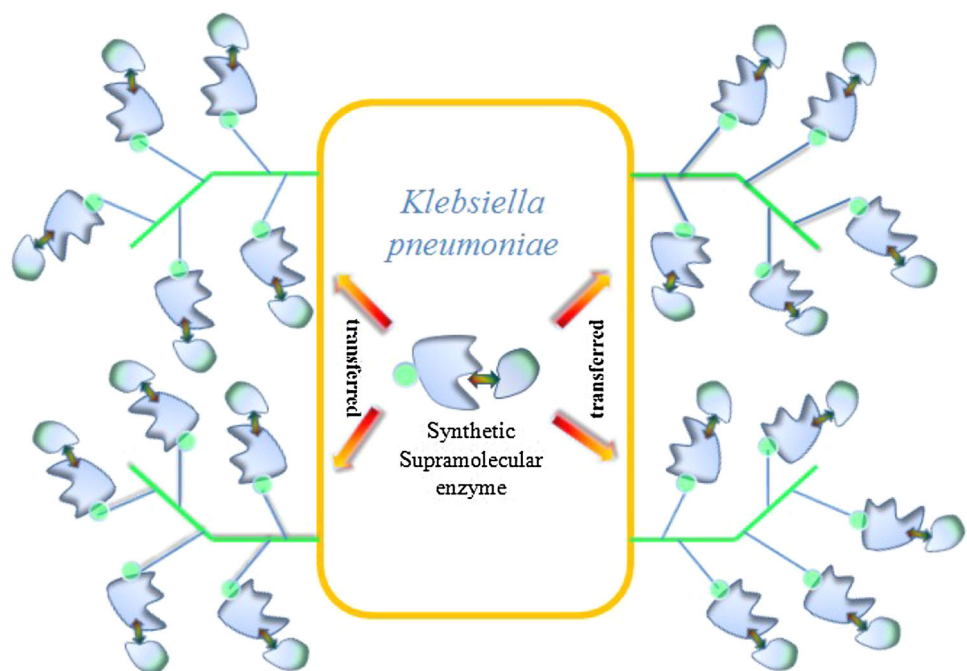
Rational Design of 3-HP Pathways

Computational methods have been developed and applied to microbial metabolic engineering. The methods such as OptKnock [47], OptStrain [48], OptFlux [49] and Genetic Design through Local Search (GDLS) [50] are effective in the rational design of *Escherichia coli* and *Saccharomyces cerevisiae* [50, 51]. To date, *K. pneumoniae* genome has been sequenced and basic research has long been conducted because this bacterium is an important clinical microbe. In addition, *K. pneumoniae* is by far a model organism biochemically similar to *E. coli*. Thus, it is the time to rationally design the networks of *K. pneumoniae* for 3-HP biosynthesis purpose. Prior work has successfully

anticipated the impacts of gene deletion on the biosynthesis of 1,3-PDO and other desired metabolites. The network BNICE [52] enables the precise prediction of 3-HP production from glucose. Despite this, 3-HP production cannot be further elevated simply by analysis of the experimental data. Computational methods in combination with experimental validations may be a wise solution to decipher 3-HP biosynthesis mechanism.

Concluding Remarks

Previously, metabolic engineering of *K. pneumoniae* for production of 3-HP was limited to the overexpression of enzymes from glycerol reduction pathway, or the deletion of genes from oxidation pathway. However, these approaches failed to overproduce 3-HP because *dha* regulon has topological rigidity. Although 3-HP cannot be highly accumulated in *K. pneumoniae* due to above mentioned bottlenecks, the burgeoning synthetic biology offers ways to overcome these limitations. Hence, in this review, the limitations on biological production of 3-HP were profoundly discussed and the strategies for refactoring *K. pneumoniae* were proposed. These strategies are multifaceted, involving cofactor regeneration, cytotoxicity attenuation, genome editing, recruitment of logic gate, alleviation of feedback inhibition, and so on. With the expending toolbox of microbial breeding, we believe that the remodeled *K. pneumoniae* strain will highly produce 3-HP, 1,3-PDO, or beyond.

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