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Analysis of energy metabolism in acetic acid bacteria during oxidative fermentation

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Research aims

Acetic acid bacteria are obligate aerobic *α*-proteobacteria with the unique ability to incompletely oxidize various alcohols and sugars to form organic acids. The ability of these bacteria to incompletely oxidize ethanol to acetate has been historically utilized for vinegar production. These organisms also cause spoilage of alcoholic beverages. Thus, acetic acid bacteria are industrially important. The incomplete oxidation reactions are catalyzed by membrane-bound pyrroloquinoline quinone (PQQ)-dependent dehydrogenases. Because the substrate specificities of these enzymes are relatively low, acetic acid bacteria expressing these enzymes are used as living biocatalysts for the production of many useful materials such as L-sorbose and 6-amino-L-sorbose, which are precursors of vitamin C and the anti-diabetic drug miglitol, respectively. Complete genome sequences of some acetic acid bacteria are now available and considerable biochemical research on these bacteria has been published. However, there are relatively few reports on molecular biological research such as gene regulation, or omics research such as transcriptome and metabolome analyses, on these bacteria compared to other industrial microorganisms. In this work, we investigated the transcriptome profiles of acetic acid bacteria under various growth conditions for a better understanding the specific regulatory features of energy and central carbon metabolism, and to develop novel applications for acetic acid bacteria.

Methods

1. *Transcriptome analysis*

Acetobacter aceti NBRC14818, which is a wild-type strain, and *Acetobacter pasteurianus* NBRC3283, which is a strain utilized for industrial vinegar production, were cultivated under various growth conditions. RNA samples were isolated from cultures and analyzed in microarray studies by using custom arrays designed and manufactured by Roche-NimbleGen.

2. *Analysis of the role of the glyoxylate pathway*

The *aceA-glcB* gene cluster encoding the glyoxylate pathway enzymes isocitrate lyase and malate synthase of *A. aceti* was knocked out by homologous recombination with the tetracycline-resistance gene. The constructed mutant strain was used to compare the growth and transcriptome profiles with its parental wild-type strain.

Results

1. *Transcriptome profile of acetic acid bacteria*

In both *A. aceti* and *A. pasteurianus*, the genes for the tricarboxylic acid (TCA) cycle enzymes were found to be significantly repressed when ethanol was present in the medium, even in the presence of glucose or acetate.[1,2] *Acetobacter* species are able to produce proton motive force that is used for ATP synthesis during incomplete oxidation of ethanol, since ethanol oxidation by PQQ-dependent alcohol dehydrogenases is coupled with oxygen reduction by respiratory quinol oxidases via ubiquinone. Our results suggest that acetic acid bacteria have a mechanism to repress the regeneration of NADH by the TCA cycle when ATP is sufficient to maintain viability.

In contrast to the TCA cycle genes, the expression levels of genes for glycolysis were not significantly changed according to the growth conditions. In *Escherichia coli*, it is known that metabolic flow through glycolysis is faster than that through the TCA cycle under aerobic conditions. Therefore, excessive amounts of pyruvate and acetyl-CoA build up, causing production of acetate from glucose. This phenomenon is called overflow metabolism. When the *Acetobacter* strains were grown in medium containing ethanol and glucose, pyruvate and acetyl-CoA were expected to accumulate intracellularly due to the overflow metabolism of glucose seen in *E. coli* (Fig. 1). Accumulation of acetyl-CoA in the cytoplasm might inhibit the utilization of acetate produced from ethanol in the periplasm. That was probably one of the major reasons for the temporal accumulation of acetate in the presence of ethanol. Some stress-responsive genes were upregulated during incomplete oxidation, probably because of production of toxic acetaldehyde as a result of overflow metabolism (Fig. 1). The glyoxylate pathway genes (*aceA-glcB*) were significantly upregulated in *A. aceti* cells when ethanol or acetate was present in the medium, suggesting that this pathway is important for acetate metabolism by *A. aceti*. Because *A. pasteurianus* does not have these genes, presence or absence of the glyoxylate pathway might affect the phenotypic difference between the strains.

2. *Role of the glyoxylate pathway*

Published genome sequences and our preliminary analy-

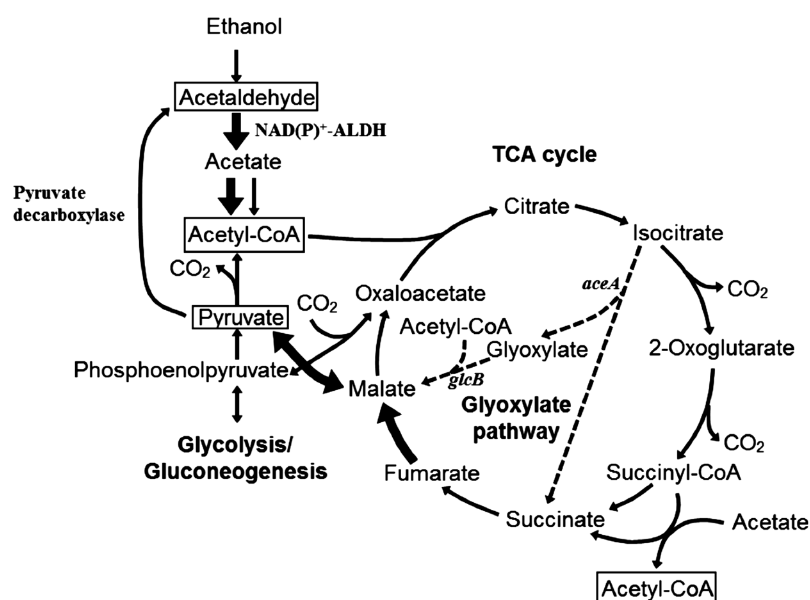

Fig. 1. Central carbon metabolic pathway of *A. aceti*. Dashed arrows indicate the glyoxylate pathway. Thick arrows indicate genes mediating reactions that were significantly upregulated in the glyoxylate pathway-deficient mutant.

ses have shown that the acetic acid bacteria used for vinegar production lack the genes for the glyoxylate pathway. This pathway consists of isocitrate lyase and malate synthase and functions as an anaplerotic shunt of the TCA cycle to supply oxaloacetate. The glyoxylate pathway is required when carbon compounds that are metabolized through acetyl-CoA as an obligate intermediate are utilized as the only carbon sources. Because TCA cycle intermediates are used as precursors for synthesis of molecules such as amino acids, nucleotides and other cofactors, the supply of oxaloacetate is necessary for function of this pathway. The *aceA-glcB* knockout mutant of *A. aceti* showed significantly slower growth in medium containing ethanol and glucose, although the glyoxylate pathway was not necessary under these conditions. Acetate productivity was not affected by the mutation, but the acetate produced was retained in the medium for a longer period of time in the mutant culture, indicating that the lack of the glyoxylate pathway is advantageous for vinegar production. Transcriptome analyses of the mutant strain suggested that the slower growth and the delay of acetate overoxidation were due to the significant repression of the TCA cycle enzymes and accumulation of acetyl-CoA in the cytoplasm (Fig. 1).[3)]

Conclusion

Transcriptome analyses of *Acetobacter* strains showed that the presence of ethanol in the medium significantly downregulates the expression of the TCA cycle enzymes, resulting in the repression of acetyl-CoA-consumption. Accumulation of acetyl-CoA in the cytoplasm might be the major reason that acetate produced by incomplete oxidation of ethanol in the periplasm is not metabolized. Acetic acid bacteria are able to produce ATP by the incomplete oxidation of alcohols and sugars, because ubiquinols reduced by PQQ-dependent dehydrogenases are reoxidized by terminal oxidases using molecular oxygen, generating proton motive force. Our results indicate that incomplete oxidation by acetic acid bacteria is the result of preferential utilization PQQ-dependent dehydrogenases substrates over the endogenous respiratory substrate NADH for energy generation. Acetic acid bacteria might have a specific mechanism to repress the regeneration of NADH by the TCA cycle for this purpose. Inactivation of the glyoxylate pathway, which also reduced metabolic flow of the TCA cycle, was advantageous for acetate production, which is consistent with the fact that strains utilized for vinegar production tend to lack this pathway.

References

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