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Multilevel engineering of microbial ethyl acetate production

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Abstract:

1. Background. This abstract will show our efforts to develop a process for the production of ethyl acetate. Ethyl acetate is produced from fossil resources at 3.5 million tonnes at a total value of $3.7 billion in 2014. To reduce CO_2 emissions a biobased process is desired. Yeasts are able to produce high amounts of ethyl acetate from sugars and ethanol. The development of an efficient fermentation process was hampered because the key enzyme was unknown.

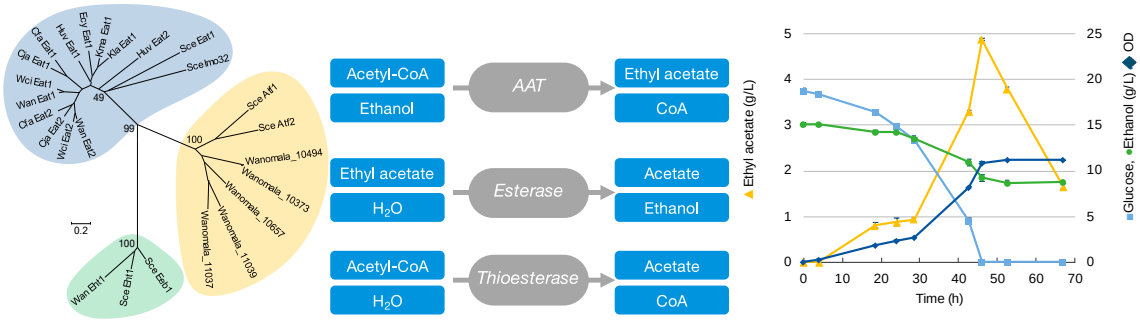
2. Methods. The Eat1 enzyme was identified by comparing the transcriptome of *Wickerhamomyces anomalus* under producing and non-producing conditions. A fusion protein of Eat1 and Gfp was made to show by light microscopy in which organelle Eat1 was expressed. The mitochondrial targeting sequence was identified using bioinformatics techniques. Eat1 was expressed in *E. coli*. Ethyl acetate production was optimized by knocking out byproduct formation, optimizing the expression level, removal of the targeting sequence, and in-situ product removal.

3. Results and discussion. We have identified this elusive enzyme. Eat1 is present in all yeasts known to produce ethyl acetate (figure 1, left). It has three activities: alcohol acetyl transferase converting ethanol and acetyl-CoA into ethyl acetate, esterase and thioesterase activity. The latter two activities have a negative effect on ethyl acetate production but were strongly suppressed when ethanol was present (Figure 1, middle)[1]. We have shown that the enzyme is located in the mitochondria, and we have identified the leader sequence responsible for mitochondrial targeting[2].

Expression of Eat1 in *E. coli* resulted in ethyl acetate production (figure 1, right)[1]. We have optimized anaerobic ethyl acetate production in *E. coli* by deleting LdhA and AckA, responsible for lactate and acetate production, respectively. This increased ethyl acetate production but also gave rise to the accumulation of pyruvate, indicating the Eat1 activity was insufficient. Eat1 activity was improved by optimizing the induction level and by removing the mitochondrial targeting sequence. This reduced

production of pyruvate but enhanced the production of acetate and ethanol. This appeared to be caused by the esterase activity of Eat1, hydrolysing ethyl acetate. By stripping the ethyl acetate from the broth, we decreased the time Eat1 could hydrolyse ethyl acetate, resulting in lower acetate and ethanol production. The final ethyl acetate yield obtained was 0.7 mol/mol, or 70% of the maximum pathway yield[3].

4. Conclusions. By multilevel engineering – bioprospecting the key enzyme, optimizing its expressing, increasing its activity by protein engineering, knocking out byproduct formation and by applying in situ product recovery - we were able to efficiently produce ethyl acetate in *E. coli*. On paper, the production costs of ethyl acetate are lower than those of bioethanol.



Figure

Left: The Eat family is a new alcohol acetyl transferase family, clearly distinct from the Atf and Eeb/Eht families.

Middle: Eat1 from *W. anomalus* has three activities.

Right: Expression of Eat1 from *W. anomalus* in *E. coli* results in ethyl acetate production.

Biography:

After studying Biology in Groningen (1984-1989) R. A. Weusthuis obtained his PhD in Microbial Biotechnology at the Delft University of Technology (1989-1994). Then he joined the WUR and headed the Bioconversion group (2004-2008). In 2007 he started working at the Wageningen University, and is active as Associate Professor Microbial Biotechnology.