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Editorial

Yeast Biotechnology 6.0

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1. Yeast Biotechnology 6.0

This Special Issue continues the "Yeast Biotechnology" Special Issue series of the MDPI journal Fermentation. This issue compiles the current state-of-the-art research and technology around "yeast biotechnology". This issue highlights prominent current research directions in the fields of yeasts as a cell factory, yeast nanobiotechnology, wine yeasts and wine fermentation, yeasts and food fermentation, and biocontainment for yeast biotechnology. We very much hope that you enjoy reading the articles contained herein and are looking forward to continuing this Special Issue series in the Topical Collection "Yeast Biotechnology".

2. Yeasts as Cell Factory

Park et al. [1] reviewed the role of CRISPR-Cas engineered nonconventional yeasts (NCYs) as emerging microbial cell factories. Conventional widely used yeasts such as *Saccharomyces cerevisiae*, which have been widely used as a microbial cell factory [2,3], have some disadvantages. Product profiles are often restricted due to the Crabtree-positive nature of *S. cerevisiae*, and ethanol production from lignocellulose is possibly enhanced by developing alternative stress-resistant microbial platforms. Alternatively, NCYs may be considered an alternative microbial platform for industrial fermentations since they have desirable metabolic pathways and regulation, and they have a strong resistance to diverse stress factors [4,5]. This review describes the current status of and recent advances in promising NCYs in terms of industrial and biotechnological applications, highlighting CRISPR-Cas9-system-based metabolic engineering strategies.

Noseda et al. [6] developed a cost-effective process for the heterologous production of SARS-CoV-2 spike receptor binding domain using *Pichia pastoris* (*Komagataella phaffii*) in a stirred-tank bioreactor. The spike protein of SARS-CoV-2 is one of the most exposed proteins [7]. The receptor-binding domain (RBD), which is a fragment of the Spike protein, interacts with the ACE2 receptors of human cells, allowing the entrance of viruses. The RBD has been proposed to be an interesting protein for the development of diagnosis tools and treatments for and the prevention of this disease [8]. A method for recombinant RBD production using *P. pastoris* as a cell factory in a stirred-tank bioreactor was developed by the cited authors. The proposed method represents a feasible, simple, scalable, and inexpensive procedure for the production of RBD.

Carneiro et al. [9] reviewed advances in *K. phaffii* engineering for the production of renewable chemicals and proteins. *K. phaffii* has been extensively used in the production of heterologous proteins [10–12] and, recently, as a cell factory to produce various chemicals through new metabolic engineering and synthetic biology tools [13]. This review summarizes *Komagataella* taxonomy, diversity, and recent approaches in cell engineering to producing renewable chemicals and proteins. Finally, strategies for optimizing and developing new fermentative processes using *K. phaffii* are discussed.

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3. Yeast Nanobiotechnology

Radonicic et al. [14] developed a rapid antifungal susceptibility testing (AFST) method based on optical nanomotion detection (ONMD) and applied it to the opportunistic fungal pathogen *Candida albicans*. In ONMD, an optical microscope is used to record the nanometric-scale movements of living cells. These recorded cellular nanomotions cease upon cell death, allowing the susceptibility of a cell or specific strain to be easily assessed. Other cellular nanomotion methods have been developed, such as methods based on AFM-cantilever sensors [15–17], plasmonic imaging of the z motion of attached bacteria [18], sensing of the attached bacteria's vibrations using the phase noise of a resonant crystal [19], tracking the x-y motion of attached uropathogenic *Escherichia coli* [20], and subcellular fluctuation imaging, which is based on total internal reflection microscopy (TIRM) [21], as well as optically tracking bacterial responses on micropillar architectures using intrinsic phase-shift spectroscopy [22]. The fast emergence of multi-resistant pathogenic *Candida* species is caused by the extensive and sometimes unnecessary use of broad-spectrum antifungals [23]. Hence, the development of rapid antifungal sensitivity tests would allow for the identification and use of more-selective antifungals, thereby reducing the spread of pathogenic fungi and their evolution toward a multi-resistant phenotype [24,25]. In this study, a microfluidic chip containing an array of microwells that were designed to trap yeast cells was developed. Yeast cell entrapment in a microwell allowed for a very rapid exchange of growth medium with the antifungal, which enabled the performance of single-cell ONMD measurements on the same cell before and after antifungal treatment. The chip was used to quantify the real-time response of individual *C. albicans* cells to the antifungal treatment in as fast as 10 min.

Villalba et al. [26] developed a new method for measuring the adhesion of the opportunistic pathogenic yeast *C. albicans* since classical methods have some drawbacks; for example, the corresponding measurement is relatively complex, requires sophisticated equipment, and, in most cases, cannot be carried out without breaking the links between the studied cell and its target [27–30]. The applied force in the new method is generated by the cell itself, whereas cellular movements are detected via optical microscopy and developed dedicated software. The authors demonstrated that the measurement was non-destructive and single-cell-sensitive and permitted observation of the evolution of adhesion as a function of time. The new cellular nanomotion-based technique was applied for different *C. albicans* strains adhering to a fibronectin-coated surface. This novel approach could significantly simplify, accelerate, and make more affordable living cell–substrate adhesion measurements.

Dekhtyar et al. [31] studied the possible influences of differently electrically charged diamond nanoparticles [32,33] on the physiological characteristics of the yeast *S. cerevisiae*. They revealed that the adverse impact of these nanoparticles can manifest not only against prokaryotes but also against eukaryotic yeast cells. The results also indicated that it is possible to reduce and, most likely, eliminate the dangerous effects of nanoparticles on cells by using special physical approaches. A comparison of non-arylated and arylated nanoparticles showed that in terms of changes in the physiological activity of cells, the selection of certain nanoparticles (non-arylated or arylated) may be necessary in each specific case, depending on the purpose of their use.

4. Wine Yeasts and Wine Fermentation

Akan et al. [34] explored the potential of NCYs in wine fermentation [35,36] with a focus on *Saccharomycopsis fermentans*. Mutant strains resistant to the toxic compound trifluoroleucine (TFL) were selected, mutations in the SfLEU4 gene were verified, and the ability of the resulting strains to contribute to fermentation bouquets was characterized. Resistance to TFL relieved feedback inhibition in the leucine biosynthesis pathway and resulted in increased leucine biosynthesis. The *S. fermentans* TFL-resistant mutants generated increased amounts of isoamyl alcohol and isovalerate during wine fermentation. The selection of TFL-resistant strains provided a generally applicable strategy for the improvement of NCYs and their utilization in co-fermentation processes for different grape must varieties.

Fernández-Fernández et al. [37] used immobilized yeasts to improve the production of sparkling wines. Verdejo sparkling wines were elaborated according to the "champenoise" method, and the second fermentation was developed with the same free or alginate-immobilized [38–41] *Saccharomyces cerevisiae bayanus* yeast strain. These sparkling wines showed no significant differences among the two typologies in terms of enological parameters (pH, total acidity, volatile acidity, reducing sugars, and alcoholic strength), effervescence, or spectrophotometric measurements. The free amino nitrogen content was significantly higher in the sparkling wines obtained from immobilized yeasts; the levels of neutral polysaccharides and total proteins were lower. No significant differences in the volatile composition were found, except for only two volatile compounds (isobutyric acid and benzyl alcohol) that were present at levels below their respective olfactory thresholds. The sensory analysis conducted by consumers showed identical preferences for both types of sparkling wines, except in terms of color acceptability. The descriptive analysis carried out by a tasting panel revealed that sensorial differences between both sparkling wines were only found regarding the smell of dough. The authors showed that using immobilized yeasts for the second fermentation of sparkling wines can reduce and simplify some enological practices such as riddling and disgorging, with no impact on quality parameters.

5. Yeasts and Food Fermentation

Bencresciuto et al. [42] evaluated starter cultures of lactic acid bacteria (*Lactobacillus plantarum* strains) and killer yeasts (*Wickerhamomyces anomalus* and *S. cerevisiae)* for the fermentation of table olives to debitter olives [43] and improve their organoleptic quality and safety [44,45]. This study aimed to assess their potential to avoid pretreatments and the use of excessive salt in the brines and preservatives. The final oleuropein levels in the olives were unaffected by the treatments, but the use of these starters did not improve the LABs' growth nor prevent the growth of *Enterobacteriaceae* and molds. The nutraceutical value of the olives could be improved due to the higher production of hydroxytyrosol.

6. Biocontainment for Yeast Biotechnology

Pavão et al. [46] reviewed biocontainment techniques and applications for yeast biotechnology. Biocontainment techniques for genetically modified yeasts (GMYs) [47] are pivotal due to the importance of these organisms in biotechnological processes and due to the design of new yeast strains using synthetic biology tools and technologies. The different biocontainment technologies currently available for genetically modified yeasts (GMYs) were evaluated. Uniplex-type biocontainment approaches (UTBAs), which rely on nutrient auxotrophies induced by gene mutation or deletion or the expression of the simple kill switches apparatus, are still the major biocontainment approaches in use for GMY. While bacteria such as *E. coli* account for advanced biocontainment technologies based on synthetic biology and multiplex-type biocontainment approaches (MTBAs), GMYs are distant from this scenario for many reasons. Therefore, a comparison of different UTBAs and MTBAs applied for GMYs and genetically engineered microorganisms (GEMs) was made, indicating the major advances in biocontainment techniques for GMYs.

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