Yeast Biotechnology 2.0

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Yeast biotechnology. For thousands of years, yeasts have been used for the making of bread and the production of fermented alcoholic drinks, such as wine and beer. *Saccharomyces cerevisiae* (bakers' and brewers' yeast) is the yeast species that is surely the most exploited by man. Nowadays, *Saccharomyces* is a cornerstone of modern biotechnology and also a top choice organism for industrial production of fuels, chemicals, and pharmaceuticals. Today, more and more different yeast species are explored for industrial applications. This Special Issue "Yeast Biotechnology 2.0" is a continuation of the first issue "Yeast Biotechnology" (https://www.mdpi.com/books/pdfview/book/324).

Yeast synthetic biology and strain engineering. Recently, important progress has been made in unlocking the key elements in the biochemical pathways involved in the synthesis of aroma compounds, as well as in methods to engineer these pathways. Recent advances in bioengineering of yeasts—including *S. cerevisiae*—to produce aroma compounds and bioflavors are reviewed in Reference [1]. This review presents yeast as a significant producer of bioflavors in a fresh context and proposes new directions for combining engineering and biology principles to improve the yield of targeted aroma compounds. In a proof-of-concept study, *Yarrowia lipolytica* was used as a whole cell factory for the *de novo* production of long chain dicarboxylic acid (LCDA-16 an -18) using glycerol as the sole carbon source [2]. The results provide basis for developing *Y. lipolytica* as a safe biorefinery platform for sustainable production of high-value LCDCAs from non-oily feedstock. It was demonstrated that a mutant strain of *Y. lipolytica* can be used to produce citric acid from renewable carbon sources such as rapeseed oil, glycerol, and glycerol-containing waste of the biodiesel industry and glucose-containing aspen waste [3]. The cost-effective production of cellulosic ethanol requires robust microorganisms for rapid co-fermentation of glucose and xylose. Therefore, a recombinant diploid xylose-fermenting *S. cerevisiae* strain was developed by integrating *Piromyces* sp. E2 xylose isomerase (*PirXylA*) and *Orpinomyces* sp. ukk1 xylose (*OrpXylA*) in the genome in multiple copies [4]. The development of a counter-selection method for phenyl auxotrophy could be a useful tool in the repertoire of yeast genetics. A fluorinated precursor, i.e., 4-fluorophenylpyruvate (FPP), was found to be toxic to several strains from *Saccharomyces* and *Candida* genera [5]. The results show that FPP could effectively be used for counter-selection, but not for enhanced phenylethanol production.

New developments in efficient biomolecule production. In recent years, interest in the industrial production of yeast β-glucan has increased since it is an immunostimulant molecule for human and animal health. The β-glucan yield was optimised during anaerobic fermentation by evaluating the effect of the carbon source (glucose) and NaCl osmotic stress [6]. A yeast isolate, selected for its lipolytic activity from a meat product, was characterized as *Pichia anomala* [7]. Submerged fermentation optimization resulted in a significantly increased production of an extracellular lipolytic enzyme.

Fermented beverages: beer, wine and honey fermentation. Nowadays, wild yeasts are explored for beer and wine making to increase the natural flavor diversity of fermented beverages. Flavor was added to beer by performing mixed fermentation using non-*Saccharomyces cerevisiae/pastorianus* yeasts [8]. For this, a total of 60 strains belonging to the genera *Candida*, *Pichia*, and *Wickerhamomyces* were evaluated. Several strains produced substantially higher amounts of aroma alcohols and esters compared to a reference lager yeast strain.

Proline is the predominant amino acid in grape juice, but it is poorly assimilated by wine yeast under the anaerobic conditions of most fermentation. A novel wine yeast mutant that was obtained through ethyl methanesulfonate (EMS) mutagenesis, showed a markedly increased proline utilization and could be used to perform fermentations in nitrogen-limited conditions [9]. Icewine is a sweet dessert wine produced from grapes naturally frozen on the vine. Since acetic acid is undesired in Icewine, the yeast cytosolic redox status and its correlation to acetic acid production was investigated [10]. Yeasts involved in veil formation during the biological aging of Sherry wines are mainly *S. cerevisiae*, which are traditionally been divided in the varieties *beticus*, *cheresiensis*, *montuliensis* and *rouxii*. A microtiter plate assay method was developed to assure the identification and classification of veil-forming yeasts during Sherry wine aging [11].

Honey fermentations are usually performed using almost exclusively yeasts in the genus *Saccharomyces*. To increase the yeast biodiversity, two strains of *Torulaspora delbrueckii* were isolated from the gut of a locally collected honey bee [12]. These wild yeast fermentations displayed better sensory characteristics than mead fermentations by a champagne yeast, and mixed fermentations of the wild and the champagne yeast resulted in a rapid industrial fermentation process.

Yeast nanobiotechnology. Clinical needs for novel antifungal agents have increased due to the increase of people with a compromised immune system, and the appearance of resistant fungi and infections by unusual yeasts. In recent years, several micro- and nanoscale approaches have been introduced for antifungal drug discovery. These are reviewed in the last contribution to this special issue [13].

In summary, this Special Issue compiles the current state-of-the-art of research and technology in the area of "yeast biotechnology" and highlights prominent current research directions in the fields of yeast synthetic biology and strain engineering, new developments in efficient biomolecule production, fermented beverages (beer, wine, and honey fermentation), and yeast nanobiotechnology. We very much hope that you enjoy reading it and looking forward to the next special issue "Yeast Biotechnology 3.0" to appear in 2019 (https://www.mdpi.com/journal/fermentation/special_issues/yeast3).

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