Enhanced organic acid production in genetic engineered strains of Aspergillus carbonarius

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Enhanced organic acid production in genetic engineered strains of *Aspergillus carbonarius*

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Introduction

The filamentous fungus *Aspergillus carbonarius* is a good citric acid producer[1] and has great abilities in utilizing a broad range of substrates as well as excellent tolerance to stress conditions. It is selected for pathway engineering of the central carbon metabolism to improve the conversion of the renewable carbon sources and the flux into selected organic acids.

The present work focuses on development of two different mutants, a *gox* mutant and a *pepck* mutant. The *gox* mutant was constructed by deleting the *gox* gene which encode glucose oxidase in *A. carbonarius*. The *pepck* mutant was constructed by inserting the gene *pepck*, which encodes the phosphoenolpyruvate carboxykinase in *Actinobacillus succinogenes*, into *A. carbonarius*.

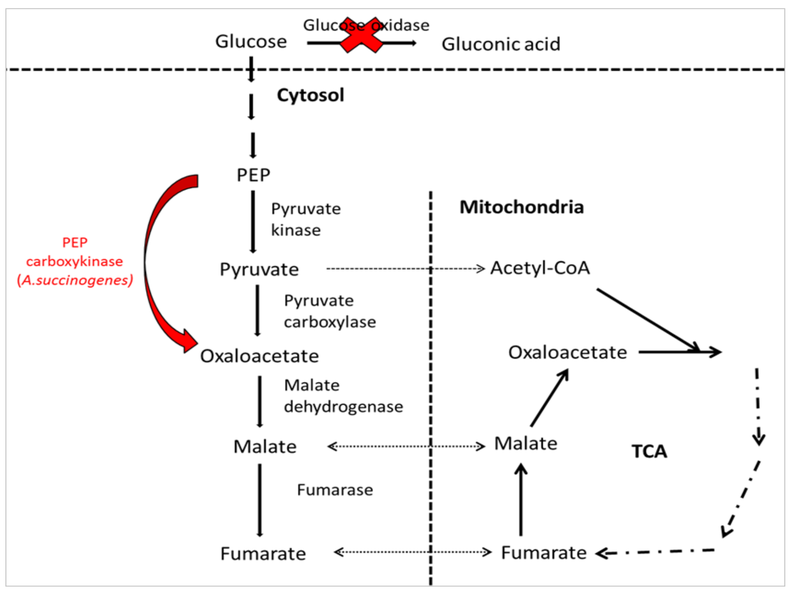
Objectives

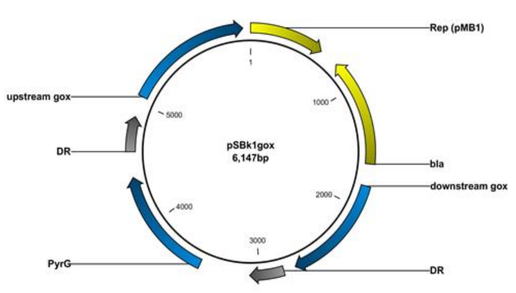
- Deletion of the *gox* gene which is assumed to encode glucose oxidase in *A.carbonarius*
- Insertion of the gene *pepck*, which encodes the phosphoenolpyruvate carboxykinase in *Actinobacillus succinogenes*, into *A. carbonarius*
- Evaluation of the effect of two genetic modifications on organic acid production in fermentation

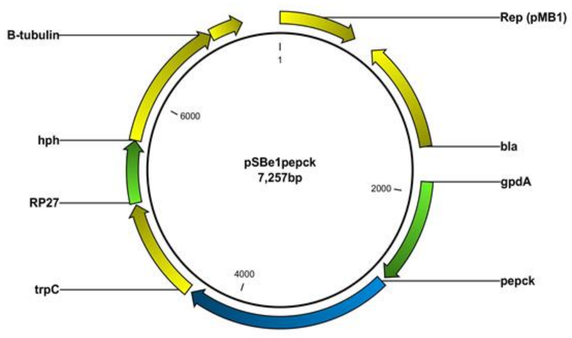
Method and Materials

1) Strains:
A. carbonarius wild-type 5010 was used to construct the pepck mutant and a Ku deficient and uracil auxotrophic transformant of the wild type strain, was used for construction of the *gox* mutant

2) Plasmids
Two plasmids pSBe1pepck and pSBk1gox built by USER cloning were used to construct pepck and gox mutant respectively .







3) Fermentation:

- Batch fermentation was carried out with the selected mutant in shaking flasks for 7 days by employing *A. carbonarius* wt 5010 and the Ku deficient strain (for comparison with the gox mutant only) as control.
- The acid production medium was as described by Goldberg[2] but supplemented with 0.1 g/L Zn.
- The concentration of gluconic acid and malic acid was analyzed by enzyme assays (Megazyme). The amount of glucose and other organic acids were analyzed by HPLC.

Results and Conclusion:

a) As shown in Figure 1 and 2, after deleting the *gox* gene, *A. carbonarius* is incapable of accumulating gluconic acid in fermentation. Furthermore, the effect of the *gox* gene deletion on production of other organic acid was evaluated. An increase of malic acid and citric acid production was obtained as well as accumulation of oxalic acid after 7 days fermentation in the *gox* mutant.

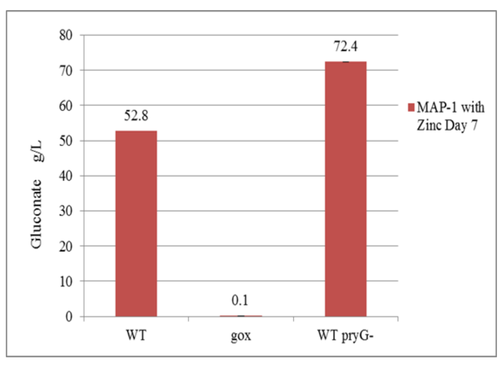


Figure 1

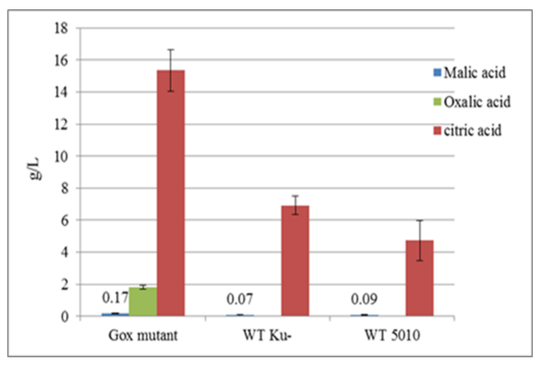


Figure 2

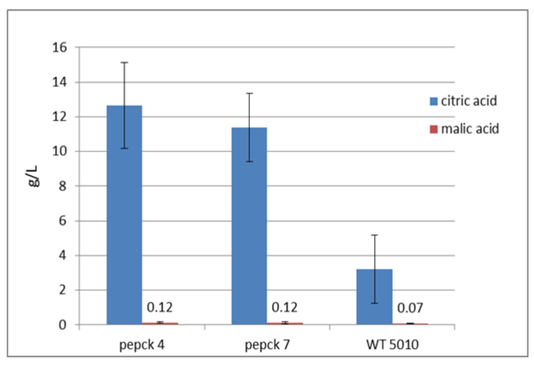


Figure 3

b) As shown in Figure 3, the two pepck mutants with highest expression level of the pepck gene showed improvement on production of malic acid and citric acid compared with wild type strain.

References:

[1]Ghareib, M. 1987, "Assimilation of galacturonate by *Aspergillus carbonarius*", Folia Microbiologica 32: 211-215

[2]Battat, E., Peleg, Y., Bercovitz, A., Rokem, J.S. & Goldberg, I. 1991, "Optimization of L-malic acid production by Aspergillus flavus in a stirred fermentor", Biotech Bioeng 37: 1108-1116.