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Mini-Review

Defense strategies for removing a highly toxic intermediate of glycerol reductive metabolism in *Klebsiella pneumoniae*

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ABSTRACT

Klebsiella pneumoniae is known to have evolved two mechanisms for preventing accumulation of the highly toxic intermediate 3-hydroxypropion aldehyde during reductive glycerol metabolism in which the toxic intermediate is converted to 3-hydroxypropionic acid (3-HP). One metabolic pathway, which involves a propanediol-utilization protein (PduP), is important under late-stage growth conditions at which concentrations of 1,3-propanediol are high. The other is a non-specific mechanism that removes intracellular toxic aldehyde molecules by reactions catalyzed by various aldehyde dehydrogenases. These microbial metabolic pathways could find application in industry for the synthesis of 3-HP, which is a useful platform chemical.

KEYWORDS: 3-hydroxypropionic acid, 3-hydroxypropion aldehyde, propanediol-utilization protein

INTRODUCTION

Glycerol is a typical non-fermentative carbohydrate that can be readily utilized aerobically by microorganisms such as *Escherichia coli*. Some microorganisms are known to have evolved a fermentative glycerol metabolism [1-6]. A representative example is *Klebsiella pneumoniae*, whose fermentative glycerol metabolism has been extensively studied (Figure 1) [7-9]. Glycerol-fermenting microorganisms have evolved a common glycerol reductive pathway that balances the intracellular redox potential during fermentative metabolism. In this pathway, glycerol is first converted to 3-hydroxypropion aldehyde (3-HPA) through the action of glycerol dehydratase, and then to 1,3-propanediol (1,3-PD) through the action of 1,3-PD oxidoreductase using reduced nicotinamide adenine dinucleotide (NADH) as a cofactor, which controls the cytosolic ratio of reduced and oxidized nicotinamide adenine dinucleotide ($NADH/NAD^+$) [10-12].

3-HPA, the intermediate metabolite of the glycerol reductive pathway, is highly toxic, and hence its accumulation must be tightly controlled. Moreover 1,3-PD oxidoreductase catalyzes a reversible reaction that converts 1,3-PD to 3-HPA under conditions of high 1,3-PD concentration, increasing the cellular amount of toxic 3-HPA. Glycerol-fermenting microorganisms have evolved a defense mechanism that prevents accumulation of this toxic material by converting it to 3-hydroxypropionic acid (3-HP) [13-17].

This mini-review introduces the microbial strategy for removing the toxic intermediate of reductive glycerol metabolism recently identified in *K. pneumoniae*.

Propanediol utilization protein-catalyzed 3-HPA-specific pathway

Recently, Luo *et al*. reported that overexpression of a propanediol utilization protein (PduP) from

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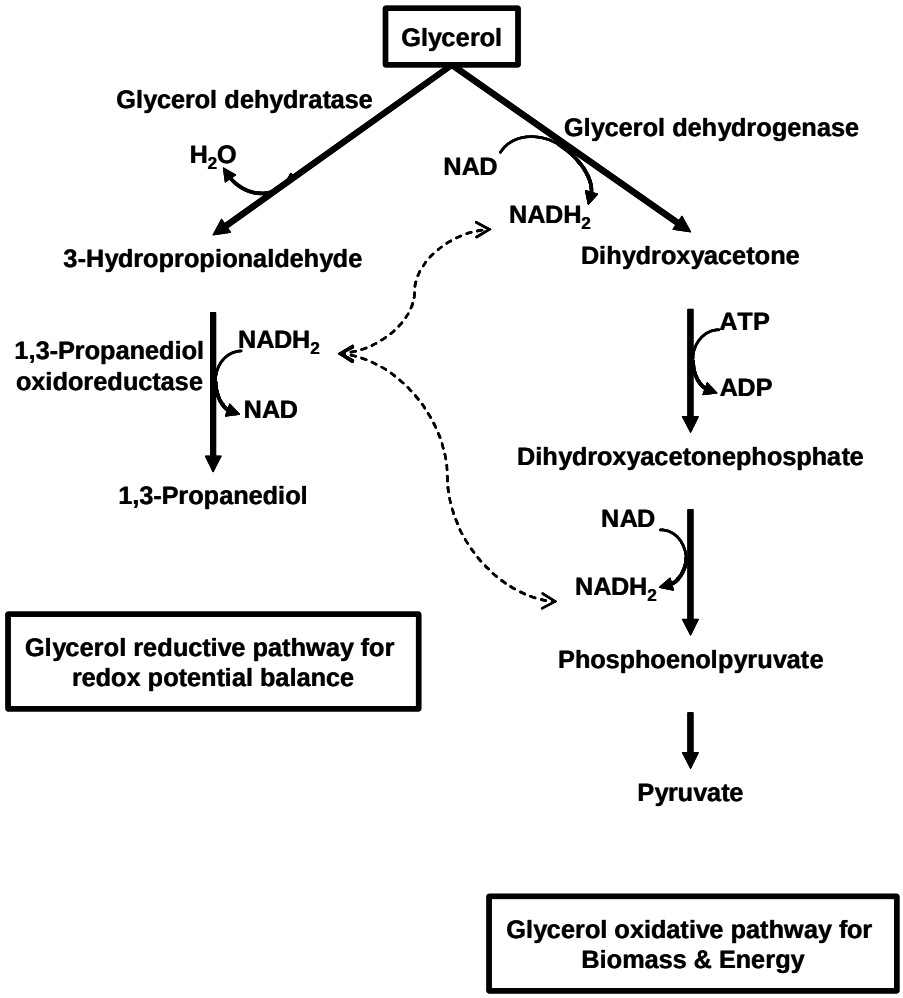

Figure 1. Glycerol oxidative and reductive metabolic pathways in *K. pneumoniae*.

Lactobacillus reuteri increased the production of 3-HP in *K. pneumonia* [18]. A homolog of PduP identified in *K. pneumoniae* similarly induced the synthesis of 3-HP, indicating the probable involvement of the propanediol utilization pathway in the conversion of 1,3-PD to 3-HP via 3-HPA (Figure 2). In agreement with this prediction, the synthesis of 3-HP diminished in a *pduP*-deficient mutant of *K. pneumoniae* specifically at late stages of growth when concentrations of 1,3-PD are high, resulting in accumulation of 3-HP and cell death [19]. This indicates that the propanediol utilization pathway is an important defense mechanism for preventing accumulation of the toxic intermediate metabolite during reductive glycerol metabolism. A further analysis at the molecular level could help elucidate the relationship between the PduP-dependent defense mechanism and the level of 1,3-PD.

Aldehyde dehydrogenase-catalyzed non-specific pathway

Interestingly, the synthesis of 3-HP in the *pduP*-deficient mutant was retained specifically at early stages of growth, indicating the involvement of another metabolic pathway in the reduction of 3-HPA in *K. pneumoniae* [19]. Luo *et al.* reported that at least three aldehyde dehydrogenases (AldHk, YdcW, and YneI) induce the reaction (Figure 2) [20]. However, deletion of each gene had no effect on the synthesis of 3-HP, indicating that the reaction converting 3-HPA into 3-HP might be a non-specific cellular defense mechanism to eliminate toxic metabolites.

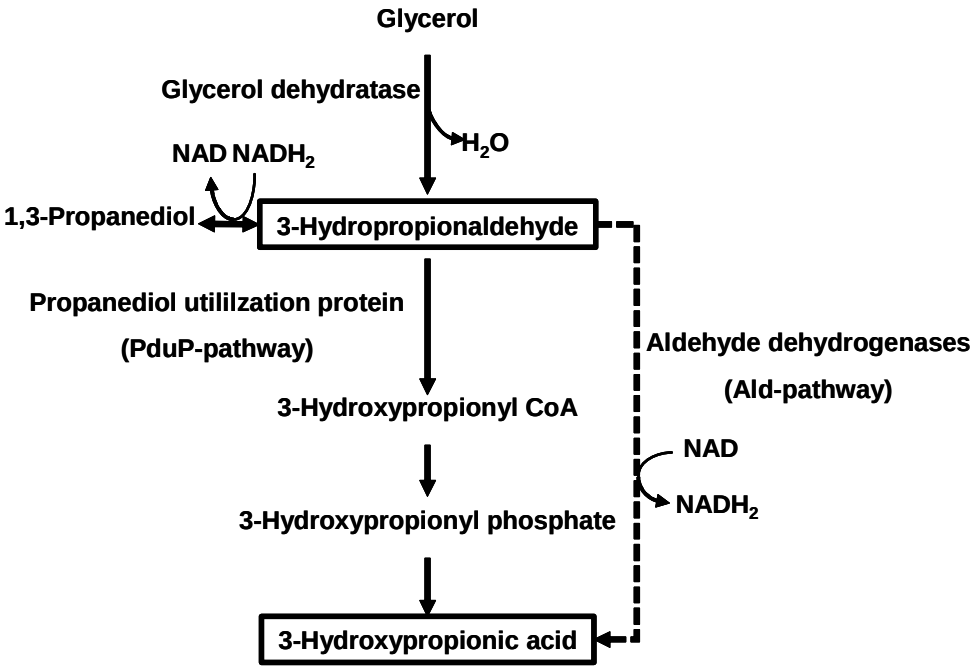

Figure 2. Proposed metabolic pathways for converting 3-hydroxypropione aldehyde to 3-hydroxypropionic acid.

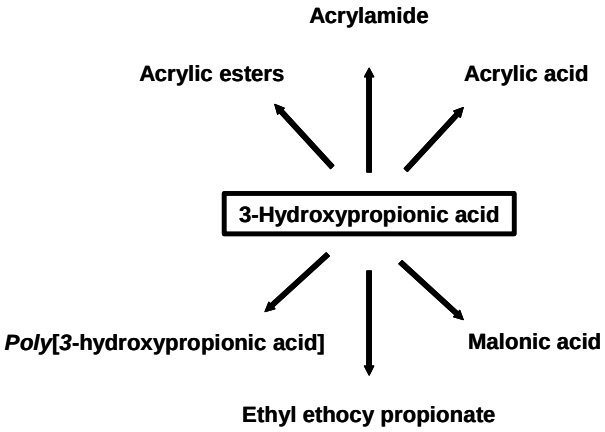

Figure 3. Industrial applications of 3-hydroxypropionic acid.

Application of microbial metabolic pathways

3-HP is a valuable C3 building block, and is used as an intermediate in the synthesis of many commercially valuable chemicals employed in the production of adhesives, fibers, and resins (Figure 3) [21]. Raj *et al*. recently reported 3-HP production in recombinant *E. coli* through the expression of a glycerol dehydratase from *K. pneumoniae* and an aldehyde dehydrogenase (*E. coli aldH* or α-ketoglutaric semialdehyde dehydrogenase from *Azospirillum brasilense*) [22]. However, the recombinant pathways required coenzyme B12 as a cofactor for the glycerol dehydratase reaction, which is expensive, thus compromising the cost-effectiveness of scale-up. *K. pneumoniae* might be applicable for the industrial production of 3-HP because the glycerol-fermenting strain has co-evolved a complex *de novo* biosynthetic pathway for coenzyme B12 [23].

CONCLUSION

K. pneumoniae is a prototypical glycerol-fermenting microorganism. To achieve this metabolic capability, the microorganism has evolved a reductive pathway that balances the cellular redox potential during fermentative metabolism and the accompanying synthesis of a highly toxic intermediate metabolite. Therefore, together with the metabolic pathway, two defense mechanisms have also evolved to prevent accumulation of the toxic intermediate in the microorganism. These delicate microbial strategies could be exploited industrially for the production of valuable platform chemicals.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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