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Direct fermentation route for the production of acrylic acid

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ABSTRACT

There have been growing concerns regarding the limited fossil resources and global climate changes resulting from modern civilization. Currently, finding renewable alternatives to conventional petrochemical processes has become one of the major focus areas of the global chemical industry sector. Since over 4.2 million tons of acrylic acid (AA) is annually employed for the manufacture of various products via petrochemical processes, this chemical has been the target of efforts to replace the petrochemical route by ecofriendly processes. However, there has been limited success in developing an approach combining the biological production of 3-hydroxypropionic acid (3-HP) and its chemical conversion to AA. Here, we report the first direct fermentative route for producing 0.12 g/L of AA from glucose via 3-HP, 3-HP-CoA, and Acryloyl-CoA, leading to a strain of *Escherichia coli* capable of directly producing acrylic acid. This route was developed through extensive screening of key enzymes and designing a novel metabolic pathway for AA.

1. Introduction

Increasing population, improving lifespan, and other demands have significantly improved the need for and growth of the acrylic acid (AA)-based superabsorbent polymer market. In particular, the rising disposable income levels of individuals, growing population, and strong economic conditions in developing countries such as China and India support promising growth in the market for ecofriendly superabsorbent polymers (Supplementary Fig. 1A and B). Currently, most of the AA is being commercially produced through the oxidation of propylene or propane (Corma et al., 2007; O'Brien et al., 1990). However, AA production from fossil fuel can adversely affect global CO_2 emissions (e.g., 575 kg/ton of CO_2 has been released in converting propane to AA by chemical processes) (Koichi, 2014) and petrochemical carbon sources are limited and not renewable (John et al., 2007; Nossin et al., 2002; Lin 2001). Additionally, the worldwide supply of propylene or propane is diminishing and pricing volatility persists for petroleum-derived chemicals. Therefore, bio-based production of AA from renewable resources such as sugar is an attractive alternative to petroleum-based chemicals. Many global players such as BASF (Germany), Dow Chemical Company (US), and Nippon Shokubai (Japan) have ventured into these markets and have significantly driven the demand for AA and its derivatives (Supplementary Fig. 1C). Novel biotechnological production routes from glucose-based renewable material have been considered but there has been no clear overview of potential biotechnological routes to produce bio-AA (Campodonico et al., 2014; Straathof et al., 2005; Chao et al., 2011; Kosec et al., 2012; Choi et al., 2015).

A method for combined fermentation/chemocatalytic production of AA has been actively developed by chemical conversion of biologically synthesized molecules such as lactic acid and 3-hydroxypropionic acid (3-HP) (Gunter et al., 1995; Danner et al., 1998; Lunelli et al., 2007; Zhou et al., 2011). Although the AA for this bio/chemocatalytic approach contains bio-based carbon, the two-step procedures for the conversion require the consumption of high amounts of energy as well as involve high production costs for the separation and use of chemical catalysts. Specifically, the separation and purification of 3-HP from the fermentation broth account for up to 32% of the production cost (Supplementary Fig. 2, separation and purification costs of 3-HP [$0.82/kg], and total costs of AA [$2.6/kg]). Another one-pot process was suggested by directly converting the produced 3-HP to AA, which is recovered via reactive distillation, without separating 3-HP (Tsobanakis et al., 2014, US 8846353 B2). This process is much simpler than the bio/chemocatalytic process, but still quite complicated process setup.

In this study, we have designed completely biosynthetic pathways for AA production from glucose (Fig. 1A and B). Especially, efficient synthetic pathway from 3-HP to AA was screened and

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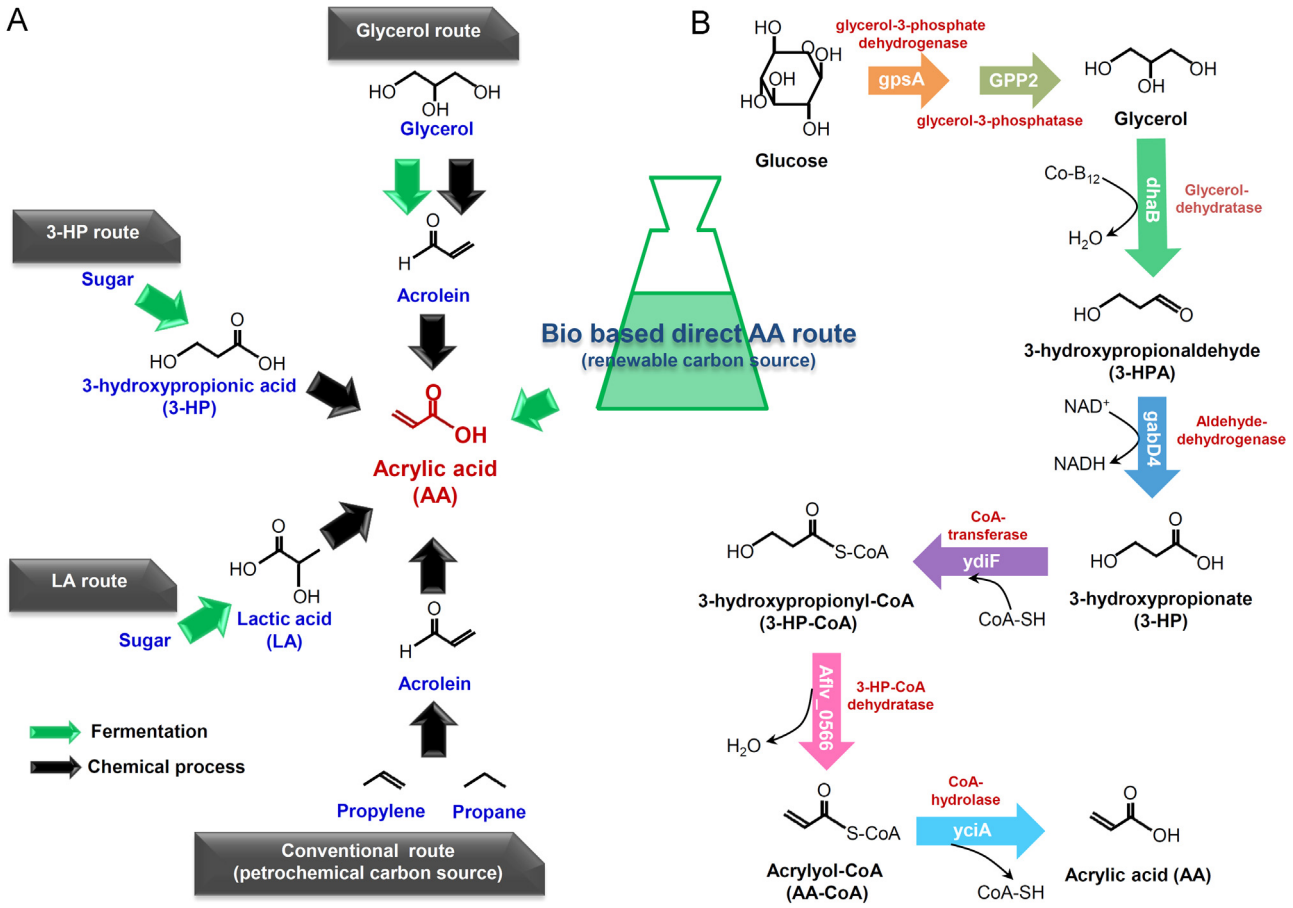

Fig. 1. Scheme of the direct production of acrylic acid (AA) and comparison with conventional approaches. (A) The chemical and biological routes for AA production. Most acrylic acid is commercially produced by the oxidation of propylene or propane. One route of glycerol transformation is its conversion to acrolein by dehydration of this by-product. 3-hydroxypropionic acid (3-HP) and Lactic acid (LA) routes from sugar were investigated by several acrylic acid producers such as Dow, Novozyme, and OPXBIO. However, this bio/chemocatalytic approach requires high energy consumption as well as high production costs for the separation and use of the chemical catalyst. (B) The direct production of acrylic acid in recombinant *Escherichia coli*. A direct pathway from glucose to acrylic acid via 3-HP, 3-HP-CoA, and acryloyl-CoA (AA-CoA) by fermentation was identified with the help of novel enzymes (i.e., *gspA*, *GPP2*, *dhaB*, *gabD4*, *Aflv*_0566, *ydiF*, and *yciA*).

integrated into our previous genetically engineered *Escherichia coli* strain producing 3-HP (Jung et al., 2014). The low titer of AA could be derived from the higher toxicity of AA than that of 3-HP and low enzymatic activity. Elevating AA resistance and further optimization for enzyme engineering could be the next hurdle as well as further optimizing this designed synthetic pathway.

2. Materials and methods

2.1. Materials

A list of the bacterial strains and plasmids used in this study is provided in Supplementary Tables 3, 4, and 5. *E. coli* strain DH5α, and the plasmids pETDuet-1 and pACYCDuet-1 were purchased from Novagen (Madison, WI, USA). Genomic DNA was purchased from DSMZ, KCTC, and KMCC. T4 DNA ligase and restriction endonucleases were obtained from New England BioLabs (Beverly, MA, USA). Plasmid DNA was purified using Spin Miniprep kits from Promega (Madison, WI, USA) and the gel extraction kit was purchased from Qiagen Inc. (Valencia, CA, USA). 3-HP standard solution was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 3-hydroxypropionaldehyde (3-HPA) was chemically synthesized from acrolein according to a previously described method (Hall and Stern, 1950). Genetic manipulations were carried out according to standard methods (Sambrook et al., 2011). All other reagents were purchased from Sigma (St. Louis, MO, USA). 3-HP-CoA and AA-CoA were synthesized according to a previously described method with slight modifications (Poteete et al., 2004) and purified using Sep-Pak column chromatography.

2.2. Construction of 3-HP-producing strains

The coding region of *dhaB* (*dhaB1*, *dhaB2*, *dhaB3*) and *gdrAB* genes (*gdrA*, *gdrB*) from *Klebsiella pneumoniae* g-DNA and *gabD4* from *Cupriavidus necator* g-DNA were amplified by polymerase chain reaction (PCR) from the genomic DNA of *K. pneumoniae* and *C. necator*, respectively. The amplified PCR fragments were ligated with the pETduet-1 vector (Novagen) using the In-Fusion® HD Cloning Kit, yielding pETduet-1_*dhaB* and pETduet-1_*gabD4*. Then, these two genes were integrated into the genomic DNA of *E. coli* W3110 by homologus recombination, resulting in the SE001 strain (Supplementary Table 1). Mutants lacking *ackA-pta* and *yqhD* were generated using the Red/ET recombination system (Datsenko and Wanner, 2000). Each PCR product containing *ackA-pta* and *yqhD* with a 45-bp homologous region at each end was recombined into the chromosome of *E. coli* W3110 using the Red plasmid pKD46 and selecting kanamycin-resistant transformants. The plasmid pCP20, which contains the thermally induced *flp* recombinase gene (Cherepanov and Wackernagel, 1995), was transformed into the *ackA-pta*::Km strain to eliminate the kanamycin resistance gene, and the double crossover mutant Δ*ackA-pta* was identified by PCR on the basis of its genotype (Supplementary Table 2). The PCR fragment containing the *yqhD* mutation was transformed into