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Recent advancements in fungal-derived fuel and chemical production and commercialization

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Due to unsustainable petroleum supply and poor yields from plant and animal sources, there is an increased effort to engineer microbial hosts for renewable chemical production. When compared to microbes such as *Escherichia coli*, fungal hosts show advantages due to their natural robust tolerance for industrial fermentation. Synthetic biology has focused on implementing heterologous pathways and manipulating native flux towards downstream products to achieve industrial productivity, titers, and yields. This review highlights recent advances in the engineering of yeasts for fuels and other molecules. As the field progresses, strains with improved productivities will begin to compete with the traditional chemical-based industrial approaches.

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Introduction

Fermentation and metabolic engineering provide a sustainable means of producing fuels, pharmaceuticals, and specialty chemicals [1]. Within this arena, fungal hosts (both conventional and non-conventional) have proven to be desirable microbial cell factories for chemical production on the basis of their industrial fermentation traits [2–4]. The expanding range of synthetic biology and metabolic engineering tools in these hosts are enabling economic and industrially relevant productivities, titers, and yields. To this end, there have been notable success stories associated with large-scale, industrial processes with fungal hosts in the past decade. For example, DuPont's omega-3-fatty acids being sold in products such as Verlasso Salmon and New Harvest EPA [5], Amyris' large scale production of semi-synthetic antimalarial drug artemisinin [6,7], and continuous improvements in archetypal ethanol production by *Saccharomyces cerevisiae* [8] highlight these successes. In each of these cases, high productivities and strain rewiring was necessary to achieve an economic advantage over traditional chemical processing.

In this review, we will highlight continued, recent advancements in this area—specifically the engineering of fungal hosts for the production of fuels, flavors and fragrances, nutraceuticals and pharmaceuticals, and commodity chemicals (Table 1). In doing so, we will specifically highlight processes that are close to industrial/commercial scale.

Short chain alcohols as fuels

Green fuel sources limit environmental impact and enable long-term sustainability [9,10]. Fungal hosts (esp. *S. cerevisiae*) have long been explored for their production of ethanol as a biofuel, but recent approaches have focused on the production of short chain alcohols that can more readily be blended with conventional fossil fuels for use with internal combustion engines [10–12]. Yeasts, especially *S. cerevisiae*, have desirable traits for this production due to natural tolerance for alcohols and fermentation conditions [13,14]. We review progress in the engineering for two short chain alcohols (n-butanol and isobutanol) here. Butanol has been explored over the past decade as a biofuel based on its high energy density and fuel pipeline compatibility [15]. As an added benefit, this molecule can also serve as an intermediate in the production of plastics, polymers, and fragrances [16•]. While naturally produced in the *Clostridium* genus (through traditional acetone-butanol-ethanol (ABE) fermentations), recent efforts have focused on expressing the butanol pathway in yeast hosts (Figure 1) [13,15].

Schadeweg & Boles recently improved upon butanol titer by increasing precursor availability [17]. Specifically, a bottleneck at the trans-2-enoyl-CoA reductase (ter) enzyme was eliminated in order to enhance reduction of crotonyl-CoA into butyryl-CoA for n-butanol production. When coupled with additional engineering strategies, the final developed strain was capable of producing 859 mg/L of n-butanol with a yield of 71 mg/g glucose. Similarly, Shi *et al.* engineered *S. cerevisiae* using a three-tiered engineering approach that: Firstly re-localized the threonine degradation pathway to the mitochondria, secondly created a citramalate synthase-mediated pathway to increase

Table 1

Recent achievements in yeast engineering. Strain achievements and production of various industrially relevant chemicals from different yeast species highlighted in this review are provided in this Table

Product	Achievement	Organism	Source
Short chain alcohols as fuels			
Butanol	Titer: 859 mg/L Yield: 0.071 g/g glucose	*S. cerevisiae*	[17]
Butanol	Titer: 1.05 g/L Yield: 52.5 mg/g glucose	*S. cerevisiae*	[16•]
Isobutanol	Titer: 1.62 g/L Yield: 16 mg/g glucose	*S. cerevisiae*	[21]
Isobutanol	Titer: 2.22 g/L Yield: 22.2 mg/g glucose	*P. pastoris*	[19•]
Isobutanol	Titer: 1.8 g/L	*S. cerevisiae*	[24]
Lipid-derived products			
Fatty acid-derivatives	Titer 40 g/L >90% lipid content	*Y. lipolytica*	[32]
Fatty acid-derivatives	Titer: 72.7 g/L Yield: .252 g/g glucose Productivity 0.97 g/L/h	*Y. lipolytica*	[29•]
Fatty Acids	Titer: 98.9 g/L Yield: 0.269 g/g glucose Productivity: 1.2 g/L/h	*Y. lipolytica*	[34••]
Biodiesel	Titer: 142.5 mg/L FAEE 23.3 mg/L fatty alkanes 2.15 g/L fatty alcohols 9.67 g/L free fatty acids 66.4 g/L TAGs Yield: 0.229 g/g glucose Productivity: 0.565 g/L/h	*Y. lipolytica*	[36•]
Biodiesel	Titer: 20.5 g/L Yield: 0.45 g/g biomass Productivity: 0.44 g/L/h	*R. toruloides*	[37]
Fatty alcohol	Titer: 1.7 g/L Yield: 28 mg/g glucose	*L. starkeyi*	[39]
Fatty alcohol	Titer: 8 g/L Yield: 40 mg/g sucrose	*R. toruloides*	[40]
Fatty alcohol	Titer: 6 g/L Yield: 20% of theoretical maximum on glucose	*S. cerevisiae*	[41]
Flavors and fragrances			
Farnesene	Titer: 130 g/kg broth	*S. cerevisiae*	[44••]
Santalene	Titer: 163 mg/L	*S. cerevisiae*	[42]
Nootkatone	Titer: 209 mg/L	*P. pastoris*	[48]
Valencene	Titer: 1.59 g/L	*S. cerevisiae*	[49]
Ethyl acetate	Titer: 10.9 g/L Yield: 0.265 g/g lactose	*K. marxianus*	[52]
Steviol glycosides	90–97% efficiency conversion from RebA to RebD and RebM	*S. cerevisiae*	[59]
Steviol glycosides	Titer: 1000– 2900 mg/L RebD 600–2,800 mg/L RebM	*S. cerevisiae*	[61]
Nutraceuticals and pharmaceuticals			
Resveratrol	Titer: 800 mg/L	*S. cerevisiae*	[64•]
Resveratrol	Titer: 5 g/L	*S. cerevisiae*	[66]
Naringenin and derivatives	Titer: 26.57 mg/L kaempferol 20.38 mg/L quercetin	*S. cerevisiae*	[68]
Breviscapine	Titer: 300 mg/L	*S. cerevisiae*	[69]