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Review

Biocatalysis with Unconventional Yeasts

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Abstract: Biocatalysis is a green and sustainable technology based on the use of natural substances to catalyze chemical reactions. Humans have been unconsciously using biocatalysis for thousands of years to produce food and alcoholic beverages, but it is only since the 19th century that we have begun to understand its fundamentals and its enormous potential. In fact, advances in our knowledge of enzymes and metabolic pathways and, in recent decades, the introduction of tools such as bioinformatics, DNA sequencing and protein engineering have made biocatalysis a key strategy in fine chemistry and for the production of active pharmaceutical ingredients. In addition, the discovery of new microorganisms adapted to adverse conditions has also been crucial in advancing this avenue. The present review focuses on the use of unconventional yeasts and their enzymes in the most interesting reactions where biocatalysis is applied. It highlights the advantages of using these microorganisms in industrial chemical processes due to their particular phenotypes, such as their ability to withstand high temperatures and pressures, as well as acidic or alkaline environments, high substrate loads, presence of organic solvents, etc. All this results in a wider range of possible substrates and higher efficiency. Examples of the most important reactions in which their use has been described are included, considering both catalysis by wild-type whole cells or their isolated enzymes and their genetically modified variants. All this information will help to understand the current relevance of unconventional yeasts and their enzymes in biocatalysis.

Keywords: biocatalysis; enzyme engineering; ester synthesis; extremotolerant yeasts; hydrolysis; immobilized enzymes; oxidation; reduction; unconventional yeasts; whole cells

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1. Introduction

1.1. Biocatalysis

Biocatalytic processes were used, albeit unconsciously, by the ancient civilizations of Mesopotamia and Egypt for the production of beer and wine. However, it was not until the mid-19th century that Schwann and Cagniard-Latour demonstrated that the alcoholic fermentation involved in these processes was carried out by yeasts, living spherical microorganisms capable of reproduction. At first, it was thought that the whole microorganism acted as a catalyst, but it was later realized that the reactions observed were produced by specific parts of it, and the term ferment was used to designate this unorganized material, which we now know as an enzyme. In the 1930s, Payen and Persoz studied diastase in detail, the first enzyme to be used in an industrial process, specifically for the production of dextrins [1].

Biocatalysis currently refers to the use of whole cells of microorganisms or purified enzymes in chemical synthesis. This methodology is very convenient and is increasingly used in the synthesis of pharmaceuticals and high-value-added molecules. Compared to conventional chemical catalysts, it is recognized to have numerous advantages, such as their high specificity and their ability to perform regio- and stereoselective transformations of multifunctional molecules, eliminating the activation or protection/deprotection

steps often required in classical organic routes [2]. In addition, whole cells and enzymes are sustainable, renewable and biodegradable catalysts that are used under very mild temperatures, pressure and pH, avoiding the use of extreme conditions that can often cause problems such as racemization, isomerization or rearrangement of the compound [3]. No heavy metals are used [4], and water, the most environmentally friendly solvent, is usually employed [5]. For all these reasons, biocatalysis is considered an essential tool of green chemistry, especially for the synthesis of chiral intermediates. The aim is to achieve environmentally friendly industrial processes with maximum use of resources and minimum generation of waste [6]. The transition to the implementation of green strategies in manufacturing processes is challenging. The introduction of new products and new drugs under investigation must be based on alternative strategies for chemical synthesis with a less environmentally invasive manufacturing approach in order to tip the balance in favor of a greener industry [7].

Given the large number of microorganisms (bacteria, fungi and protists) that exist, the number of potentially active biocatalysts and the opportunities for research in this field are enormous [8]. Biocatalysis is highly valued by the pharmaceutical industry and has been established as an indispensable clean tool to achieve chirality in Active Pharmaceutical Ingredient (API) synthesis. In many racemic drugs, the therapeutic activity is due to one of the two enantiomers, and replacing the racemic entity with the chiral drug is a guarantee to improve the therapeutic index and gain in efficacy and safety. It is noteworthy that between 2010 and 2020, the U.S. Food and Drug Administration (FDA) approved less than 10 racemates, and the vast majority of permitted substances were in the form of pure enantiomers [9]. The intermediates of many important therapeutic agents (sitagliptin, pregabalin, ragaglitazar, paclitaxel, epothilone, abacavir, etc.) have been successfully synthesized via biocatalysis [3,10–13].

Most natural biocatalysts are not suitable for industrial-scale processes, and the chemical/pharmaceutical industry faces several challenges in implementing biocatalytic steps. One of these is achieving the typical product concentration of a conventional chemical process (at least 50–100 g/L). Since enzymes in nature operate at millimolar substrate levels, enzyme and microorganism modification techniques are required to achieve such concentrations. Enzymes can be overexpressed and their stability and half-life can be increased by genetic engineering or immobilization on supports. Immobilized enzymes have some advantages over their soluble counterparts, such as the ability to be used in continuous processes and/or in repetitive batch reactions, their easy separation from reaction mixtures, and their reuse and utility in multistep cascade reactions [13–16]. Although many publications on biocatalysis focus on isolated enzymes, the use of whole cells as biocatalysts is a simpler and generally equally effective method for achieving high yields and enantiomeric excess. Moreover, whole-cell studies are often the first step in the search for new enzymes with applications in enantioselective transformations [8]. The use of whole cells has many advantages over the use of purified or immobilized enzymes: the cost of the catalyst is lower and does not require expensive purification, the addition of cofactors is not necessary, and the stability of the catalyst is higher due to the residual cell wall compounds. Some disadvantages, such as limitations in mass transfer, can be overcome either by modification of the cell wall or by engineering tools. On the other hand, the use of non-conventional reaction media containing compatible ionic liquids as sustainable solvents is very interesting in increasing the concentration of organic compounds. Another problem associated with the use of whole cells is the generation of by-products due to other cellular enzymatic activities; to solve this problem, it is convenient to use strains mutated in the genes encoding these enzymes. Whole-cell catalysts can exhibit productivities far beyond industrial benchmarks if the microorganisms are genetically modified and/or immobilized [17].

1.2. Non-Conventional Yeasts and Their Application in Biotechnological Processes

Yeasts are eukaryotic microorganisms, generally defined as microscopic unicellular or dimorphic fungi with a primary unicellular stage in the environment [18]. They reproduce

asexually by budding or fission, producing single cells, and have sexual structures that are not enclosed in a fruiting body [19]. Undoubtedly, the most studied and important yeast from a basic and applied point of view is *Saccharomyces cerevisiae*, traditionally used in various fermentation processes for the production of bread, beer, wine and some other fermented foods [20,21]. The fission yeast *Schizosaccharomyces pombe*, which shares genetic characteristics with humans, has become a model organism for understanding the regulation and maintenance of the eukaryotic cell cycle [22]. However, there are other yeast species, known as non-conventional yeasts (NCYs), which, in many cases, possess properties such as the utilization of complex carbon sources or high tolerance to harsh conditions. For this reason, they are becoming increasingly important and have known or potential biotechnological applications [23–26]. Figure 1 provides a description of some of these NCYs.

Due to their immense physiological variability, yeasts are found all over the world. In the last 20 years, several under-explored habitats have been extensively studied, resulting in a significant number of new species, but although the discovery of new yeasts is increasing, it is unlikely that there will be documentation for all of them.

The kingdom Fungi is one of the most widespread groups of organisms in the world and contains five true phyla: Chytridiomycota, Zygomycota, Ascomycota, Basidiomycota, and Glomeromycota. Non-*Saccharomyces* yeasts include both members of the Ascomycota (to which *S. cerevisiae* belongs) and the Basidiomycota [20,21,27].

Yeast ascomycetes are involved in a wide range of processes such as food and feed production, ethanol generation and heterologous production of proteins and enzymes. Moreover, they are model organisms for biomedical research, used in biocatalytic processes to obtain chiral compounds of interest, and widely employed in agriculture as control and bioremediation agents [24].

The use of basidiomycetous yeasts in biotechnological processes has been more delayed and they currently have limited industrial utility. They are mainly utilized in the production of enzymes for chemical and pharmaceutical synthesis, in the generation of certain classes of primary and secondary metabolites such as terpenoids and carotenoids, in the aerobic degradation of complex carbon sources, and in the bioremediation of environmental and xenotoxic pollutants [25].

Figure 2 and Tables S1 and S2 summarize the main applications of these microorganisms in biotechnological processes other than those directly related to biocatalysis. Despite all these benefits, it is important to note that both types of yeast can have negative effects, leading to food spoilage and, consequently, significant economic losses [28].

Candida

- It is a very heterogeneous genus that includes ascomycetes and basidiomycetes.
- These yeasts grow and produce biomass on a wide variety of substrates (in some cases in the presence of organic solvents such as hexane and cyclohexane) and waste streams.
- These species are very common in the natural environment, especially in humid conditions that are rich in organic compounds, including organic acids and ethanol.
- Some species are pathogenic to humans. The formation of biofilms and the secretion of enzymes with hydrolytic activity facilitates this trait.

Debaryomyces hansenii

- It is an "oleaginous" yeast, capable of producing high amounts of lipids.
- It is an osmotolerant and halotolerant yeast (it can grow in media containing 10% (w/v) NaCl or 5% (w/v) glucose).
- Several common foods such as cheese and sausage are the source of many isolates.
- It presents ambiguous CUG decoding, which can explain increased protein diversity.

Klyveromyces

- *K. marxianus* and *K. lactis* are thermotolerant yeasts.
- These two species have a role in the production of dairy products.
- *K. lactis* was the first species, after *S. cerevisiae*, defined as Generally Regarded As Safe (GRAS). It has allowed studies of lactose metabolism and has become a NCY model.
- *K. marxianus* has features such as the ability to utilize a broad range of sugars (including lactose and inulin), secretion of lytic enzymes, and higher growth rate than other eukaryotes.

Methylotrophic yeasts

- They include genera *Komagataella* (*Pichia*) and *Ogataea* (*Hansenula*).
- They are capable of using methanol as their sole carbon and energy source. The promoter of the gene encoding methanol oxidase has been used for high-level expression of native and heterologous genes.
- *K. phaffii* (formerly *P. pastoris*) is very useful for the purification of recombinant proteins due to their proper posttranslational modifications, folding and secretion, together with its limited production of endogenous secretory proteins.
- It grows rapidly on inexpensive media containing methanol, glucose, glycerol or ethanol as the sole carbon source.

Meyerozyma guilliermondii

- It can utilize various carbon sources including typical hydrophilic and hydrophobic materials.
- It has high secretion capability for proteins with low glycosylation modification.
- As *D. hansenii* and *Candida*, it is member of the CTG clade.
- It is a rare pathogen of immunocompromised people.

Rhodotorula

- These species are other example of "oleaginous" yeasts. They can accumulate up to 70% of its dry cell weight as lipid content under nutrient-limited conditions (nitrogen, phosphate and sulphate).
- It can utilize a wide range of substrates, including low-cost sugars and can grow in various waste streams.
- Accessible genetic tools are available for *R. toruloides*, including transformation methods, RNAi machinery for gene downregulation and editing using the CRISPR-Cas9 System.
- It can be an important human pathogen, particularly in immunocompromised hosts.

Yarrowia lipolytica

- It accumulates lipids above 20% of its dry cell weight under natural conditions, mainly consisting of triglycerides and sterol esters.
- It has a strict respiratory metabolism. It can use a wide range of carbon sources (polyalcohols, organic acids, and long-chain hydrocarbons) and has a strong tolerance to changes in pH and salt concentration.
- The size of the yeast genome is not constant in all wild and laboratory strains.
- It is capable of producing high quantities of heterologous proteins.

Zygosaccharomyces

- *Z. rouxii* is a highly osmotolerant yeast. It can grow in the presence of 60-70% (w/v) glucose concentrations.
- it is also resistant to other stress conditions such as high concentrations of organic acids and inorganic salts.
- *Z. bailii* is highly tolerant to weak organic acids such as lactic, ascorbic and acetic acids.
- *Z. bailii* produces zygocin, a dsRNA-encoded toxin that disrupts the plasma membrane integrity of many filamentous fungi and several yeasts (including *S. cerevisiae*, *K. lactis*, most strains of *Z. bailii*, and the pathogenic species *C. glabrata* and *C. albicans*), and has broad lethal activity.

Figure 1. Characteristics of some NCYs used in biotechnological processes ([24,25] and references therein).

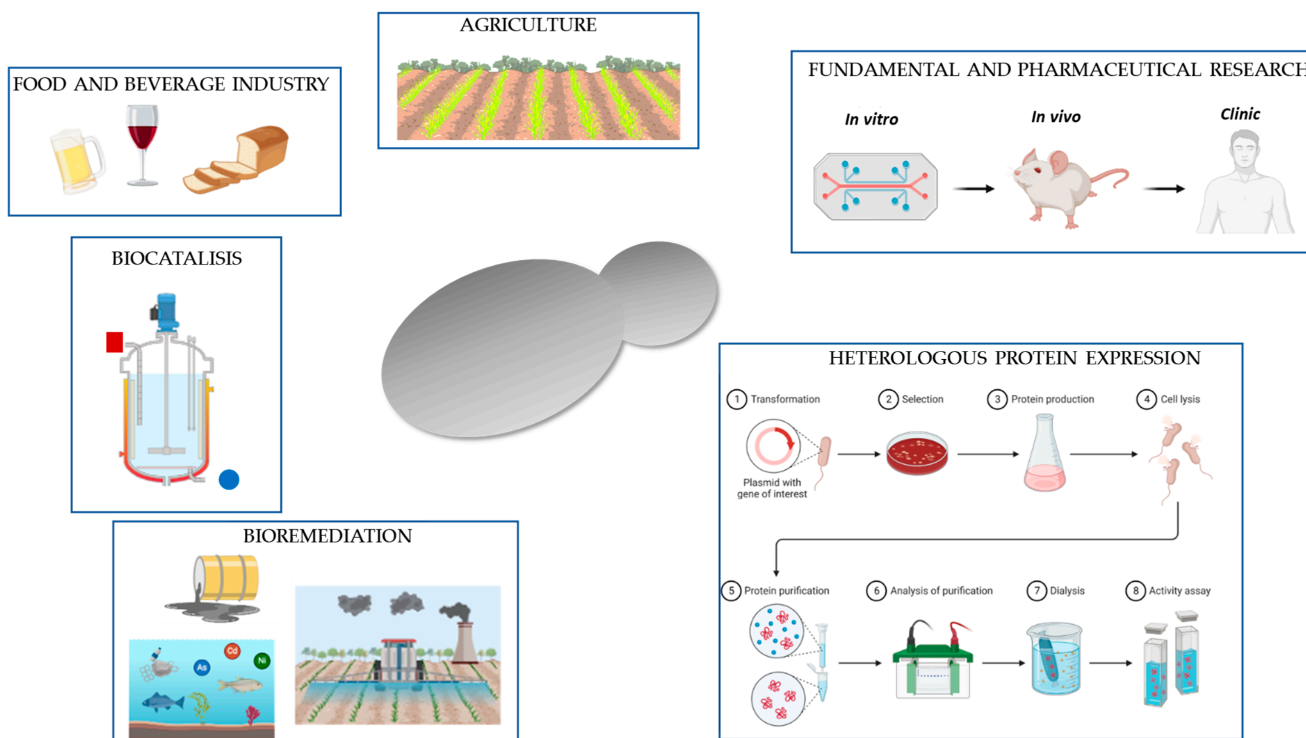


Figure 2. Some biotechnological applications of NCYs (figure constructed with BioRender app).

1.3. Biocatalysis with Non-Conventional Yeasts

The use of enzymes or whole cells in chemical synthesis may require the improvement of their characteristics and properties through genetic engineering to adapt them to harsh reaction conditions, a costly and tedious task. In this sense, the use of unconventional wild yeasts adapted to adverse environments represents an opportunity to overcome these difficulties. Yeasts that live in extreme environments (such as stratospheric air, hot springs, cold and deep seas, polar regions, glaciers, deserts, acidic and alkaline habitats, dry rocks, etc.), with conditions that make them uninhabitable for humans, are known as extremophiles or extremotolerant. Some organisms, the polyextremophiles/polyextremotolerant, have evolved different molecular mechanisms that allow them to grow in different harsh conditions [18,29]. Buzzini et al. (2018) provide a list of the most important yeast species with thermophilic/thermotolerant, psychrophilic/psychrotolerant, halophilic/halotolerant, osmophilic/osmotolerant, xerotolerant, acidophilic/acidotolerant, alkali-tolerant or polyextremphilic/extremotolerant characteristics [29]. It should be noted that some yeasts included in Tables S1 and S2, because of their interesting biotechnological applications, are considered extremophilic/extremotolerant organisms according to this list. For instance, *Debaryomyces hansenii*, *Glaciocyma antarctica*, *Papiliotrema laurentii*, *Yarrowia lipolytica*, *Zygosaccharomyces rouxii*, and some species of *Candida*, *Cystofilobasidium*, *Mrakia*, *Rhodotorula* and *Sporobolomyces* are poliextremophilic/poliextremotolerant.

These microorganisms and their derived enzymes (extremozymes) are an attractive alternative to tailor a given biocatalyst for a specific industrial application. They may be able to catalyze their respective reactions in non-aqueous environments, water/solvent mixtures, at extremely high pressures, acidic and alkaline pH, at temperatures up to 140 °C, or near the freezing point of water [30,31]. In addition, the discovery of new microorganisms extends the scope of the reaction to substrates on which it was previously not possible.

To date, several thousand different yeast species have been identified and described, of which only a small number have been characterized in detail and are commonly used as cell factories, including *Y. lipolytica*, *Komagataella phaffii* (*Pichia pastoris*), *Hansenula/Ogataea polymorpha*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Zygosaccharomyces bailii* and

Rhodotorula toruloides (formerly *Rhodosporidium toruloides*) (Figure 1). The genomes of a few hundred species of unconventional yeasts have been sequenced and their growth phenotypes determined, and, in many cases, they show interesting characteristics.

Non-conventional yeasts are relevant not only because of their own catalytic activities; although not covered in this review, it is important to note that some of them are also used to express enzymes from other organisms [32–36]. Because of their ability to perform post-translational modifications, eukaryotic cells are often preferred for the production of complex enzymes and biopharmaceuticals. *K. marxianus*, *K. lactis*, *Y. lipolytica*, *P. pastoris*, *Scheffersomyces stipitis*, *H. polymorpha*, and *R. toruloides* have been developed as eukaryotic hosts because of their desirable phenotypes, including thermotolerance, assimilation of diverse carbon sources, and high protein and lipid secretion [32,33]. Among these yeasts, *P. pastoris* has become the most popular. The advantages of protein production using the *P. pastoris* system include the ability to grow to extremely high cell densities, proper protein folding in the endoplasmic reticulum, and efficient secretion of the recombinant product into the external environment of the cell. In addition, protein glycosylation is very similar to that of mammalian cells, which is a desirable feature when expressing proteins from these organisms [34,35]. *P. pastoris* is an endotoxin-free host system and has been granted GRAS (Generally Recognized As Safe) status by the U.S. Food and Drug Administration. Another unconventional yeast, the oleaginous *Y. lipolytica*, has attracted attention for its high protein-secretion capacity and advanced secretory pathway. The most common strategies to improve protein secretion in *Y. lipolytica* include codon optimization, increasing gene copy number, engineering regulatory elements, optimizing culture conditions, allowing inducible expression, and secretory tag engineering [36].

The aim of this review is to summarize the existing information in the literature on non-conventional yeasts and their use in biocatalytic processes for the synthesis of fine chemicals and drug intermediates. We will consider the most interesting reactions in which biocatalysis is employed. During the last decades, these yeasts and their biotechnological applications have been intensively studied, resulting in a large number of interesting publications in this field, including reviews (referenced herein) that focus on some non-conventional yeasts and their biocatalytic applications or on a particular reaction or the synthesis of a certain type of compound of interest. However, to the best of our knowledge, there has not been a comprehensive review that focuses on the broad potential and diversity of reactions that non-conventional yeasts can facilitate. In this review, we also aim to highlight the general characteristics of these microorganisms and the advantages they offer for their application in large-scale chemical synthesis. We hope that this approach will provide an overview of the utility of these microorganisms in catalysis.

In recent years, articles have appeared mainly on the use of isolated enzymes, immobilized or not, often with genetic manipulation to increase their applicability. In this respect, we have made a review of the last five years. However, we have also collected some bibliographic citations from previous years that we consider essential.

We have organized the following sections according to the main types of reactions in which unconventional biocatalysts have been used (carbonyl reduction, reduction of activated alkenes, oxidation reactions, nitrile hydrolysis, and reactions catalyzed by lipases). In each case, a general description of the catalytic mechanism and the enzymes involved is given, followed by some examples highlighting the advantages of using these biocatalysts. In order to make the presentation clearer, these examples have been classified according to whether they are whole cells, wild isolated enzymes, or those with some kind of genetic modification.

2. Carbonyl-Reduction

Chiral secondary alcohols can be used in the preparation of various drugs such as Isuprel, Prozac, Neobenodine or the recently approved Iptacopan. The asymmetric reduction of prochiral ketones by alcohol dehydrogenases (ADHs), also known as carbonyl reductases (CRs) or ketoreductases (KREDs), is a well-known method for the production of chiral

alcohols and is the most thoroughly investigated class of biotransformations. Based on their 3D structures and amino acid sequences, ADHs have been classified into three families: short-chain dehydrogenase/reductase (SDR), medium-chain dehydrogenase/reductase (MDR), and aldo-keto reductase (AKR). SDRs are the ADHs with the shortest chain length (250–350 residues) and are not metal ion-dependent. MDRs have a longer amino acid length (about 350 residues), and most of them from eukaryotes typically require the assistance of Zn^{2+} to exert their catalytic functions. Finally, AKRs (about 320 amino acids) are non-metal dependent dehydrogenases. In higher eukaryotes, ADHs are usually dimeric, whereas those in prokaryotes and lower eukaryotes (yeast) are tetrameric. A detailed description of these enzymes can be found in the review by Zhang et al. (2024) [37].

Unlike most chemical redox catalysts, ADHs are capable of catalyzing both the oxidation of primary and secondary alcohols to aldehydes and ketones, respectively, and the reduction of these carbonyl compounds to their alcohol derivatives. The oxidation or reduction reactions are accompanied by the reduction or oxidation of a stoichiometric co-substrate: the nicotinamide cofactor $[NAD(P)]^+$ acts as a hydride acceptor in the oxidation of a substrate, and NAD(P)H acts as a hydride donor in a reduction process. The preference for NAD^+ or $NADP^+$ depends on the enzyme [37].

In the reduction of prochiral ketones, the hydride can be transferred from the *re*-face, yielding an alcohol with (*S*) configuration, or from the *si*-face, yielding an (*R*) alcohol. Since the nicotinamide cofactor is chiral, one of these pathways is preferred. The enantiopreference of most ADHs produces the (*S*)-alcohol, and these are termed Prelog-selective ADHs, while those that produce the (*R*)-alcohol are termed *anti*-Prelog ADHs [38]. The substrate binding site in these enzymes contains a large (L′) and a small (s′) pocket, which helps to control the stereoselectivity of the product. The large (L) and small (s) substituents of the substrate fit into the large and small pockets, respectively. Provided that the large group has higher priority, most ADHs generate *S*-configured alcohols according to the Cahn–Ingold–Prelog rules (Figure 3), and only a few stereocomplementary ketoreductases are known. At present, genome libraries, online databases and screening of natural enzymes are the most useful resources to identify stereocomplementary enzymes [39].

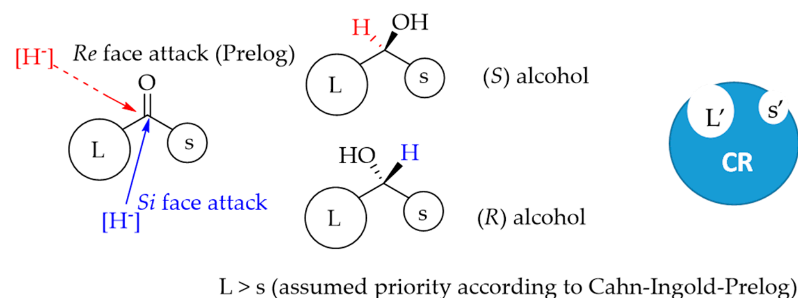


Figure 3. Stereochemistry of the alcohol produced by asymmetric ketone reduction according to the Prelog rule.

Since the reaction requires the use of cofactors, methods must be used that allow their recycling, or, alternatively, whole cells must be used as biocatalysts. This is often the best alternative, but an important limitation when using wild-type yeast cells is the presence of different dehydrogenases with opposite stereoselectivities or other enzymes that may catalyze side reactions leading to unwanted products. In addition, the productivity of the bioprocess may be hampered by the poor water solubility of the substrate and its toxicity to microbial cells. When using a purified enzyme preparation, two enzymatic approaches can be utilized to regenerate the cofactor: (1) use of a coupled enzyme and (2) use of a coupled substrate. In the first case, a second enzyme, such as glucose dehydrogenase, and a sacrificial hydride donor, such as glucose, are employed. In the second alternative, an enzyme is used for both substrate reduction and cofactor regeneration. For example, ADH is employed to synthesize a high-value compound by reducing the primary substrate, and

2-propanol is used as a sacrificial hydride donor that oxidizes to the volatile by-product acetone, allowing cofactor regeneration. Such systems typically require high concentrations of the sacrificial alcohol to drive the equilibrium toward the desired product. This, in turn, leads to a loss of activity in the main target reaction as a result of competition between substrates and cosubstrates for the same active sites on the enzyme [40].

2.1. Carbonyl Reduction with NCYs Using Whole Cells

As in other processes, *S. cerevisiae* has been widely used in stereoselective carbonyl reduction. However, some unconventional yeasts have also proved very useful and are an important resource, often giving better results than *S. cerevisiae*. Scheme 1 shows some examples where whole cells of some of these microorganisms are used.

Some strains of *Candida arborea*, *Issatchenkia scutulata*, and especially *K. lactis* and *Candida parapsilosis*, were used for the large-scale production of (*R*)-1,3-butanediol from 4-hydroxy-2-butanone. *K. lactis* IFO 1267 gave a high yield and good enantiomeric excess of (*R*)-1,3-butanediol, while *C. parapsilosis* IFO 1396 was the best for (*S*)-1,3-butanediol production. Both enantiomeric alcohols are chiral starting materials for organic synthesis [41].

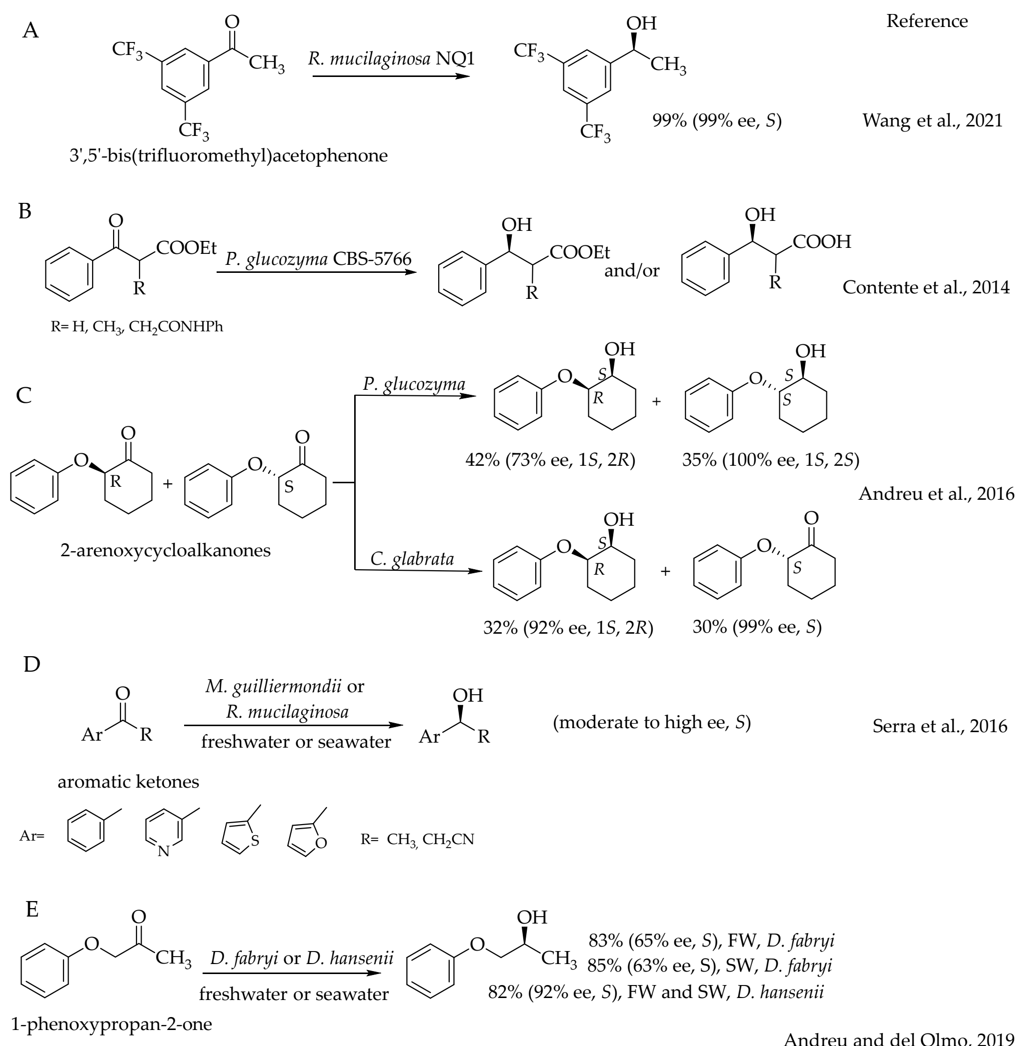
The asymmetric biocatalytic reduction of bulky prochiral carbonyl compounds is difficult and with low catalytic efficiencies. Both enantiomers of 1-(3,5-bis(trifluoromethyl)phenyl)-ethanol are important building blocks for the synthesis of APIs. These compounds can be obtained by stereoselective reduction of 3′,5′-bis(trifluoromethyl)acetophenone. Recently, a strain isolated from a simple soil, identified and named *Rhodotorula mucilaginosa* NQ1, was shown to be very effective in the bioreduction of 3′,5′-bis(trifluoromethyl)acetophenone to (*S*)-1-(3,5-bis(trifluoromethyl)phenyl)-ethanol with an excellent stereoselectivity (Scheme 1A). After optimization of the key reaction parameters involved in the whole-cell catalyzed reduction process, the preparative scale asymmetric reduction of 3′,5′-bis(trifluoromethyl)acetophenone using whole cells of strain NQ1 was performed in a bioreactor at 30 °C, and the substrate was converted with over 99% yield and over 99% e.e. The application of *R. mucilaginosa* NQ1 was also extended to 10 other substrates with bulky groups under the above-mentioned conditions and gave corresponding bulky chiral hydroxyl compounds with excellent conversions and enantiomeric excess [42].

Other unconventional yeasts such as *Pichia glucozyma* CBS 5766 (now *Ogataea glucozyma*) also reduced acetophenone, propiophenone, butyrophenone and valerophenone with moderate to high conversions and always with high enantioselectivity to the corresponding (*S*)-alcohol (Scheme 1B). This microorganism also showed high catalytic activities towards aromatic β- and γ-ketoesters; in this case, the competitive ester hydrolysis reaction by esterases can be modulated by the appropriate cosubstrate concentration: ketoreductase activity is predominant when it is high, while esterase activity is mostly observed in its absence [43]. The yeast strain *Candida zeylanoides* P1 was also able to reduce various substituted acetophenones to the corresponding chiral secondary alcohols with high enantioselectivity on the gram scale [44].

Whole cells of the yeast strains *P. glucozyma* and *Candida glabrata* have also been used to catalyze the reduction of several 2-arenoxycycloalkanones to produce chiral 2-arenoxycycloalcohols with good to excellent enantioselectivity. These compounds are cyclic rigid analogs of β-aryloxy alcohols, which are interesting building blocks that form part of β-adrenergic antagonists and may be useful in obtaining more selective drugs. *P. glucozyma* allowed the conversion of both enantiomers of the starting material to produce 2-arenoxycycloalcohols with (1*S*,2*R*) and (1*S*,2*S*) configurations. The reaction with *C. glabrata* almost always allowed the kinetic resolution of the starting ketone, yielding (*S*)-2-arenoxycycloalkanone and (1*S*,2*R*)-2-arenoxycycloalcohol (Scheme 1C) [45].

Recently, new marine microorganisms with potential biotechnological applications have been characterized to perform bioprocesses under saline conditions [46]. Seawater is an economical and widely available resource and has been proposed as a biosustainable alternative to the use of freshwater. *Meyerozyma guilliermondii* and *R. mucilaginosa* strains isolated from different depths of deep seafloor sediments in New Zealand were able to re-

duce aromatic ketones with high molar conversions and moderate to high enantioselectivity in both freshwater and seawater (Scheme 1D) [46].



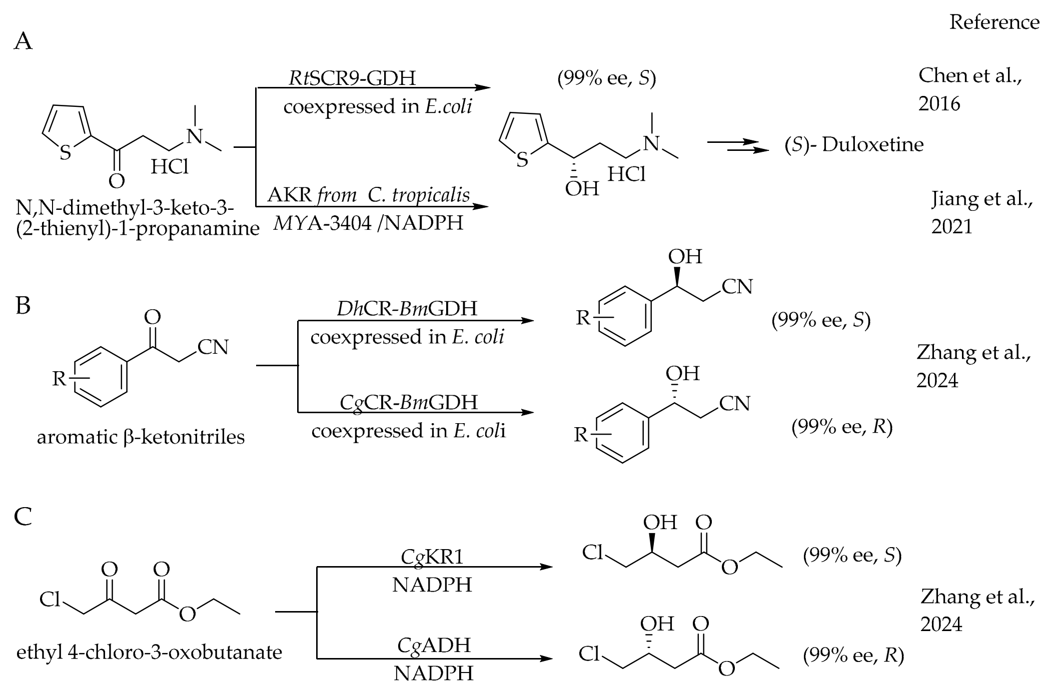
Scheme 1. Stereoselective reduction of various ketones by whole cells of NCYs in the presence of glucose. (**A**) Adapted from [42]; (**B**) Adapted from [43]; (**C**) Adapted from [45]; (**D**) Adapted from [46]; (**E**) Adapted from [47]. FW and SW refer to freshwater and seawater, respectively.

The potential of several halotolerant *Debaryomyces* yeast strains as cell factories for biocatalytic processes using seawater has been demonstrated [47,48]. *D. hansenii*, *D. hansenii var. fabryi*, *Schwanniomyces etchellsii* and *Schwanniomyces polymorphus var. polymorphus* were more resistant to several organic solvents in seawater than in freshwater and also showed greater thermoresistance. Several prochiral ketones were reduced more efficiently in the former case, which also allowed higher substrate loading and catalyst recycling for at least three rounds (Scheme 1E) [47,48].

2.2. Carbonyl Reduction with Wild and Engineering Modified Reductases from NCYs

Ketoreductases from some non-*Saccharomyces* yeasts have also been heterologously expressed in *Escherichia coli* or in other microorganisms (several examples are shown in Scheme 2). For example, the KRED *Rt*SCR9 from the red yeast *R. toruloides* was used for the asymmetric carbonyl reduction of *N*,*N*-dimethyl-3-keto-3-(2-thienyl)-1-propanamine to produce (*S*)-3-(dimethylamino)-1-(2-thienyl)-1-propanol, a chiral alcohol precursor of the antidepressant duloxetine (Scheme 2A). This enzyme was co-expressed with glucose dehydrogenase (GDH) for cofactor recycling in *E. coli* and showed excellent activity and

enantioselectivity [49]. The same compound was obtained by the aldo-keto reductase from *Candida tropicalis* MYA-3404 in the presence of NADPH. This enzyme was expressed and isolated from *E. coli* BL21 (DE3). The purified enzyme was then characterized and showed a broad temperature and pH optimum, high substrate specificity and excellent solvent tolerance. A 3D model of this enzyme docking with coenzyme NADPH was constructed and the catalytic and binding sites were identified [50].



Scheme 2. Some examples of esteroselective reduction of ketones with purified NCY ketoreductases expressed in *E. coli*. (**A**) Adapted from [49,50]; (**B**) Adapted from [37]; (**C**) Adapted from [37].

The chiral 3-hydroxypiperidine moiety is an important synthon in the pharmaceutical industry because it is found in many bioactive compounds. It is often prepared by chemical approaches from chiral starting materials. The NADPH-dependent carbonyl reductase YGL039W from *K. marxianus* ATCC 748, expressed at high levels as a soluble recombinant protein in *E. coli*, was shown to be a potential biocatalyst for the synthesis of (*R*)-*N*-Boc-3-hydroxypiperidine (>99% ee) from *N*-BOC-piperidine-3-one. A cofactor regeneration system based on GDH-catalyzed glucose oxidation was used to ensure an adequate supply of NADPH. Optimization of temperature, pH, metal ions, substrate concentration, etc., allowed the bioreduction of an extremely high substrate concentration with 99% ee [51].

A conjugated polyketone reductase from *C. glabrata* (*Cgl*CPR) was highly expressed in *E. coli*. The purified enzyme was characterized in detail and used for the production of D-(−)-pantolactone, a key intermediate in the production of D-(+)-pantothenic acid, using ketopantoyl lactone as the starting material in the presence of glucose and a recombinant *E. coli* harboring a glucose dehydrogenase gene for NADPH cofactor regeneration. The reaction was optimized and ultimately performed using a strategy that combined fed-batch addition of whole-cell catalyst with semi-continuous substrate feeding, resulting in a higher final concentration of substrate loading. *Cgl*CPR possessed the typical TIM barrel fold of the AKR superfamily, as determined by homology modeling and docking analysis. Furthermore, it conformed to the anti-prelog rule, justifying the generation of D-(−)-pantolactone with excellent stereoselectivity [52].

It is interesting to find carbonyl reductases with complementary stereopreferences. For example, *Dh*CR from *D. hansenii* and *Cg*CR from *C. glabrata* showed high activity and complementary stereoselectivity for the asymmetric reduction of aromatic β-ketonitriles. The co-expression of *Dh*CR or *Cg*CR and glucose dehydrogenase from *Bacillus megaterium*

(*Bm*GDH) in *E. coli* allowed the application in scale-up synthesis of complementary chiral β-hydroxy nitrile with excellent yields and enantiomeric excess (Scheme 2B). Very important was also the discovery in the same unconventional yeast *C. glabrata* of two robust ketoreductases, *Cg*KR1 and *Cg*ADH, with 78% sequence similarity but with completely opposite stereoselectivity towards the reduction of some ketones, such as the prochiral ethyl 4-chloro-3-oxobutanate (Scheme 2C). *Cg*KR1 produced the reduced products with the (*S*) configuration, whereas *Cg*ADH produced the (*R*) alcohol. Considering their high sequence and three-dimensional structure similarity, these two natural enzymes evolved stereocomplementarily and are interesting in understanding the molecular basis of stereoselectivity control of ADHs [37].

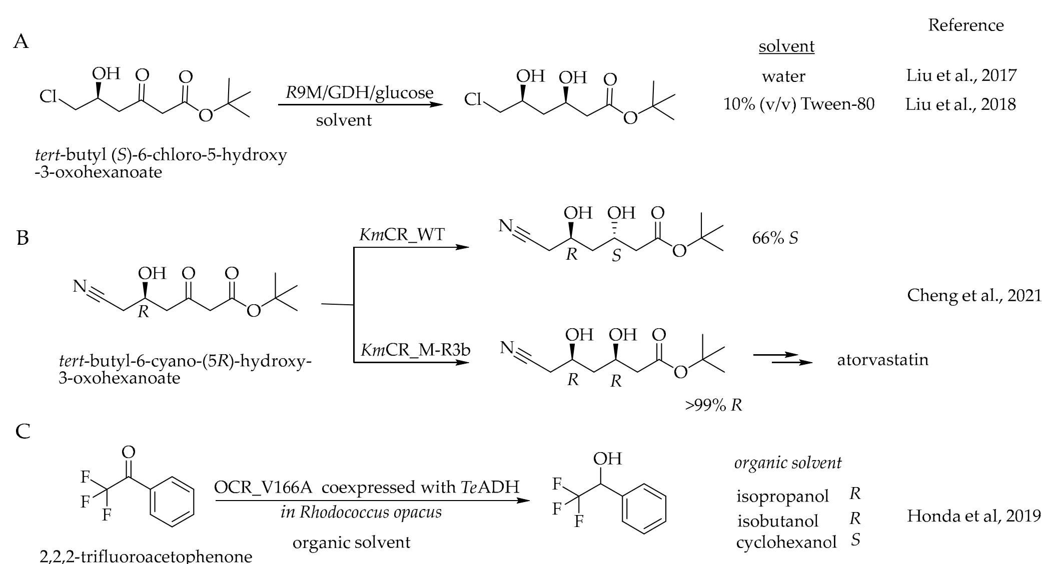
Protein engineering strategies are powerful tools for modifying the catalytic properties of biocatalysts, improving substrate scope and stereoselectivity, as well as tolerance to harsh reaction conditions and modifying stereoselectivity. Natural enzymes can be adapted by protein engineering to improve their properties. Two main techniques are used: (1) Semirational design, which consists of making specific mutations, insertions or deletions in the coding sequence. Selection is based on structural and functional information about the target biomolecule, which is often unavailable; (2) Directed evolution, which attempts to mimic the process of natural evolution in the laboratory on a much shorter time scale. In nature, beneficial mutations are selected iteratively over many generations, but in the laboratory the goal is to create molecules that meet the needs defined by the researcher [53,54]. Random mutations are introduced into the parent gene, resulting in a vast library of mutants that must be screened for improvements in the desired property. The selected variant then serves as the parent for the next round of evolution. This process is very time- and resource-consuming, but if some structural or mechanistic information is available, mutation positions can be constrained and smaller libraries can be constructed [53,54]. Protein engineering has been applied to many enzymes. Here are some examples of this strategy in reductases (Scheme 3).

Tert-butyl (3*R*,5*S*)-6-chloro-3,5-dihydroxyhexanoate is a key chiral intermediate used primarily in the synthesis of the chiral side chain of the cholesterol-regulating drugs atorvastatin and rosuvastatin. Its chemical synthesis is possible but requires the use of expensive and environmentally unfriendly reagents and organic solvents. It is also difficult to achieve enantiomeric purity in the final product. This compound was prepared from *tert*-butyl (*S*)-6-chloro-5-hydroxy-3-oxohexanoate as starting material, using the carbonyl reductase *Rt*SCR9 from the oleaginous yeast *R. toruloides*, with low cost, high enantioselectivity and broad functional group tolerance. The activity of this carbonyl reductase was improved by random and site-saturation mutagenesis, yielding three positive mutants with improved catalytic properties. Among them, mutant Ile144Lys (R9M) showed the highest fold and specific activity improvement in the asymmetric reduction of *tert*-butyl (*S*)-6-chloro-5-hydroxy-3-oxohexanoate (Scheme 3A) [55]. *E. coli* cells expressing mut-Ile144Lys were later used in a whole-cell biosynthesis process in monophasic media containing 10% (v/v) Tween-80 as cosolvent and in a water–octanol biphasic reaction medium. In both cases, but especially in the first, the bioreduction efficiency was increased compared to water as the reaction medium [56].

Another key component of atorvastatin, *tert*-butyl-6-cyano-(3*R*,5*R*)-dihydroxyhexanoate, can also be produced by the asymmetric reduction of *tert*-butyl-6-cyano-(5*R*)-hydroxy-3-oxohexanoate catalyzed by carbonyl reductases. As mentioned above, ADH enzymes often exhibit a Prelog preference (Figure 3), and carbonyl reductases with anti-Prelog stereoselectivity are relatively rare in nature. It is, therefore, important to find strategies to control their stereopreference with the desired selectivity. Since the enantioselectivity of an ADH is a property that is closely related to the geometry of the active site, engineering the substrate binding site can help to change the stereoisomer of the product [39]. The wild-type carbonyl reductase from *K. marxianus* ZJB14056 (KmCR_WT) showed excellent tolerance to the substrate *t*-butyl-6-cyano-(5*R*)-hydroxy-3-oxohexanoate but moderate stereoselectivity, resulting in an undesirable Prelog selectivity (66% *S*) (Scheme 3B). To reverse the

stereoselectivity towards (*R*)-alcohols, the four key residues regulating the stereopreference of this *Km*CR_WT were identified by semi-rational engineering and structure analysis of structure-available CRs and *Km*CRs. The construction of a small library with modifications in these key residues yielded the mutant *Km*CR_M-R3b with a strict anti-Prelog stereopreference. When it catalyzed the reduction of *t*-butyl-6-cyano-(5*R*)-hydroxy-3-oxohexanoate, complete *t*-butyl-6-cyano-(3*R*,5*R*)-dihydroxyhexanoate was obtained. In addition, the stereopreference of 11 CRs sharing 20% to 40% sequence identity with *Km*CR was successfully predicted by the in silico method and verified experimentally [57].

The low solubility of substrates in water sometimes requires the use of organic solvents. Honda et al. (2019) obtained a mutant (OCR_V166A) of the yeast *Ogataea minuta* carbonyl reductase with improved tolerance to them through directed evolution experiments. This mutant was expressed in *Rhodococcus opacus* cells, a bacterium that is easily dispersed in organic solvents due to its highly lipophilic surface. The alcohol dehydrogenase from *Thermoanaerobacter ethanolicus* (*Te*ADH) was co-expressed for NADPH regeneration. Whole cells carrying these enzymes were used to reduce 2,2,2-trifluoroacetophenone in several alcoholic organic solvents, and it was found that each specific organic solvent determined the conversion and enantioselectivity, as well as the stability of the catalyst (Scheme 3C) [58].



Scheme 3. Reactions catalyzed by protein engineering modified reductases. (**A**) Adapted from [55,56]; (**B**) Adapted from [57]; (**C**) Adapted from [58].

In addition to traditional biocatalysts selected from nature, the rapid development of modern bioinformatics tools and genome-mining from genomic databases has been proven to be a very effective and promising way to discover and identify new previously unknown enzymes [59]. An example of this is the strain *R. mucilaginosa* LSL, a robust extrachemophilic yeast capable of reducing a wide range of ketones with excellent stereoselectivity. The carbonyl reductase repertoire of *R. mucilaginosa* LSL was screened by sequence homology with the Basic Local Alignment Search Tool (BLAST), using as queries sequences of already characterized reductases from yeasts belonging to the genus *Rhodotorula*. A new aldo keto reductase, named AKR3B4, was discovered. The gene was cloned, and the enzyme was expressed and characterized, showing a highly selective substrate profile and very good stability at moderate temperatures over a wide pH range and in the presence of organic solvents [60].

3. Reduction of Carbonyl-Activated Alkenes

In contrast to asymmetric cis-hydrogenations carried out by traditional synthetic routes using metals, chiral ligands and molecular hydrogen, the ene-reductase (ER) enzymes belonging to the family of old yellow enzymes containing flavin (OYE) (EC 1.6.99.1) catalyze

the regio- and stereoselective trans-hydrogenation of electron-poor alkenes (conjugated enals, enones, α,β-unsaturated carboxylic acids, nitroalkenes, etc.) [61,62]. Its ability to generate up to two stereocenters is a highly sought-after feature in the field of asymmetric synthesis. Since the discovery of the first member (OYE1) from *Saccharomyces pastorianus* in 1933, several intracellular ER homologs have been isolated from plants, bacteria, yeasts and filamentous fungi, providing a source of chirality in the synthesis of fine chemicals and APIs [61,62].

In the active center of the enzyme, the substrate is fixed by the formation of strong H-bonds between the electron-withdrawing group (EWG) and two donor residues that help to position the substrate and make it more reactive to the 1,4-hydride addition. These donor residues are His191 and Asn194 in OYE1, which are almost universally conserved throughout the family except for a limited number of homologs that carry two His residues instead. The $FMNH_2$ prosthetic group promotes the hydride addition to the β-position, and then a proton is transferred to the α-position from the acidic residue Tyr196 in OYE1, which is also universally conserved. This results in a formal hydrogenation of the C=C bond with anti-stereospecificity. After the product is released, a molecule of the nicotinamide cofactor NAD(P)H binds to reduce FMN to $FMNH_2$, and the catalytically active form is restored to start a new reduction cycle. Therefore, in biocatalytic applications, ERs are routinely coupled with an appropriate cofactor regeneration system to recycle $NAD(P)^+$ using an inexpensive reducing agent such as a combination of glucose/GDH (Figure 4) [61,62]. The need to regenerate the reducing cofactor has also been addressed by the use of whole-cell biocatalysts, but in these cases, the presence of carbonyl reductases limits chemoselectivity and generates by-products that reduce yields.

The enzyme can bind to the substrate in two different orientations that differ by about 180° of rotation. These two binding modes are called "classical" and "flipped", and one or the other depends on the structure of the substrate. Depending on the binding mode, the same prochiral substrate yields products with opposite stereochemical configurations. If the substrate does not have a preferred binding mode, the result is a low enantioselectivity in the hydrogenated product. Sometimes it is interesting to somehow change the binding mode to access the other stereoisomer [62].

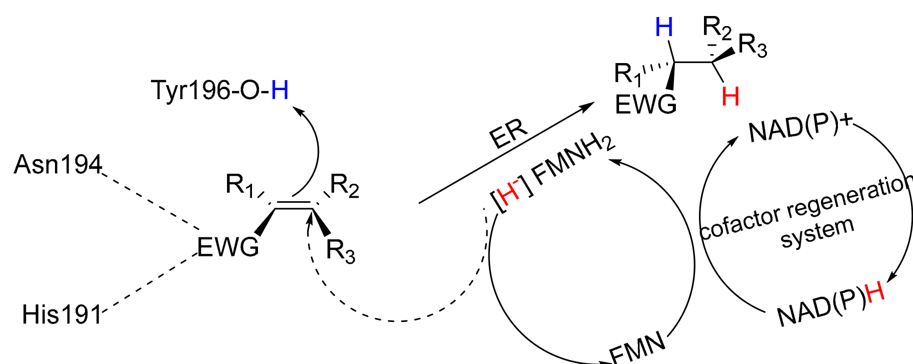


Figure 4. Catalytic mechanism in the ER for the reduction of activated alkenes. In this case, the substrate is bound to the active site in the classical mode. In the flipped mode, the substrate would be rotated 180° so that the substituents EWG and R_3 are behind the plane and R_1 and R_2 are in front.

3.1. Hydrogenation of Carbonyl-Activated Alkenes with NCYs Whole Cells

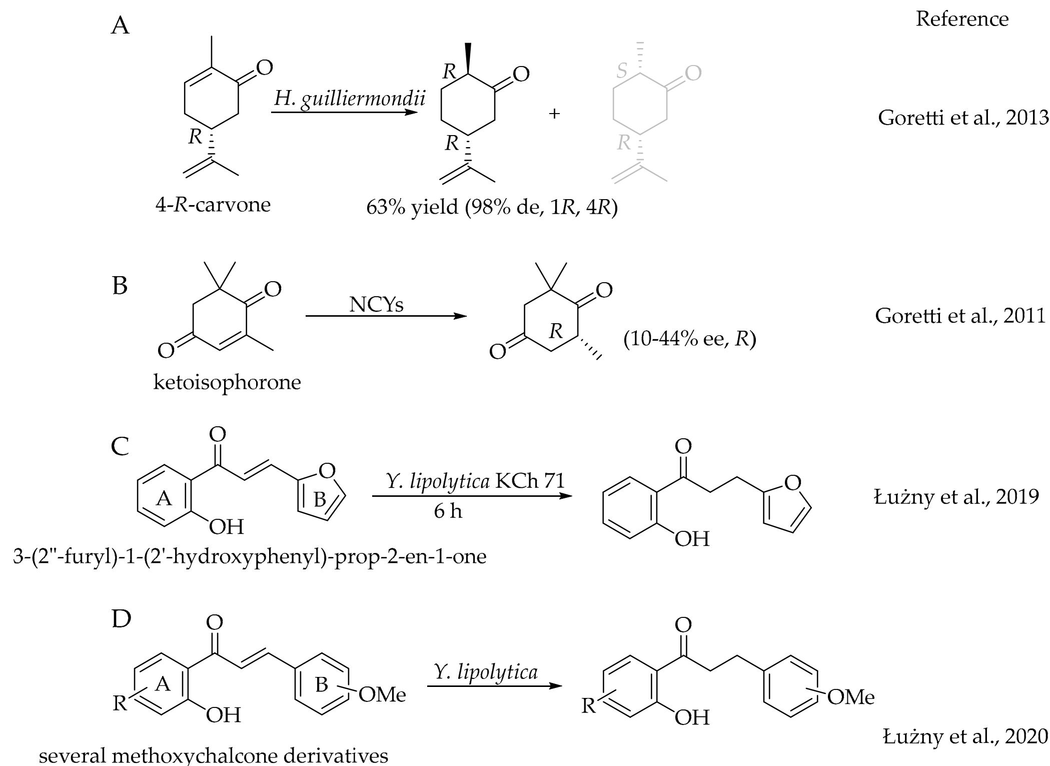
OYEs have been used in some industrial processes for the synthesis of valuable products such as pregabalin or menthol, but their industrial application is still very limited. The synthetic utility of the first generation of OYEs (wild-type enzymes from some microorganisms) had two major shortcomings: (1) the relatively size-restricted active sites of most OYEs, limiting their substrate range to small alkenes, and (2) the identical stereoselectivity of OYEs from different sources, producing the same stereoisomers. Furthermore, the modest reaction rate and the resulting low total number of conversions of most known ERs

drastically limit their application, and there is great interest in the search for new and robust ERs that perform highly efficient reactions [63]. In this perspective, non-conventional yeasts still represent an unexplored source of biodiversity that deserves to be explored for the identification of OYE enzymes with novel catalytic performances and/or different chemo- or stereoselectivity. Scheme 4 shows some examples of hydrogenation of electron-poor alkenes catalyzed by whole cells of NCYs, which will be explained in the following paragraphs.

Flavors and fragrances are widely used in the food, cosmetic and medical industries. Their enzymatic synthesis has several advantages over natural extraction or chemical preparation, including high yield, stable quality, mildness and environmental friendliness. On the other hand, different enzymes can produce different configurations of these compounds, resulting in significantly different olfactory properties. Several enzymes have been used to produce these compounds, such as lipase to synthesize ester flavors or oxidase primarily to produce aldehyde and ketone flavors. In recent years, ERs have also been used as biocatalysts for the synthesis of these compounds [64]. The biocatalytic conversion of carvone (2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one) and other terpene ketones by various NCYs has been studied and reported to competitively reduce C=C and C=O double bonds to produce mixtures of saturated ketones, saturated alcohol and, rarely, the allylic alcohol. With the NCYs tested, the reduction of (4*R*)-carvone was often much faster than the reduction of (4*S*)-carvone. The predominant catalytic activity of whole cells of the genera *Candida*, *Cryptococcus*, *Debaryomyces*, *Hanseniaspora*, *Kazachstania*, *Kluyveromyces*, *Lindnera*, *Nakaseomyces*, *Vanderwaltozyma*, and *Wickerhamomyces* was ER [65]. The bioreduction of (4*R*)-carvone produced a mixture of (1*R*,4*R*)- and (1*S*,4*R*)-dihydrocarvone, with a clear preference for the production of the (1*R*,4*R*)-diastereomer. The best bioconversion yield coupled with excellent diastereomeric excess was found with *Hanseniaspora guilliermondii* (synonym of *M. guilliermondii*) (Scheme 4A). Only traces of dihydrocarbeols, formed by the reduction of the carbonyl group of dihydrocarvone, were found [65].

Bioreduction of other carbonyl-activated alkenes to aroma compounds has also been described. For example, the bioreduction of the α,β-unsaturated ketones ketoisophorone (2,2,6-trimethyl-cyclohexene-1,4-dione), 2-methyl- and 3-methyl-cyclopentenone has been reported by NCYs belonging to the genera *Candida*, *Cryptococcus*, *Debaryomyces*, *Hanseniaspora*, *Kazachstania*, *Kluyveromyces*, *Lindnera*, *Nakaseomyces*, *Vanderwaltozyma* and *Wickerhamomyces* (Scheme 4B). Very high bioconversions, via asymmetric reduction of the conjugated C=C bond, catalyzed by a few NCYs, have been found especially for ketoisophorone and 2-methylcyclopentenone with moderate stereoselectivity for *R*-isomers, usually with low competing carbonyl reductases [66].

Chalcones are bioactive open-chain flavonoids containing α,β-unsaturated carbonyl groups in the backbone. Structurally, they are composed of two aryl groups linked by a three-carbon chain containing an α,β-unsaturated carbonyl system. Some dihydrochalcones, obtained by regioselective reduction of the C=C double bond, have been found to exhibit various activities that could be of interest to the pharmaceutical and cosmetic industries (antiviral, anti-inflammatory, antioxidant, UV-protective, pro-health, etc.). Filipucci et al. (2020) have identified nine strains of NCYs (*Cyberlindnera amylophila* DBVPG 6346, *Goffeauzyma gastrica* DBVPG 4709, *H. guilliermondii* DBVPG 6790, *Kazachstania exigua* DBVPG 6469, *Kazachstania spencerorum* DBVPG 6746, *K. lactis* DBVPG 6854, *Naganishia diffluens* DBVPG 6237, *Scheffersomyces shehatae* DBVPG 6850 and *Wickerhamomyces canadensis* DBVPG 6211) with ER activity, which convert several chalcones with some structural differences with good yields, excellent regioselectivity and reproducibility. The bioreduction capacity of whole cells of these NCYs was influenced by both the structure of the chalcones and the logP value of the substrates tested, with better results obtained with compounds characterized by higher lipophilicity, probably due to their greater ability to cross the yeast cell membrane [67].



Scheme 4. Several compounds derived from whole-cell ER activity in unconventional yeasts. (**A**) Adapted from [65]; (**B**) Adapted from [66]; (**C**) Adapted from [68]; (**D**) Adapted from [69].

The many restrictions on the sweeteners available on the market have created a need to search for new compounds that are more beneficial to human health and more attractive to industry. Replacing some of the popular sweeteners with other compounds without side effects is an increasingly common practice in the food market. Some dihydrochalcones are known for their sweet taste and have great potential as synthetic sweeteners. The best known of these is neohesperidine dihydrochalcone, a methoxy dihydrochalcone glycoside found naturally in sweet oranges [68]. There is also a group of dihydrochalcone analogs that have a heteroatom on the B-ring and do not have a sweet taste. This group includes the hydrogenated derivative 3-(2″-furyl)-1-(2′-hydroxyphenyl)-propan-1-one, which can have different tastes depending on the concentration, from none (at concentrations of 1 ppm) to bitter, licorice, slightly sweet, etc. (at concentrations up to 100 ppm). Chalcones containing heterocycles in their structure have also been substrates for several unconventional yeasts. The biotransformation of 3-(2″-furyl)- and 3-(2″-thienyl)-1-(2′-hydroxyphenyl)-prop-2-en-1-one, tested with eight yeast strains, showed that the most promising were *S. cerevisiae* KCh 464 and especially the unconventional yeast *Y. lipolytica* KCh 71. Both started to produce the dihydrogenated product after the first hour of biotransformation, and *Y. lipolytica* reached >99% conversion efficiency after three and six hours in the thiophene- or furan-containing chalcone, respectively (Scheme 4C) [68]. The results showed no difference in the reduction of these compounds compared to 2-hydroxychalcone and that the presence of the heteroatom in the B-ring had virtually no effect on the conversion efficiency. The same research group demonstrated the ability of several NCYs, in particular the GRAS microorganism *Y. lipolytica* KCh 71, to hydrogenate the double bond of some methoxychalcone derivatives. They found that methoxychalcones (containing the methoxy group in the B-ring) were effectively hydrogenated to the specific dihydrochalcones (Scheme 4D). However, as the number of methoxy substituents in the chalcone substrate increased, the rate and efficiency of conversion to the dihydrogenated product decreased [69]. These compounds are also very interesting because of the known potent inhibitory effect of chalcones and dihydrochalcones on acetylcholinesterase. It was found that 2′,6′-dihydroxy-

4′-methoxydihydrochalcone significantly improved learning and memory in experimental mice with induced Alzheimer's disease [70].

Łużny, et al. (2022) also reported the susceptibility of bromochalcones to hydrogenation to the corresponding dihydrobromochalcones. These compounds are interesting because of their susceptibility to substitution reactions, which allow them to obtain compounds with different properties. The bromine atoms make them lipophilic and facilitate their passage through cell membranes, a useful property in the case of derivative drugs. Again, the most effective non-conventional yeast in this reaction was the strain *Y. lipolytica* KCh 71, which was very suitable for biotransformation on a preparative scale [71].

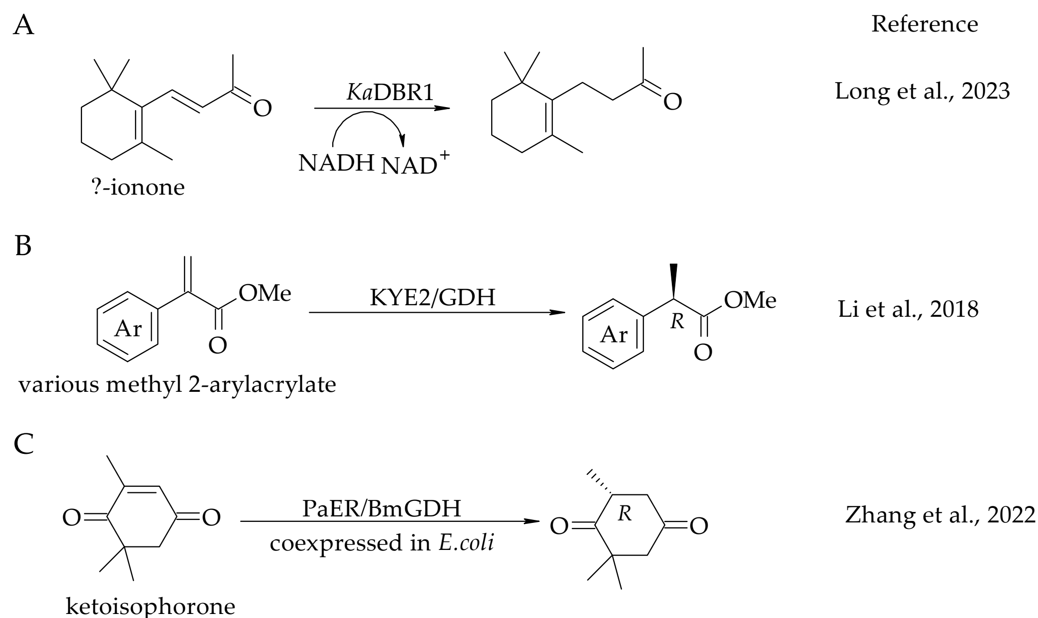
Deep eutectic solvents appear to be a promising alternative to conventional organic solvents and are bursting onto the green chemistry scene. Recently, Chipała et al. (2024) described the use of these solvents to obtain 4′-dihydrochalcones. They used cultures of the yeasts *Y. lipolytica* KCh 71, *D. hansenii* MI1a, and several *Rhodotorula* strains as biocatalysts at the optimized temperature of 35 °C. The most suitable deep eutectic solvent contained choline chloride as a hydrogen acceptor and glycerol as a hydrogen donor [72].

3.2. Hydrogenation of Carbonyl-Activated Alkenes with Wild and Engineering Modified Ene Reductases from NCYs

Several ene reductases from NCYs have been identified, characterized and used as purified enzymes (Scheme 5). One of them is *Ka*DBR1, from the yeast *K. exigua* HSC6. The enzyme showed high activity for the conversion of β-ionone to dihydro-β-ionone, exhibited optimum activity at 60 °C and pH 6.0 and preferred NADH over NADPH as a cofactor (Scheme 5A). Dihydro-β-ionone is widely used in the flavor and fragrance industry and has potential applications in health care and pharmaceuticals [73].

Another ene-reductase (*Mg*ER from *M. guilliermondii* ATCC6260) was identified by a BLAST search using the amino acid sequence of *S. pastorianus* Old Yellow Enzyme 1 as a template. Furthermore, sequence homology analysis showed that *Mg*ER shares a maximum amino acid sequence identity of 57% with OYE2.6 from *S. stipitis* (formerly *Pichia stipitis*). *Mg*ER was heterologously expressed in *E. coli* BL21 (DE3), isolated, purified, and characterized for its biochemical and biocatalytic properties. Under the optimized conditions, (*R*)-carvone was completely converted to (2*R*,5*R*)-dihydrocarvone with excellent yield and optical purity using *Mg*ER as a catalyst [74]. Similarly, an OYE from the genome of *K. marxianus* CBS4857 (named KYE2) was identified and characterized. This new OYE belongs to the classical family and has a broad substrate spectrum. Multiple sequence alignment of KYE2 with nine representative OYEs revealed the conservation of the substrate positioning residues (His192 and Asn195) and the proton source (Tyr197). In addition, five residues known to interact with the flavin cofactor are also conserved. The encoding gene was expressed in *E. coli* BL21, and the ER enzyme produced was characterized and applied to the chemoenzymatic synthesis of various profen methyl esters. Successful stereoselective synthesis of (*R*)-flurbiprofen methyl ester (>95% *ee*) was achieved on a semi-preparative scale (Scheme 5B) [75].

FMN-binding ene reductase (*Pa*ER) from *Pichia angusta* was heterologously expressed in *E. coli* BL21 (DE3). The recombinant enzyme exhibited optimal activity at 40 °C and pH 7.5 and was quite stable below 30 °C over a wide pH range. *Pa*ER had a good ability to reduce the C=C bond of various α,β-unsaturated compounds in the presence of NADPH with a high reduction rate and excellent stereoselectivity. The engineered whole cells of *E. coli* were used as a preparative tool to obtain (*R*)-levodione from ketoisophorone, showing great potential for practical synthesis (Scheme 5C) [76].



Scheme 5. Reduction of some activated alkenes by purified ER enzymes obtained from heterologous expression or by engineered whole cells of *E. coli* expressing these enzymes. (**A**) Adapted from [73]; (**B**) Adapted from [75]; (**C**) Adapted from [76].

The stereoselectivity of ERs is almost always the same, and it would be desirable to find enantiocomplementary alkene reductase partners and thus increase the potential of their applications in organic synthesis. Unfortunately, most efforts to identify OYEs with reversed stereoselectivity have not yielded the desired results. Protein engineering has been applied to several microorganisms in order to alter the stereoselectivity of alkene reductase. When the structure is known, the mutation of residues around the binding pocket (random or rational) has sometimes yielded variants with improved properties (reviewed by Shi et al., 2020) [77]. *P. stipitis* OYE 2.6 is known to be more stable than *S. pastorianus* OYE1. Unlike this and most of its homologs, the native sequence of OYE 2.6 contains an Ile at the site of the highly conserved active site Trp116. In addition, OYE 2.6 differs from *S. pastorianus* OYE1 in the length of two active site loops and in its complementary stereoselectivity in some cases. Therefore, it was chosen to develop variants with complementary enantioselectivities. The crystal structure of *P. stipitis* OYE 2.6 revealed the presence of 13 residues with side chains close to the center of the active site, and these were selected for site saturation mutagenesis. Three Baylis–Hillman adducts were selected as test substrates (Figure 5). Wild-type *P. stipitis* OYE 2.6 reduced all these compounds with very high enantioselectivities to the corresponding useful chiral intermediates with configuration *S*. The molecular biology methods were optimized to ensure that all libraries were sufficiently diverse and contained all the targeted amino acid substitutions. In the case of 2-(hydroxymethyl) cyclopenten-2-en-1-one, the enantioselectivity switch from (*S*)-selectivity to (*R*)-selectivity was straightforward with only one key change (Tyr78Trp), but this was not the general trend, and, in some cases, no significant movement towards (*R*)-stereoselectivity was observed after three generations. This and other examples are reviewed by Amato and Stewart in 2015 [78].

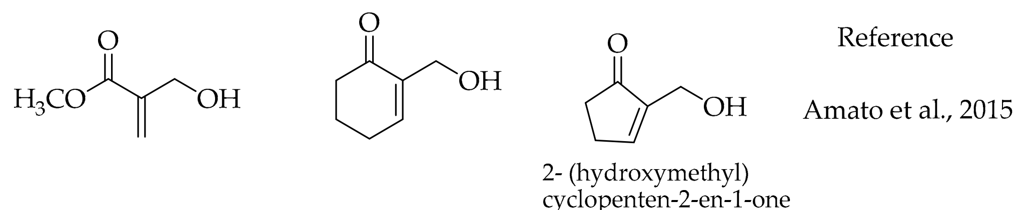


Figure 5. Baylis–Hillman adducts selected as test substrates for reaction with wild or mutant *P. stipites* [78].

4. Oxidation Reactions

There are three classes of enzymes used for alcohol oxidation: alcohol dehydrogenases (ADHs—EC 1.1.1.1), flavin-dependent alcohol oxidases (FAD-AOXs—EC 1.1.3.13), and copper radical alcohol oxidases (CRO-AlcOx—EC 1.1.3.13). Klepach et al., 2021, Ribeaucourt et al., 2022, Schober et al., 2023 and their references provide interesting overviews of these enzymes [79–81]. Some of this information is summarized here. The well-studied ADHs (or ketone reductases—KRED) catalyze the reversible reaction using nicotinamide cofactor ($NAD(P)^+/NAD(P)H$). Less studied FAD-AOXs and CRO-AlcOx catalyze the irreversible oxidation of the alcohol to the corresponding aldehyde, using molecular O_2 as the final electron acceptor. The best-known FAD-AOXs are located in the peroxisomes of methylotrophic yeasts such as *P. pastoris*, *Candida boidinii* or *Hansenula* sp. They play a key role in methanol metabolism and their native structure corresponds to a homooctamer. Long-chain alcohol oxidases (LCAOs) or fatty alcohol oxidases (FAOs) (EC 1.1.3.20) have also been described and isolated from alkane-producing yeasts, fungi and plants. In general, all FAD-AOXs share a conserved FAD-binding domain that contains both the GxGxxG/A sequence motif and a Rossmann fold. The C-terminal substrate binding domain is more variable and can accommodate different types of substrates. As part of their catalytic cycle, FAD-AOXs release H_2O_2, which, if accumulated, is harmful to the enzyme and must be eliminated. Catalases (EC 1.11.1.6) are widely used for this purpose. CROs are monocopper metalloenzymes and catalyze the formation of aldehydes by the two-electron aerobic oxidation of alcohols. No cofactors are involved in the catalytic mechanism of these enzymes. Most of the CROs characterized so far correspond to filamentous fungi [79,80]. Scheme 6 shows some examples of oxydations catalyzed by NCYs.

4.1. Alcohol Oxidation by NCYs

Whole cells and, more frequently, isolated enzymes from NCYs have been used for alcohol oxidation with different purposes. Among flavor compounds, aldehydes are a prominent class of molecules that are widely used as food additives and perfume ingredients. In particular, the so-called fatty aldehydes (C6 to C13 saturated aldehydes) play a central role in this field [80]. Studies on the chemical properties of natural volatile flavor compounds have shown that their antioxidant, anticancer, anti-inflammatory and anti-obesity activities may have potential applications in human health. AOXs from various microorganisms are capable of oxidizing alcohols to aldehydes. Focusing on unconventional yeasts, examples include the AOXs from the methylotrophic yeast *K. phaffii* with high activity on short-chain aliphatic alcohols, or a thermotolerant aryl alcohol oxidase from *Moesziomyces antarcticus* (syn. *Candida antarctica*) (*Ma*AAOX) that accepts a wide range of primary benzyl alcohols, aliphatic allylic alcohols and furan derivatives [81]. The AOX of *Ogataea* (*Hansenula*) *polymorpha* has some differences with respect to that of other methylotrophic yeasts, such as a higher optimum temperature, a wide range of optimum pH, and resistance to the inhibitory effect of chloride anion [79]. However, the use of free soluble AOX in industrial processes is not economically attractive due to its poor stability, and sometimes its immobilization has been necessary. Alcohol oxidase from the thermotolerant methylotrophic yeast *Ogataea thermomethanolica* (*OthAOX*) was covalently immobilized on barium ferrite ($BaFe_{12}O_{19}$) magnetic microparticles. The immobilized enzyme exhibited

improved thermostability and higher catalytic efficiency for the oxidation of methanol and ethanol than the free enzyme. It also retained its activity after successive batch cycles [82].

Studies with AOX from *P. pastoris* and other wild-type AOXs have shown that these enzymes have a higher affinity and reaction rate for methanol than for ethanol. Three-dimensional structural analyses have provided an explanation for this fact, showing that the substrate channel leading to the active site is narrow due to the presence of bulky aromatic side chains, mainly corresponding to residues of Phe98, Phe402 and Trp566. Considering the conservation of this channel structure between *P. pastoris* and the *H. polymorpha* enzyme, Li et al. (2024) determined by in silico studies that the Phe99 residue of the latter protein is the amino acid of the substrate channel with the greatest effect on enzymatic activity. These authors found that the F99V substitution resulted in a significant increase in enzyme activity and catalytic efficiency for ethanol due to an increase in channel size, which facilitates substrate access to the active site of the enzyme [83].

The overoxidation of aldehydes to carboxylic acids cannot be performed directly by these enzymes. In fact, in most cases, their observed oxidation is directly dependent on the propensity of the aldehyde to undergo hydration to form the corresponding geminal diol and the subsequent ability of the enzyme to accept the latter as a substrate [80].

Applications of biocatalytic oxidation of secondary alcohols are not common because they involve the destruction of chirality when sp3-hybridized alcohols are converted to sp2-hybridized carbonyl groups. However, stereoselective oxidation of a single enantiomer of the racemic alcohol is one way to obtain enantiomerically pure alcohols. Thus, whole cells of *C. albicans* CCT 0776 were employed to convert racemic 1-(4-substituted phenyl)-1,2-ethanediols to (*S*)-1-(4-substituted phenyl)-1,2-ethanediols in a one-pot, two-step process. First, the (*R*)-stereoisomers were completely oxidized to the corresponding substituted α-hydroxyacetophenones, which were later completely reduced to the (*S*)-1-(4-substituted phenyl)-1,2-ethanediols in good isolated yield and high enantiomeric excess (Scheme 6A) [84]. A similar transformation has been described with the unconventional yeast *C. parapsilosis*, which can perform efficient kinetic resolution of various racemic *sec*. alcohols: aliphatic and aromatic diols, α- and β-hydroxy esters, allylic alcohols and aryl ethanols. In these cases, whole cells and isolated enzymes were used (reviewed by Chadha et al., 2016) [85].

Hydroxylactones such as chlorolactone are intermediates in industrial processes for the manufacture of several statin drugs. The hydroxylactones with aliphatic side chains are also used as intermediates for flavor and fragrance lactones. The chemical oxidation of hydroxylactols to hydroxylactones using conventional chemical reagents is not selective, but it is possible to perform regioselective oxidation of the target substrates enzymatically. In this sense, Bartsch et al. (2020) described the utility of the enzyme ADHA, an ADH from *Candida magnoliae* DSMZ 70638, for this purpose [86]. Pure oxygen was used as the oxidant, and an engineered NAD(P)H oxidase from *Streptococcus mutans* was employed for cofactor recycling in this reaction; the process was scaled up to industrial pilot plant scale and was efficient and safe in some cases. However, the wild-type ADHA enzyme was not productive enough in chlorolactol oxidation, and an engineered enzyme was designed using a full-site saturation mutagenesis approach combined with a multi-parameter design. The optimized ADHA (ADHA-0398) showed higher oxidative activity (17-fold) and increased thermostability (26 °C) compared to the wild-type enzyme and can be used to develop an efficient chlorolactol oxidation process (Scheme 6B).

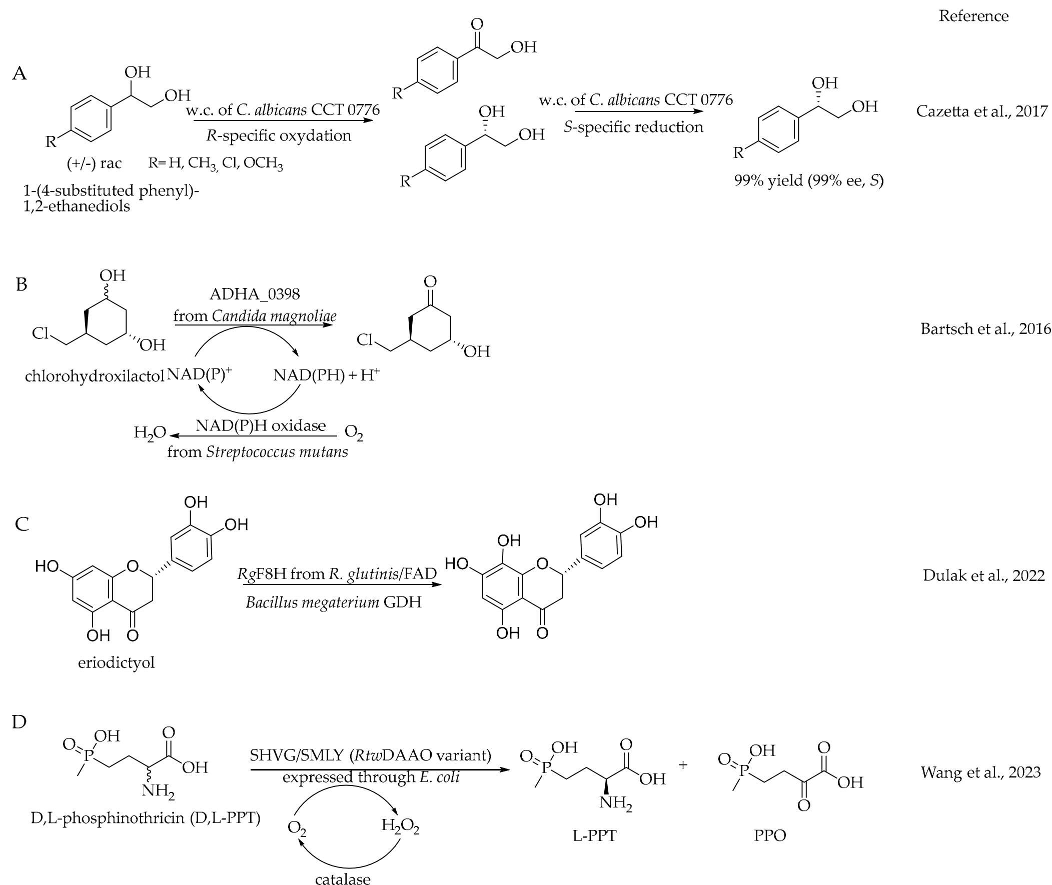
4.2. Other Oxidation Reactions Catalyzed by NCYs

Non-conventional yeasts can also carry out oxidations of several types of non-alcoholic substrates, although these are less common than those described above. Examples include the Baeyer–Villiger oxidation of ketones to esters or lactones with Baeyer–Villiger monooxygenases (BVMOs) or the oxygenation of heteroatoms (sulfur, nitrogen, phosphorus, boron or selenium) by the same enzymes [87]. Chiral sulfoxides are very important building blocks because they are used as chiral auxiliaries in organic synthesis and also in the produc-

tion of pharmaceuticals such as esomeprazol or armodafinil. Biocatalytic sulfoxidation is an important alternative to the chemical synthesis of chiral sulfoxides, although it can also lead to the undesired oxidation of the sulfoxide to the achiral sulfone. Several putative BVMOs were identified from the yeast *Y. lipolytica* and recombinantly expressed in *E. coli* [87]. One of them, YMOA, was not active with any of the ketone substrates tested but showed activity and high stereoselectivity in the oxidation of sulfides to sulfoxides. Unfortunately, YMOA was also able to oxidize sulfoxides to sulfones by removing their chirality. A mutational study of YMOA yielded variants with increased and decreased sulfone yields, indicating relevant amino acid positions for controlling sulfoxidation activity, which may provide insight into how to avoid sulfone production in BVMO-catalyzed reactions. The unique properties of YMOA and a phylogenetic analysis placed this enzyme in a distinct branch from previously characterized BVMOs, and it is the first example of a new class of fungal monooxygenases [87].

It is also worth mentioning that non-conventional yeasts contain enzymes capable of regioselective hydroxylation of phenolic compounds. Among these compounds, flavonoids are of particular interest because of their antioxidant capacity. Dulak et al. (2022) identified the enzyme in *Rhodotorula glutinis* KCh735 that is responsible for the C-8 hydroxylation of flavones and flavanones (Scheme 6C). This protein, *Rg*F8H, belongs to the family of flavin-dependent monooxygenases, and these authors showed that the form expressed and purified from *E. coli*, in combination with a GDH for cofactor regeneration, acts on several compounds. The C8-hydroxylase activity of this enzyme is associated with the presence of a C-7 hydroxyl group [88].

In this review, we cannot neglect the D-amino acid oxidases (DAAOs), flavoenzymes that catalyze the oxidative deamination of the D-isomer of polar, aliphatic and aromatic amino acids with no side-chain charge to the corresponding alpha-keto acid and ammonium (reviewed in Pollegioni et al., 2008) [89]. Because of their stereoselectivity and the wide variety of substrates on which they can act, they are very interesting enzymes for the synthesis of drugs and fine chemicals, including the chiral herbicide phosphinothricin (L-PPT), with significant market potential. Microbial DAAOs have interesting kinetic properties that have made them the subject of interest in recent years. DAAO variants of the unconventional yeast *Rhodotorula taiwanensis* (*Rtw*DAAO) with improved catalytic potential towards D-PPT were created by protein engineering. For example, a semirational design approach used to generate a mutation library based on tunnel-pocket engineering allowed obtaining the variant M3rd-SHVG, which exhibited a >2000-fold increase in relative activity, but its poor thermostability limits its further industrial application [90]. This variant was later used as a starting strain to obtain a new strain with improved thermal stability. A detailed computational design approach for protein stability engineering was performed, resulting in positive substitutions at specific sites (A43S, T45M, C234L, E195Y). The new variant, SHVG/SMLY, showed a significant synergistic effect, which was also confirmed experimentally. The mutant expressed in *E. coli* showed an apparent melting temperature 10 degrees higher than that of the parent enzyme and a half-life at 50 °C 20 times higher. The SHVG/SMLY form of the enzyme was able to perform the asymmetric catalytic oxidation of D,L-PPT with excellent enantioselectivity in shorter times than the parent form of the enzyme (Scheme 6D) [91].



Scheme 6. Oxidation reactions biocatalyzed by whole cells (w.c.) or isolated enzymes from NCYs. (**A**) Adapted from [84]; (**B**) Adapted from [86]; (**C**) Adapted from [88]; (**D**) Adapted from [91].

5. Nitrile Hydrolysis

Nitriles are widespread in nature, and synthetic nitriles are an important group of chemicals used as starting materials in industry. Their conventional chemical hydrolysis requires harsh reaction conditions and leads to the formation of unwanted by-products and large amounts of waste. Enzymatic hydrolysis of nitriles is an accepted method for obtaining a wide range of useful amides and carboxylic acids (reviewed in [92–94]). Two types of enzymes can be involved in nitrile catabolism: nitrilases (EC 3.5.5.1), which convert nitriles directly to the corresponding carboxylic acids and NH_3, and nitrile hydratases (NHases; EC 4.2.1.84), which catalyze the formation of the corresponding amides from nitriles. Amidases (EC 3.5.1.4) can then hydrolyze the amides to carboxylic acids and NH_3.

Nitrilases and amidases belong to a superfamily found in both prokaryotes and eukaryotes. Despite differences in sequence identity, the superfamily is characterized by having a homodimeric building block with an αββα–αββα sandwich fold and an active site containing highly conserved residues (Cys, Glu, and Lys). Their high chemical specificity and frequent enantioselectivity make them attractive biocatalysts for the production of fine chemicals [92–94].

Nitrile Hydrolysis by Whole Cells of NCYs

More than 60 nitrile metabolizing yeasts, including species of *Candida*, *Pichia*, *Saccharomyces*, *Hanseniaspora*, *Debaryomyces*, *Geotrichum*, *Williopsis*, *Torulopsis*, *Exophiala*, *Kluyveromyces*, *Aureobasidium*, *Cryptococcus*, and *Rhodotorula*, have been isolated from cyanide treatment bioreactors, fermented foods, and the soil. Most yeasts have nitrilohydratase and amidase activities, while a few, such as *C. glabrata* (previously *Torulopsis candida*) and *Exophiala oligosperma* R1, have only nitrilase activity. *Cryptococcus* sp. UFMG-Y28 has nitrilase activity

in addition to nitrile hydratase and amidase. Since, in most cases, two intracellular enzymes (nitrile hydratase and amidase) are often involved, it is very convenient and economical to perform the transformation reactions using free or immobilized whole cells [95].

Gong et al. [96] found that nitrilase activity and biomass formation are influenced by culture composition (e.g., carbon and nitrogen sources, the presence of inducers and metal ions) and bioconversion conditions (e.g., temperature, pH, enzyme modifiers, and organic solvents). These authors compile information on some of these nitrile-hydrolyzing organisms or enzymes and their uses. For example, one possible application is the conversion of α-amino- and α-hydroxynitriles into chiral amino acids and α-hydroxycarboxylic acids, which are products of interest to the chemical industry. In this sense, cells from the strain *T. candida* GN405 were used to synthesize optically active α-hydroxy acids from D,L-α-hydroxynitrile compounds. This biotransformation tends to occur under acidic conditions, where hydroxynitriles are more stable. Also of interest is the nitrilase system of *Cryptococcus* sp. UFMG-Y28, which could be induced in the presence of benzonitrile as the sole nitrogen source. This strain also produces nitrile hydratase (NHase) and amidase enzymes when grown on other nitriles as nitrogen sources [96].

As mentioned above, the black yeast *Exophiala oligosperma* R1 is another nitrilase microorganism. It was isolated in a medium at pH 4.0, and its growth conditions were optimized for the production of cellular material and the induction of nitrile-converting activity. Cell-free extracts of this strain were also used to isolate acids corresponding to the hydrolysis of phenylacetonitrile and various substituted derivatives. Whole cells of *E. oligosperma* R1 showed acid tolerance and significant long-term stability, which could be of biotechnological interest. Indeed, the reaction rates were almost constant over a pH range of 1.5 to 9 [97].

Nitrile hydratase and amidase activity of *Kluyveromyces thermotolerans* MGBY 37 yeast was reported using resting cells. In this strain, the hydrolysis of amide to acid is mediated by two amidases, one of which is constitutive and has an affinity for N-heterocyclic aromatic, unsaturated and saturated aliphatic amides, while the other is inducible, especially by formamide, and has an affinity for aromatic amides [98].

Several strains of *Candida guilliermondii* (teleomorph *M.* (*P.*) *guilliermondii*) have shown nitrile hydrolyzing enzyme activity. *C. guilliermondii* UFMG-Y65, isolated from a gold mine, was able to hydrolyze acrylonitrile and benzonitrile in media at pH 7.0–8.0 and a temperature of 25–30 °C. This activity was shown to be inducible and intracellular [99]. The yeast *M. guilliermondii* CGMCC12935 was isolated and identified from environmental samples using 3-hydroxypropionitrile as the sole nitrogen source. This microorganism contains a nitrile hydrolyzing enzyme with broad substrate specificity for nitriles, particularly 3-hydroxypropionitrile, aminoacetonitrile and 3-cyanopyridine. This strain has been shown to be a promising biocatalyst for the sustainable production of valuable 3-hydroxypropionic acid [100]. An extremophilic microorganism with nitrilase activity and very poor amidase activity was recently reported by Serra et al. The salt-resistant yeast isolated from the sea floor *M. guilliermondii* LM2 (UBOCC-A-214008), was able to hydrolyze several nitriles with high molar conversions. Its activity was induced by cyclohexanecarbonitrile and showed interesting enantioselectivity towards racemic compounds and high activity towards aromatic substrates. This yeast was used in media with different salinities and was highly active up to 1.5 M NaCl concentration. The hydrolysis of nitriles was also efficient in seawater-based media [101].

6. Reactions Catalyzed by Lipases

Lipase enzymes are serine hydrolases of the triacylglycerol ester hydrolase family (EC 3.1.1.3). They catalyze the hydrolysis of the ester linkage of long-chain mono-, di-, and triglycerides to fatty acids and glycerol, but they can accept a wide range of compounds as substrates ([102] and references therein). Lipases differ from esterases (EC 3.1.1.1) in their ability to hydrolyze triglycerides at the lipid–water interface. They can also exhibit other activities such as interesterification, esterification, transesterification, aminolysis,

alcoholysis, etc., and have useful properties such as easy availability and the capacity to catalyze reactions in heterogeneous media and in organic solvents. In fact, the same enzyme is capable of catalyzing the hydrolysis of esters in water and their synthesis in an organic solvent. Due to their versatility in catalyzing different reactions, this subsection will review these enzymes and some of the reactions that they can catalyze and that are relevant in the industry. In fact, lipases are a versatile choice for applications in the food, flavoring, pharmaceutical, cosmetic and other industries [102,103].

Microbial lipases are more valuable than plant and animal lipases because they are easy to genetically manipulate, can be produced in high yields using inexpensive culture media, and have high substrate specificity and stability in an organic solvent [103]. Various yeasts and filamentous fungi are microorganisms recognized as sources of extracellular lipases: *Candida* spp., *Y. lipolytica*, *Rhodotorula* spp., *Pichia* spp., *Saccharomycopsis crataegensis*, *Torulospora globosa*, *Trichosporon asteroides*, *Mucor* spp. and *Aspergillus* spp. [104].

The solvent used in the lipase reactions can affect their efficiency. In general, their activity decreases in polar organic solvents, but non-polar organic solvents also affect them by inducing excessive rigidity. Therefore, it is essential to dispose of lipases that are solvent-tolerant to use more suitable solvents. In this regard, ionic liquids or deep eutectic solvents have been gradually introduced for various lipase-catalyzed reactions, and well-known strategies such as immobilization, screening of novel enzymes and protein engineering have been developed to improve the catalytic properties of these enzymes [105,106]. Undoubtedly, lipases produced by extremophiles are of interest for industrial bioprocessing due to their higher stability in organic solvents and at high temperatures.

6.1. Ester Hydrolysis by Isolated Wild-Type and Mutant Lipases

CALB is a commercial lipase produced by the yeast *C. antarctica*. It exhibits high substrate specificity and enantio-, regio- and chemoselectivity in the resolution of secondary substrates such as alcohols and amines. CALB has been widely used in the pharmaceutical industry for the synthesis of drug delivery carriers, anticancer drugs, prodrugs or vitamins. The use of CALB avoids the risk of potential toxicity of conventional chemical catalysts and shows satisfactory results. In the resolution of chiral drugs, the stereoselectivity and regioselectivity of CALB make it a natural choice for the production of high-purity enantiomers [107].

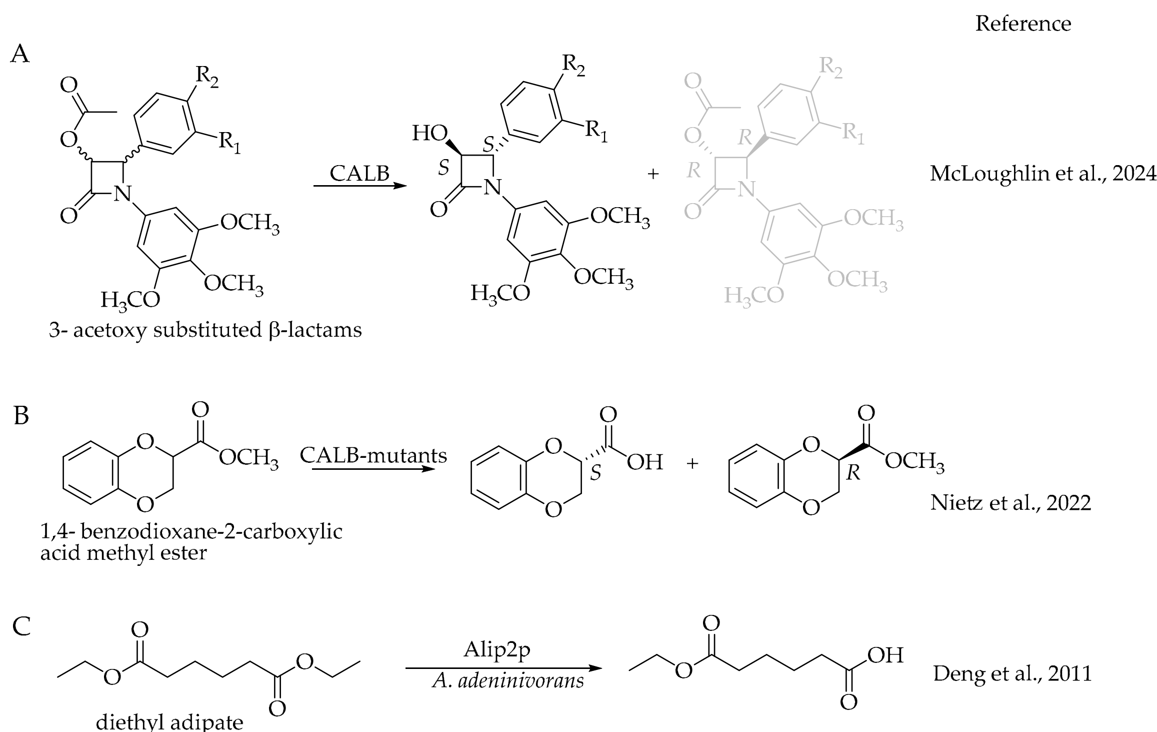
Some recent specific applications of this lipase are discussed here (Scheme 7). CALB has been used in the kinetic resolution of 3-acetoxy-substituted β-lactams bearing a variety of para and meta B-ring substitutions. Enantioselective hydrolysis of 3-acetoxy racemates in the presence of methanol led to the 3-hydroxyl enriched 3*S* and 4*S* eutomers with high ee without opening the β-lactam ring (Scheme 7A) [7]. Another application of CALB is the synthesis of chiral motifs of 2,3-dihydro-1,4-benzodioxane, which are widely used in various drugs such as prosympal, dibozane, piperoxan or doxazosin. The screening of 38 CALB covariant residues allowed to find two mutants (A225F and A225F/T103A) that catalyze the kinetic resolution of the substrate 1,4-benzodioxane-2-carboxylic acid methyl ester (Scheme 7B). The best results allowed for optimal resolution and were obtained using 20% n-butanol as cosolvent. Structure analysis of A225F and A225F/T103A revealed that mutation sites 225 and 103 are located far from the active center, implying that covariant amino acids interacting remotely with the substrate also regulate enzyme catalysis and provide new insight into protein evolution. These mutants thus provide a new environmentally benign and promising biosynthesis method for chiral 2,3-dihydro-1,4-benzodioxane motifs [108].

In the search for new lipases with interesting properties for ester hydrolysis, non-traditional yeasts continue to be analyzed. The genome of the thermotolerant and osmotolerant yeast *Blastobotrys* (*Arxula*) *raffinosifermentans* (*adeninivorans*) LS3 was screened using a computed set of concatenated orthologs and a super-tree approach for putative lipases with the ability to produce monoesters. The corresponding genes were overexpressed in the yeast itself, and crude extracts or culture supernatants were screened. The highest

activities and monoethyl adipate production yields were found for the intracellularly localized enzyme encoded by the *A. adeninivorans* lipase 2 gene (*ALIP2*). The putative lipase Alip2p was purified and characterized, and its potential for the production of this and other monoesters by hydrolysis of their symmetrical dicarboxylic acids was evaluated. In the case of monoethyl adipate, commonly used as a monomeric spacer for functional polymers, a 96% yield was obtained in only 30 min (Scheme 7C). None of the commercial lipases tested during this period were able to produce higher yields of monoethyl adipate [109].

6.2. Ester Synthesis by Wild-Type and Mutant Lipases

Short and medium alkyl esters are important flavor and aroma enhancers used in the food industry. These molecules are typically synthesized from acids and alcohols with two to eight carbon atoms and are obtained by conventional synthesis using toxic solvents and catalysts with no substrate selectivity and with the formation of by-products that must be removed. Wax esters are found in natural sources (e.g., jojoba oil, carnauba wax or sheep wool) and have a high economic relevance as they can be used in areas such as cosmetics, printing inks, lubricants and food (Figure 6). They consist of long-chain fatty acids and alcohols linked by an ester bond. These esters are also mainly produced by chemical synthesis. Flavor and wax esters can also be obtained from vegetable sources, but this requires large amounts of raw materials and is expensive for large-scale applications. As an alternative, enzymatic synthesis using lipases in a non-aqueous medium could be of interest to both. For example, lipase from *Candida* sp. 99–125 was used for cetyl oleate synthesis in a solvent-free medium [110], and Novozyme 435 from *C. antarctica* enabled cetyl octanoate synthesis in supercritical carbon dioxide [111].



Scheme 7. Some hydrolysis reactions catalyzed by lipases from unconventional yeasts. (**A**) Adapted from [7]; (**B**) Adapted from [109]; (**C**) Adapted from [110].

Lipases obtained by solid-state fermentation (SSF) have the advantage of using agro-industrial wastes and by-products as a support and substrate for microbial growth. In this regard, lipase from the non-conventional yeast *Y. lipolytica* produced by SSF using soybean meal has been applied to the synthesis of various flavor esters and biolubricant wax esters, achieving high substrate conversions for ethyl octanoate, cetyl stearate and stearyl

palmitate synthesis in the presence of non-polar solvents at 38 °C with 10–15% (*w*/*v*) of biocatalyst [112]. *Candida rugosa* lipase immobilized on the hydrophobic and macroporous polypropylene polymer Accurel MP 1000 (AMP) showed high values of hydrolytic and specific activity in an organic medium. This enzyme was used in an effective wax ester synthesis with good conversions. In addition, the immobilized enzyme showed enhanced stability and recyclability [113].

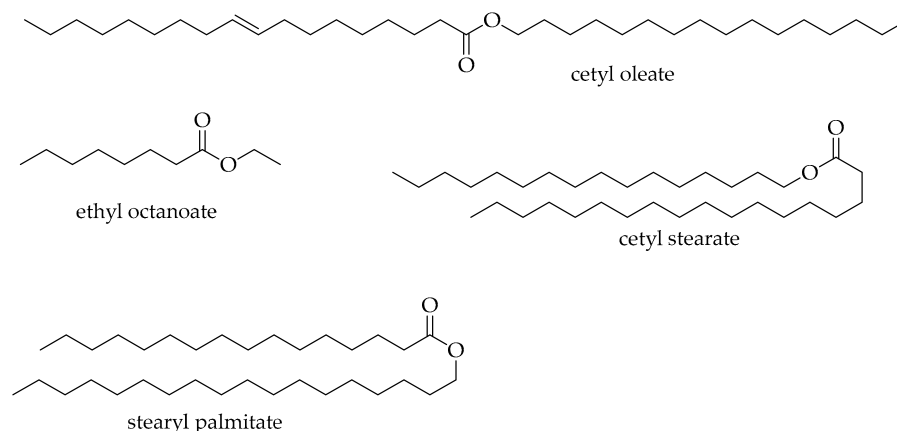


Figure 6. Wax esters synthetized by lipases.

Chiral tertiary alcohols are important organic compounds valuable as pharmaceutical agents or intermediates. Kinetic resolution by enantioselective esterification could be an attractive approach for their preparation. However, their complex structure and steric hindrances compared to primary and secondary alcohols make their production difficult. Lipase A from *C. antarctica* (CALA) catalyzes the enantioselective esterification of several tertiary alcohols with excellent enantioselectivity, but the wild-type enzyme shows moderate activity. Recently, the application of rational protein engineering to enlarge the active pocket for the sterically demanding tertiary alcohols was used to improve the activity of CALA. After docking studies, seven potential positions were replaced by smaller amino acids or those whose orientation would create more space in the active pocket. The mutants were expressed in *P. pastoris* X-33 on a preparative scale, immobilized on Lewatit® supports to provide a water-free environment during the reaction, and tested against a tertiary alcohol model substrate using vinyl *n*-butyrate as an acyl donor in diisopropyl ether. Of particular interest were the double mutant V278S and S429G, which showed 2–4 times higher conversions than wild-type CALA and excellent selectivity [114].

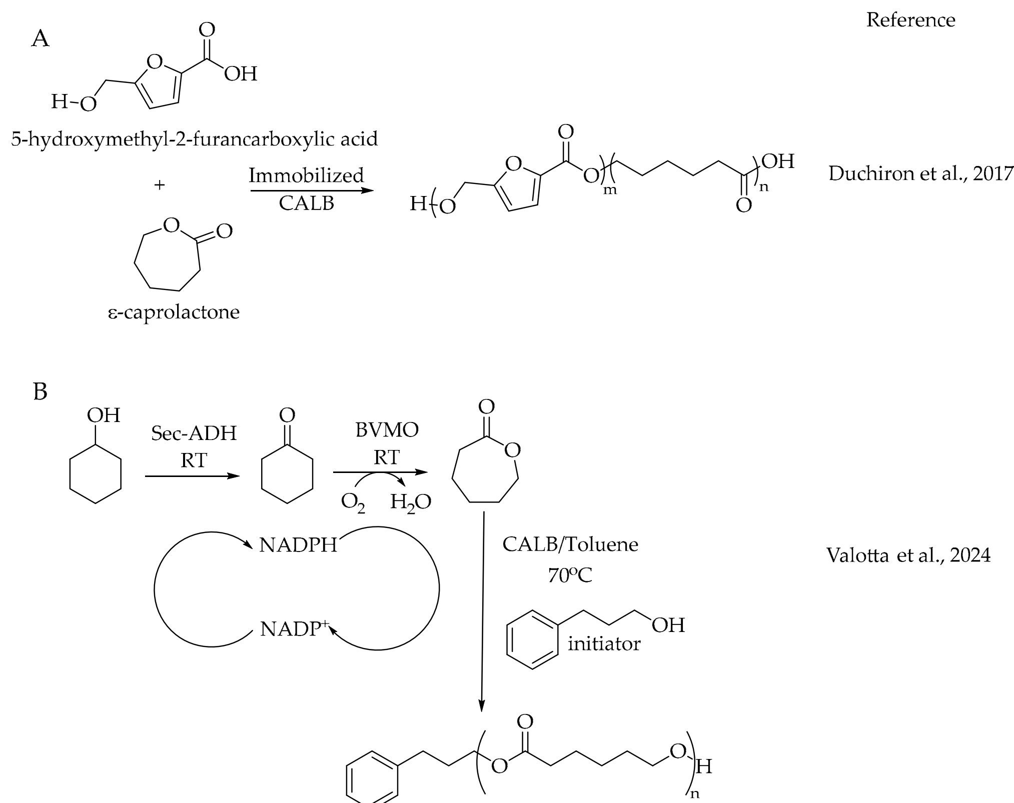
Most enzymes have optimal activity within a narrow window (20 to 45 °C) that is not suitable for industrial processes, and the engineering of thermostable lipases from biological sources for use in industrial reactors is an important goal. *Y. lipolytica* lipase LIP2 is used in many industrial applications as a biocatalyst for hydrolysis and esterification reactions. However, this enzyme is sensitive to thermal and interfacial denaturation and only works under mild conditions, so improving the thermostability of LIP2 is highly desirable. A rational design approach based on identifying the cause of the structure–function relationship of the enzymes was used to obtain a thermostable mutant of LIP2 [115]. In general, thermophilic enzymes are more rigid and have lower Root Mean Square Deviation (RMSD) values than their mesophilic counterparts. Based on this hypothesis, stabilization strategies based on the introduction of disulfide bonds, salt bridges or the enhancement of intramolecular interactions will result in lower RMSD values of an enzyme. Molecular dynamics simulations at different temperatures are necessary to predict the weak points of an enzyme and to verify its rigidity. The application of this method to LIP2 allowed the identification of the most variable side chains, which are likely to be thermally sensitive. Residues S115 or V213 were selected for mutations to improve the thermostability of the enzyme, and, following the proline rule, the target residues were replaced by prolines and

the stability of the mutants was validated. The V213P mutant was found to have higher thermostability showing an optimum temperature of about 5.0 °C higher than the wild type [115].

6.3. Ring-Opening Polymerization (ROP) Catalyzed by Lipases from NCYs

Poly(ε-caprolactone) is a polymer with good mechanical properties, biocompatible and fully biodegradable. These properties make it very useful, for example, in the manufacture of controlled-release drugs. However, biodegradable polyester has some drawbacks, such as poor thermal properties and low resistance to chemical solvents. The most conventional synthesis of these polymers consists of lactone ring-opening polymerization (ROP) catalyzed by organometallic compounds, but they are known for their cytotoxicity to cellular systems [116]. In addition, they are often difficult to remove from polymeric products, which could be detrimental to biomedical applications; enzyme-catalyzed ROP is a promising tool to avoid these problems. Lipases, particularly *C. antarctica* lipase B (CALB), have been used in a variety of polymer-forming reactions [116] (Scheme 8).

Todea and colleagues (2019) obtained cyclic and linear copolymers of ε-caprolactone and 5-hydroxymethyl-2-furancarboxylic acid (Scheme 8A). This material has improved thermal properties and also biodegrades faster than ε-caprolactone polymers due to the insertion of the furan units. Three commercially available immobilized lipases were used for its synthesis: Novozyme 435 (lipase B from *C. antarctica* immobilized on acrylic resin), Lipozyme *Cal*B (from *C. antarctica*) and GF-*Cal*B-IM (lipase B from *C. antarctica* immobilized on microporous ion exchange resin). Among them, the latter gave the best results at low water content. Moreover, it was stable after four batch reaction cycles, although a gradual decrease in the average molecular weight of the polymer was observed [117].



Scheme 8. Lactone ring-opening polymerization (ROP) catalyzed by lipases from NCYs. (**A**) Adapted from [116]; (**B**) Adapted from [118].

Recently, Valotta et al. (2024) proposed an elegant three-step semi-continuous production of polycaprolactone using a fully biocatalytic process (Scheme 8B). First, caprolactone

is produced in batches from cyclohexanol by a coenzymatic cascade involving an alcohol dehydrogenase (ADH) using $NADP^+$ as a cofactor and a Baeyer–Villiger monooxygenase (BVMO) in the presence of molecular oxygen. Second, the continuous extraction of caprolactone into an organic solvent required for the polymerization step was optimized. Finally, the ring-opening polymerization of caprolactone to polycaprolactone was catalyzed by CALB in the presence of 3-phenylpropanol as an initiator [118].

7. Other Reactions Catalyzed by NCYs

7.1. C-C Bond Formation: R-(−)-Phenylacetylcarbinol Synthesis

The formation of stereoselective C–C bonds is one of the most important transformations in organic synthesis. Pyruvate decarboxylase (PDC, E.C. 4.1.1.1), a key thiamine diphosphate (ThDP)-dependent enzyme, plays a fundamental role in alcoholic fermentation by catalyzing the decarboxylation of pyruvate to acetaldehyde. When benzaldehyde or other related aldehydes are present in the medium, the activated acetaldehyde binds to them, leading to the stereoselective synthesis of 2-hydroxyketones. Condensation between benzaldehyde and pyruvate catalyzed by yeast produces *R*-(−)-phenylacetylcarbinol (1-hydroxy-1-phenylpropan-2-one, (*R*)-PAC), which is a chiral precursor of the drug ephedrine (Scheme 9A). This was one of the first biotransformation processes to be commercialized using whole cells. Andreu and del Olmo (2014) reported the use of whole cells of *S. etchellsii* as biocatalysts in acyloin condensation. The conditions for efficient biotransformation of benzaldehyde and minimization of by-product formation were optimized (pH of the reaction medium, use of additives, temperature, time, substrate concentration and dosage) [119]. Later, these authors proposed the use of seawater as a mild solvent and demonstrated several improvements compared to the use of freshwater, such as a higher conversion of the starting material to (*R*)-PAC with minimal formation of by-products, the possibility to increase the benzaldehyde load in the reaction medium by at least 2-fold, and the maintenance of cell activity after several rounds of recycling, making (*R*)-PAC production a simple and economical process [120].

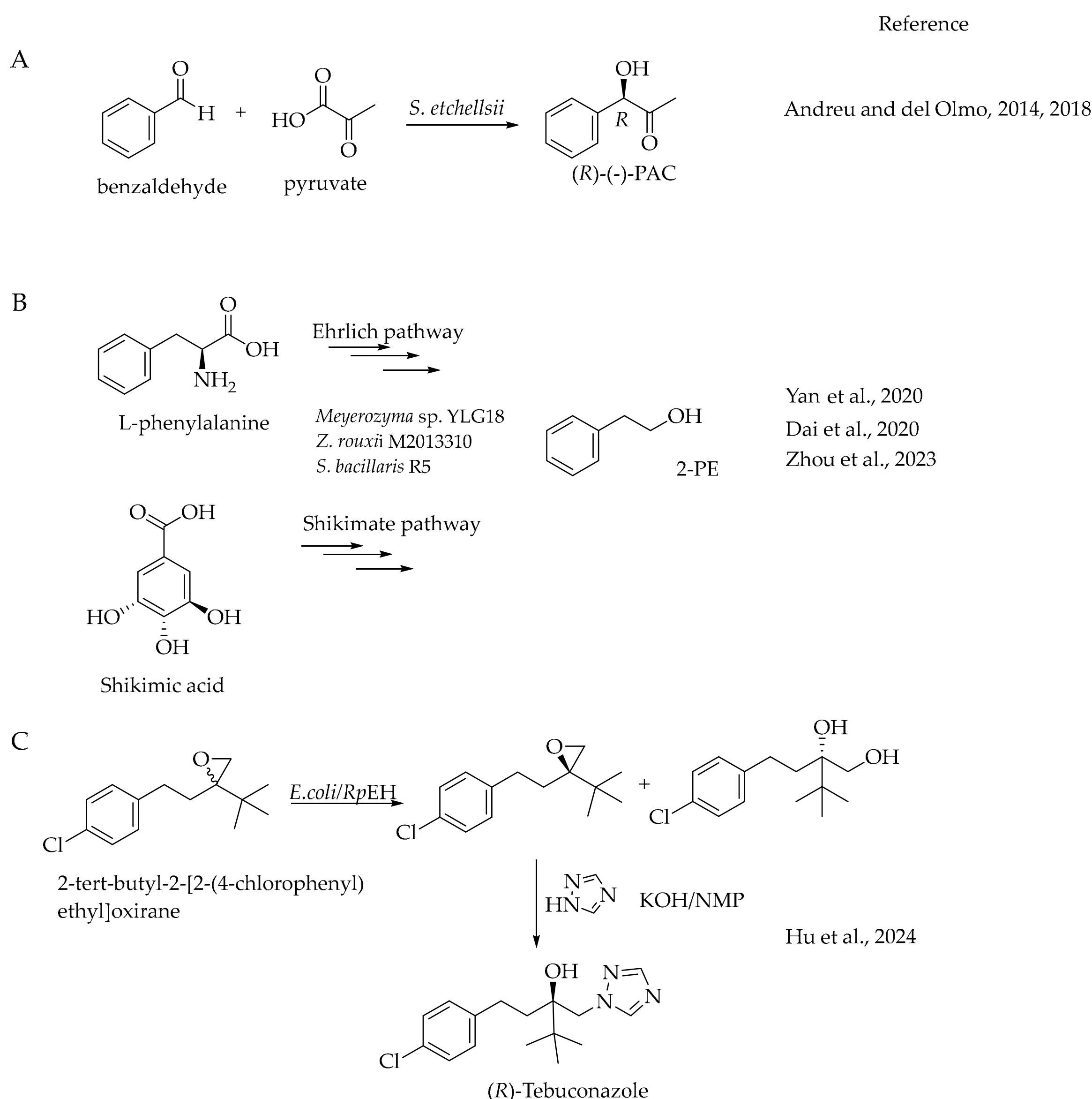
7.2. Phenylethanol Production

2-Phenylethanol (2-PE) is an aliphatic alcohol widely used in the cosmetic and food industries for its rose-like aroma, and its derivatives, such as phenylethyl acetate and *p*-hydroxyphenylethanol, are used in the pharmaceutical and fine chemical industries ([121–123] and references therein). 2-PE can be extracted from essential oils of plants, but its concentration is extremely low. It can also be chemically synthesized, but its quality is severely compromised by the harsh conditions and toxic reagents used. Many wild-type microorganisms, including some unconventional yeasts (*K. marxianus*, *K. lactis*, *Pichia fermentans*, *Candida glycerinogenes* and *Z. rouxii*), have been shown to be capable of producing 2-PE. This alcohol can be obtained either from L-phenylalanine via the three-step catalysis of the Ehrlich pathway or de novo from glucose via the multistep shikimate pathway (Scheme 9B). The main disadvantage of these biocatalytic strategies is that the lipophilic property of 2-PE makes it toxic to cells, as the lipid membrane structure is a preferential binding target [121–123]. It was found that *Meyerozyma* sp. strain YLG18, isolated using L-Phe as the sole nitrogen source, was able to produce 2-PE via both the Ehrlich and shikimate pathways and had a higher 2-PE tolerance level than other strains. After determining the influencing factors, optimizing the process using Response Surface Methodology (RSM) and reducing the toxicity of 2-PE with In Situ Product Removal (ISPR) techniques, strain YLG18 was able to produce more 2-PE [121]. Similar results were obtained with the allodiploid yeast M2013310, isolated from chili sauce and closely related to *Z. rouxii*. Investigation of the pathway by which this strain synthesizes 2-PE revealed that it can also use either the shikimate or Ehrlich pathway [122]. On the other hand, *Starmerella bacillaris* (syn. *Candida zemplinina*) strain R5, isolated from pear pellets, could also synthesize 2-PE from L-phe or glucose via the Ehrlich or shikimate pathways, although the biotransformation of L-phe was more efficient than that of glucose. Strain

R5 also showed tolerance to high concentrations of 2-PE [123]. These results indicate the potential of all of these strains for the production of 2-PE.

7.3. Epoxyde Hydrolysis

Epoxide hydrolases (EHs, EC 3.3.2.-) catalyze the asymmetric hydrolysis of *rac*-epoxides and have proven useful in the preparation of optically pure epoxides and/or diols. Tebuconazole (2-*tert*-butyl-2-[2-(4-chlorophenyl)ethyl]oxirane) is a triazole fungicide widely used in agriculture as a racemic mixture. However, (*R*)-tebuconazole is the most active enantiomer, and the recombinant epoxide hydrolase from *Rhodotorula paludigensis* (*Rp*EH) expressed in *E. coli* BL21 (DE3) was used for its preparation (Scheme 9C). This enzyme showed enantiopreference for the hydrolysis of the (*S*)-enantiomer of 2-*tert*-butyl-2-[2-(4-chlorophenyl)ethyl]oxirane, the bulky oxirane precursor of tebuconazole. Optimization of the reaction conditions using whole cells of *E. coli*/*Rp*EH allowed the kinetic resolution of the racemic mixture, and the (*R*)-enantiomer was prepared on a gram scale with excellent enantioselectivity [124].



Scheme 9. Biocatalytic preparation of (*R*)-(-)-PAC, 2-PE and tebuconazole. (**A**) Adapted from [119,120]; (**B**) Adapted from [121–123]; (**C**) Adapted from [124].

Unconventional yeasts or their natural or modified enzymes produce other key compounds important to the pharmaceutical and chemical industries through other reactions not discussed in the previous sections. Some examples are given in Table 1.

Table 1. Some less common NCY-catalyzed reactions.

Enzyme/Yeast	Reaction	Reference
Dye-decolorizing peroxidase of *Pleurotus sapidus* (*Psa*POX)	Cleavage of alkenes for the production of aldehydes with olfactory properties for the fragrance and flavor industry	[125,126]
Genetically modified *Y. lipolytica*	Production of phospholipids (PL) used in replacement therapy for enrichment damaged cell membranes, food, cosmetic and pharmaceutical products and as lipid liposome components	[127]
Genetically modified *Y. lipolytica*	Synthesis of Gastrodin (4-hydroxybenzyl alcohol-4-*O*-β-D-glucopyranoside), which is widely used in the treatment of neurogenic and cardiovascular diseases	[128]
Recombinant *P. pastoris*	Production of malic acid from methanol. Malic acid is one of the top 12 building block chemicals, used extensively in the chemical, pharmaceutical, food, and agricultural industries	[129]
Rhodotorula bogorensis	Production of sophorolipids, glycolipid biosurfactants that have good biodegradability and low ecotoxicity. They are produced based on renewable resource substrates and are useful for various industries	[130]
Immobilized Phenylalanine ammonia-lyase (*Rg*Pal) from *R. glutinis* JN-1	Production of optically pure D-phenylalanine through asymmetric resolution of the racemic DL-phenylalanine	[131]
R. mucilaginosa UFMG-CM-Y3647	Ferulic acid (FA) consumption and conversion into aroma compounds such as vanillin, vanillic acid (VA), and 4-vinylguaiacol (VG)	[132]
C. antarctica lipase B (CALB)	"Click" Michael addition catalyzed between fluorescein *o*-acrylate and thiol-functionalized poly(ethylene glycol)s	[133]
CrS enoate reductase from *Thermus scotoductus* SA-01	Synthesis of (1*R*,3*R*)-3-hydroxycyclopentanemethanol (intermediate of the antiviral agent carbocyclic-ddA)	[134]

8. Conclusions and Future Prospects

Although *S. cerevisiae* is the traditional yeast used in a variety of biocatalytic processes, non-conventional yeasts have also proven to be very useful tools. Many NCYs have been isolated in extreme and uninhabitable environments for humans, such as cold glaciers, polar regions, cold seafloors, deserts, acidic or alkaline environments, etc. These extremophilic microorganisms have a phenotype adapted to these extreme conditions, which are often similar to the harsh conditions in which chemical reactions take place. Sometimes, they are able to catalyze reactions in non-aqueous environments, in solvent mixtures, at extremely high pressures and temperatures, in acidic and alkaline pH, etc., and for this reason, they represent an excellent alternative for use as industrial biocatalysts, either as whole cells or by using their isolated enzymes. Sometimes, their enzymes have been genetically engineered to produce mutant versions with improved properties. In fact, these modified enzymes have made it possible to further increase the range of reaction temperature, pH and substrate loading, as well as the possibility of obtaining stereocomplementary compounds to those provided by wild-type enzymes. In addition, several NCYs have been classified as GRAS microorganisms, making them useful in the food and pharmaceutical industries.

In this review, the main reactions of industrial importance that can be catalyzed by these microorganisms have been presented. Their advantages over traditional chemical synthesis methods and also over traditional yeasts have been demonstrated, as well as their promising potential as catalysts in more sustainable and environmentally friendly processes.

The main limitation to the use of suitable unconventional yeasts in biocatalysis is their discovery and knowledge of their molecular and physiological properties. The vast majority

of existing yeast species have not yet been isolated or identified, so yeast biodiversity can still be considered an untapped resource for biocatalytic applications. Many habitats on Earth have not yet been sampled for yeasts. For example, some cold regions and some natural forests harboring specific yeast communities need to be studied before they may disappear due to climate change and deforestation. On the other hand, the study of isolated unconventional yeasts is an emerging field thanks to the recent technological revolution in synthetic biology, genomic sequencing, OMIC technologies, and CRISPR/Cas9. However, for most NCYs, metabolic engineering tools are still lacking or underdeveloped due to the limited availability of analysis and manipulation methods such as in silico models, well-characterized genetic components and optimized genome engineering tools [26]. As the genome sequence of many of these yeasts and information about their proteome become available, advances in their genetic manipulation will be made in the coming years, which may further increase their potential.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/catal14110767/s1; Table S1: Main biotechnological applications of non-*Saccharomyces* ascomycetous yeast species [18,24,25,135–138]; Table S2: Main biotechnological applications of non-*Saccharomyces* basidiomycetous yeast species [18,24,25,135–137].

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References

1. Buchholz, K.; Collins, J. The Roots—A Short History of Industrial Microbiology and Biotechnology. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 3747–3762. [CrossRef]
2. Sheldon, R.A.; Pereira, P.C. Biocatalysis Engineering: The Big Picture. *Chem. Soc. Rev.* **2017**, *46*, 2678–2691. [CrossRef] [PubMed]
3. Jiang, W.; Fang, B. Synthesizing Chiral Drug Intermediates by Biocatalysis. *Appl. Biochem. Biotechnol.* **2020**, *192*, 146–179. [CrossRef] [PubMed]
4. Hernáiz, M.J.; Alcántara, A.R.; García, J.I.; Sinisterra, J.V. Applied Biotransformations in Green Solvents. *Chem. Eur. J.* **2010**, *16*, 9422–9437. [CrossRef]
5. Ni, Y.; Holtmann, D.; Hollmann, F. How Green Is Biocatalysis? To Calculate Is To Know. *ChemCatChem* **2014**, *6*, 930–943. [CrossRef]
6. Alcántara, A.R.; Domínguez De María, P.; Littlechild, J.A.; Schürmann, M.; Sheldon, R.A.; Wohlgemuth, R. Biocatalysis as Key to Sustainable Industrial Chemistry. *ChemSusChem* **2022**, *15*, e202102709. [CrossRef]
7. McLoughlin, E.C.; Twamley, B.; O'Boyle, N.M. *Candida antarctica* Lipase B Mediated Kinetic Resolution: A Sustainable Method for Chiral Synthesis of Antiproliferative β-Lactams. *Eur. J. Med. Chem.* **2024**, *276*, 116692. [CrossRef] [PubMed]
8. Garzón-Posse, F.; Becerra-Figueroa, L.; Hernández-Arias, J.; Gamba-Sánchez, D. Whole Cells as Biocatalysts in Organic Transformations. *Molecules* **2018**, *23*, 1265. [CrossRef]
9. Hancu, G.; Modroiu, A. Chiral Switch: Between Therapeutical Benefit and Marketing Strategy. *Pharmaceuticals* **2022**, *15*, 240. [CrossRef]
10. Patel, R.N. Biocatalysis for Synthesis of Pharmaceuticals. *Bioorg. Med. Chem.* **2018**, *26*, 1252–1274. [CrossRef]
11. Kinner, A.; Nerke, P.; Siedentop, R.; Steinmetz, T.; Classen, T.; Rosenthal, K.; Nett, M.; Pietruszka, J.; Lütz, S. Recent Advances in Biocatalysis for Drug Synthesis. *Biomedicines* **2022**, *10*, 964. [CrossRef] [PubMed]
12. Rossino, G.; Robescu, M.S.; Licastro, E.; Tedesco, C.; Martello, I.; Maffei, L.; Vincenti, G.; Bavaro, T.; Collina, S. Biocatalysis: A Smart and Green Tool for the Preparation of Chiral Drugs. *Chirality* **2022**, *34*, 1403–1418. [CrossRef] [PubMed]
13. Remonatto, D.; Lerin, L.A. Biocatalysis and Bioactive Molecules: Future and Development. *Int. J. Mol. Sci.* **2023**, *24*, 5571. [CrossRef]
14. Dalby, P. Engineering Enzymes for Biocatalysis. *Recent Pat. Biotechnol.* **2007**, *1*, 1–9. [CrossRef] [PubMed]
15. Pollard, D.J.; Woodley, J.M. Biocatalysis for Pharmaceutical Intermediates: The Future Is Now. *Trends Biotechnol.* **2007**, *25*, 66–73. [CrossRef]
16. De Miranda, A.S.; Milagre, C.D.F.; Hollmann, F. Alcohol Dehydrogenases as Catalysts in Organic Synthesis. *Front. Catal.* **2022**, *2*, 900554. [CrossRef]
17. Wachtmeister, J.; Rother, D. Recent Advances in Whole Cell Biocatalysis Techniques Bridging from Investigative to Industrial Scale. *Curr. Opin. Biotechnol.* **2016**, *42*, 169–177. [CrossRef]

18. Segal-Kischinevzky, C.; Romero-Aguilar, L.; Alcaraz, L.D.; López-Ortiz, G.; Martínez-Castillo, B.; Torres-Ramírez, N.; Sandoval, G.; González, J. Yeasts Inhabiting Extreme Environments and Their Biotechnological Applications. *Microorganisms* **2022**, *10*, 794. [CrossRef]
19. Kurtzman, C.P.; Sugiyama, J. 1 Saccharomycotina and Taphrinomycotina: The Yeasts and Yeastlike Fungi of the Ascomycota. In *Systematics and Evolution*; McLaughlin, D.J., Spatafora, J.W., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; pp. 3–33, ISBN 978-3-662-46010-8.
20. Kurtzman, C.P.; Fell, J.W.; Boekhout, T. *The Yeasts: A Taxonomic Study*, 5th ed.; Elsevier: Amsterdam, The Netherlands, 2011; ISBN 978-0-444-52149-1.
21. Kurtzman, C.P.; Boekhout, T. Yeasts as Distinct Life Forms of Fungi. In *Yeasts in Natural Ecosystems: Ecology*; Buzzini, P., Lachance, M.-A., Yurkov, A., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 1–37, ISBN 978-3-319-61574-5.
22. Vyas, A.; Freitas, A.V.; Ralston, Z.A.; Tang, Z. Fission Yeast *Schizosaccharomyces pombe*: A Unicellular "Micromammal" Model Organism. *Curr. Protoc.* **2021**, *1*, e151. [CrossRef]
23. Spencer, J.; Ragout De Spencer, A.; Laluce, C. Non-Conventional Yeasts. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 147–156. [CrossRef]
24. Johnson, E.A. Biotechnology of Non-*Saccharomyces* Yeasts—The Ascomycetes. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 503–517. [CrossRef]
25. Johnson, E.A. Biotechnology of Non-*Saccharomyces* Yeasts—The Basidiomycetes. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 7563–7577. [CrossRef]
26. Geijer, C.; Ledesma-Amaro, R.; Tomás-Pejó, E. Unraveling the Potential of Non-Conventional Yeasts in Biotechnology. *FEMS Yeast Res.* **2022**, *22*, foab071. [CrossRef]
27. Boekhout, T.; Amend, A.S.; El Baidouri, F.; Gabaldón, T.; Geml, J.; Mittelbach, M.; Robert, V.; Tan, C.S.; Turchetti, B.; Vu, D.; et al. Trends in Yeast Diversity Discovery. *Fungal Divers.* **2022**, *114*, 491–537. [CrossRef]
28. Hernández, A.; Pérez-Nevado, F.; Ruiz-Moyano, S.; Serradilla, M.J.; Villalobos, M.C.; Martín, A.; Córdoba, M.G. Spoilage Yeasts: What Are the Sources of Contamination of Foods and Beverages? *Int. J. Food Microbiol.* **2018**, *286*, 98–110. [CrossRef]
29. Buzzini, P.; Turchetti, B.; Yurkov, A. Extremophilic Yeasts: The Toughest Yeasts Around? *Yeast* **2018**, *35*, 487–497. [CrossRef] [PubMed]
30. Elleuche, S.; Schröder, C.; Sahm, K.; Antranikian, G. Extremozymes—Biocatalysts with Unique Properties from Extremophilic Microorganisms. *Curr. Opin. Biotechnol.* **2014**, *29*, 116–123. [CrossRef]
31. Ibrahim, N.E.; Ma, K. Industrial Applications of Thermostable Enzymes from Extremophilic Microorganisms. *Curr. Eng. J.* **2017**, *4*, 75–98. [CrossRef]
32. Löbs, A.-K.; Schwartz, C.; Wheeldon, I. Genome and Metabolic Engineering in Non-Conventional Yeasts: Current Advances and Applications. *Synth. Syst. Biotechnol.* **2017**, *2*, 198–207. [CrossRef]
33. Patra, P.; Das, M.; Kundu, P.; Ghosh, A. Recent Advances in Systems and Synthetic Biology Approaches for Developing Novel Cell-Factories in Non-Conventional Yeasts. *Biotechnol. Adv.* **2021**, *47*, 107695. [CrossRef]
34. Karbalaei, M.; Rezaee, S.A.; Farsiani, H. *Pichia pastoris*: A Highly Successful Expression System for Optimal Synthesis of Heterologous Proteins. *J. Cell. Physiol.* **2020**, *235*, 5867–5881. [CrossRef]
35. De Brabander, P.; Uitterhaegen, E.; Delmulle, T.; De Winter, K.; Soetaert, W. Challenges and Progress towards Industrial Recombinant Protein Production in Yeasts: A Review. *Biotechnol. Adv.* **2023**, *64*, 108121. [CrossRef]
36. Wang, W.; Blenner, M.A. Engineering Heterologous Enzyme Secretion in *Yarrowia lipolytica*. *Microb. Cell Factories* **2022**, *21*, 134. [CrossRef]
37. Zhang, L.; Sun, Z.; Xu, G.; Ni, Y. Classification and Functional Origins of Stereocomplementary Alcohol Dehydrogenases for Asymmetric Synthesis of Chiral Secondary Alcohols: A Review. *Int. J. Biol. Macromol.* **2024**, *270*, 132238. [CrossRef]
38. Prelog, V. Specification of the Stereospecificity of Some Oxido-Reductases by Diamond Lattice Sections. *Pure Appl. Chem.* **1964**, *9*, 119–130. [CrossRef]
39. Shanbhag, A.P. Stairway to Stereoisomers: Engineering Short- and Medium-Chain Ketoreductases To Produce Chiral Alcohols. *ChemBioChem* **2023**, *24*, e202200687. [CrossRef] [PubMed]
40. Wang, X.; Saba, T.; Yiu, H.H.P.; Howe, R.F.; Anderson, J.A.; Shi, J. Cofactor NAD(P)H Regeneration Inspired by Heterogeneous Pathways. *Chem* **2017**, *2*, 621–654. [CrossRef]
41. Matsuyama, A.; Kobayashi, Y.; Ohnishi, H. Microbial Production of Optically Active 1,3-Butanediol from 4-Hydroxy-2-Butanone. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 348–349. [CrossRef] [PubMed]
42. Wang, N.; Xu, Y.; Peng, C.; Wang, X.; Wei, Y.; Li, K.; Wang, S.; Xu, A.; Gao, J. Identification of a Newly Isolated *Rhodotorula mucilaginosa* NQ1 and Its Development for the Synthesis of Bulky Carbonyl Compounds by Whole-cell Bioreduction. *Lett. Appl. Microbiol.* **2021**, *72*, 399–407. [CrossRef]
43. Contente, M.L.; Molinari, F.; Zambelli, P.; De Vitis, V.; Gandolfi, R.; Pinto, A.; Romano, D. Biotransformation of Aromatic Ketones and Ketoesters with the Non-Conventional Yeast *Pichia glucozyma*. *Tetrahedron Lett.* **2014**, *55*, 7051–7053. [CrossRef]
44. Şahin, E.; Dertli, E. Highly Enantioselective Production of Chiral Secondary Alcohols with *Candida zeylanoides* as a New Whole Cell Biocatalyst. *Chem. Biodivers.* **2017**, *14*, e1700121. [CrossRef] [PubMed]
45. Andreu, C.; Peña, M.; Del Olmo, M. Biocatalytic Reduction of Racemic 2-Arenoxycycloalkanones by Yeasts *P. glucozyma* and *C. glabrata*: One Way of Achieving Chiral 2-Arenoxycycloalcohols. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 4865–4873. [CrossRef] [PubMed]

46. Serra, I.; Guidi, B.; Burgaud, G.; Contente, M.L.; Ferraboschi, P.; Pinto, A.; Compagno, C.; Molinari, F.; Romano, D. Seawater-Based Biocatalytic Strategy: Stereoselective Reductions of Ketones with Marine Yeasts. *ChemCatChem* **2016**, *8*, 3254–3260. [CrossRef]
47. Andreu, C.; del Olmo, M. Improved Biocatalytic Activity of the *Debaryomyces* Species in Seawater. *ChemCatChem* **2019**, *11*, 3085–3092. [CrossRef]
48. Andreu, C.; Del Olmo, M. Whole-Cell Biocatalysis in Seawater: New Halotolerant Yeast Strains for the Regio- and Stereoselectivity Reduction of 1-Phenylpropane-1,2-Dione in Saline-Rich Media. *ChemBioChem* **2020**, *21*, 1621–1628. [CrossRef] [PubMed]
49. Chen, X.; Liu, Z.-Q.; Lin, C.-P.; Zheng, Y.-G. Chemoenzymatic Synthesis of (S)-Duloxetine Using Carbonyl Reductase from *Rhodosporidium toruloides*. *Bioorg. Chem.* **2016**, *65*, 82–89. [CrossRef]
50. Jiang, W.; Fu, X.; Wu, W. Gene Mining, Codon Optimization and Analysis of Binding Mechanism of an Aldo-Keto Reductase with High Activity, Better Substrate Specificity and Excellent Solvent Tolerance. *PLoS ONE* **2021**, *16*, e0260787. [CrossRef]
51. Chen, L.-F.; Zhang, Y.-P.; Fan, H.-Y.; Wu, K.; Lin, J.-P.; Wang, H.-L.; Wei, D.-Z. Efficient Bioreductive Production of (R)-N-Boc-3-Hydroxypiperidine by a Carbonyl Reductase. *Catal. Commun.* **2017**, *97*, 5–9. [CrossRef]
52. Zhu, F.-Y.; Zhong, J.; Shen, Q.; Jia, D.-X.; Ma, S.-J.; Du, J.; Wu, H.; Yang, Q.; Cao, M.; Liu, Z.-Q.; et al. Development of an *Escherichia coli* Whole Cell Catalyst Harboring Conjugated Polyketone Reductase from *Candida glabrata* for Synthesis of D-(−)-Pantolactone. *Process Biochem.* **2022**, *112*, 223–233. [CrossRef]
53. Sellés Vidal, L.; Isalan, M.; Heap, J.T.; Ledesma-Amaro, R. A Primer to Directed Evolution: Current Methodologies and Future Directions. *RSC Chem. Biol.* **2023**, *4*, 271–291. [CrossRef] [PubMed]
54. Nguyen, D.T.; Mitchell, D.A.; Van Der Donk, W.A. Genome Mining for New Enzyme Chemistry. *ACS Catal.* **2024**, *14*, 4536–4553. [CrossRef]
55. Liu, Z.-Q.; Wu, L.; Zhang, X.-J.; Xue, Y.-P.; Zheng, Y.-G. Directed Evolution of Carbonyl Reductase from *Rhodosporidium toruloides* and Its Application in Stereoselective Synthesis of *Tert*-Butyl (3*R*,5*S*)-6-Chloro-3,5-Dihydroxyhexanoate. *J. Agric. Food Chem.* **2017**, *65*, 3721–3729. [CrossRef]
56. Liu, Z.-Q.; Wu, L.; Zheng, L.; Wang, W.-Z.; Zhang, X.-J.; Jin, L.-Q.; Zheng, Y.-G. Biosynthesis of Tert-Butyl (3R,5S)-6-Chloro-3,5-Dihydroxyhexanoate by Carbonyl Reductase from *Rhodosporidium toruloides* in Mono and Biphasic Media. *Bioresour. Technol.* **2018**, *249*, 161–167. [CrossRef] [PubMed]
57. Cheng, F.; Chen, Y.; Qiu, S.; Zhai, Q.-Y.; Liu, H.-T.; Li, S.-F.; Weng, C.-Y.; Wang, Y.-J.; Zheng, Y.-G. Controlling Stereopreferences of Carbonyl Reductases for Enantioselective Synthesis of Atorvastatin Precursor. *ACS Catal.* **2021**, *11*, 2572–2582. [CrossRef]
58. Honda, K.; Ono, T.; Okano, K.; Miyake, R.; Dekishima, Y.; Kawabata, H. Expression of Engineered Carbonyl Reductase from *Ogataea minuta* in *Rhodococcus opacus* and Its Application to Whole-Cell Bioconversion in Anhydrous Solvents. *J. Biosci. Bioeng.* **2019**, *127*, 145–149. [CrossRef] [PubMed]
59. Zallot, R.; Oberg, N.; Gerlt, J.A. Discovery of New Enzymatic Functions and Metabolic Pathways Using Genomic Enzymology Web Tools. *Curr. Opin. Biotechnol.* **2021**, *69*, 77–90. [CrossRef]
60. Anello, A.L.; Aguilera, L.; Kurina-Sanz, M.; Juri Ayub, M.; Mascotti, M.L. Broadening the Repertoire of Microbial Aldo-Keto Reductases: Cloning and Characterization of AKR3B4 from *Rhodotorula mucilaginosa* LSL Strain. *Enzyme Microb. Technol.* **2020**, *132*, 109415. [CrossRef]
61. Kumar Roy, T.; Sreedharan, R.; Ghosh, P.; Gandhi, T.; Maiti, D. Ene-Reductase: A Multifaceted Biocatalyst in Organic Synthesis. *Chem. Eur. J.* **2022**, *28*, e202103949. [CrossRef]
62. Parmeggiani, F.; Brenna, E.; Colombo, D.; Gatti, F.G.; Tentori, F.; Tessaro, D. "A Study in Yellow": Investigations in the Stereoselectivity of Ene-Reductases. *ChemBioChem* **2022**, *23*, e202100445. [CrossRef] [PubMed]
63. Dobrijevic, D.; Benhamou, L.; Aliev, A.E.; Méndez-Sánchez, D.; Dawson, N.; Baud, D.; Tappertzhofen, N.; Moody, T.S.; Orengo, C.A.; Hailes, H.C.; et al. Metagenomic Ene-Reductases for the Bioreduction of Sterically Challenging Enones. *RSC Adv.* **2019**, *9*, 36608–36614. [CrossRef]
64. Fan, X.-Y.; Yu, Y.; Yao, Y.; Li, W.-D.; Tao, F.-Y.; Wang, N. Applications of Ene-Reductases in the Synthesis of Flavors and Fragrances. *J. Agric. Food Chem.* **2024**, *72*, 18305–18320. [CrossRef] [PubMed]
65. Goretti, M.; Turchetti, B.; Cramarossa, M.; Forti, L.; Buzzini, P. Production of Flavours and Fragrances via Bioreduction of (4R)-(-)-Carvone and (1R)-(-)-Myrtenal by Non-Conventional Yeast Whole-Cells. *Molecules* **2013**, *18*, 5736–5748. [CrossRef]
66. Goretti, M.; Ponzoni, C.; Caselli, E.; Marchegiani, E.; Cramarossa, M.R.; Turchetti, B.; Forti, L.; Buzzini, P. Bioreduction of α,β-Unsaturated Ketones and Aldehydes by Non-Conventional Yeast (NCY) Whole-Cells. *Bioresour. Technol.* **2011**, *102*, 3993–3998. [CrossRef]
67. Filippucci, S.; Tasselli, G.; Kenza Labbani, F.-Z.; Turchetti, B.; Cramarossa, M.R.; Buzzini, P.; Forti, L. Non-Conventional Yeasts as Sources of Ene-Reductases for the Bioreduction of Chalcones. *Fermentation* **2020**, *6*, 29. [CrossRef]
68. Łużny, M.; Krzywda, M.; Kozłowska, E.; Kostrzewa-Susłow, E.; Janeczko, T. Effective Hydrogenation of 3-(2″-Furyl)- and 3-(2″-Thienyl)-1-(2′-Hydroxyphenyl)-Prop-2-En-1-One in Selected Yeast Cultures. *Molecules* **2019**, *24*, 3185. [CrossRef] [PubMed]
69. Łużny, M.; Kozłowska, E.; Kostrzewa-Susłow, E.; Janeczko, T. Highly Effective, Regiospecific Hydrogenation of Methoxychalcone by *Yarrowia lipolytica* Enables Production of Food Sweeteners. *Catalysts* **2020**, *10*, 1135. [CrossRef]
70. Gonçalves, A.E.; Malheiros, Â.; Casarin, C.A.; De França, L.; Palomino-Salcedo, D.L.; Ferreira, L.L.G.; Andricopulo, A.D.; Dávila-Rodríguez, M.J.; Lacava, L.C.; De Oliveira, A.S.; et al. 2′,6′-Dihydroxy-4′-Methoxy Dihydrochalcone Improves the Cognitive Impairment of Alzheimer's Disease: A Structure-Activity Relationship Study. *Curr. Top. Med. Chem.* **2021**, *21*, 1167–1185. [CrossRef] [PubMed]

71. Łużny, M.; Kaczanowska, D.; Gawdzik, B.; Wzorek, A.; Pawlak, A.; Obmińska-Mrukowicz, B.; Dymarska, M.; Kozłowska, E.; Kostrzewa-Susłow, E.; Janeczko, T. Regiospecific Hydrogenation of Bromochalcone by Unconventional Yeast Strains. *Molecules* **2022**, *27*, 3681. [CrossRef]
72. Chlipała, P.; Janeczko, T.; Mazur, M. Bioreduction of 4′-Hydroxychalcone in Deep Eutectic Solvents: Optimization and Efficacy with Various Yeast Strains. *Int. J. Mol. Sci.* **2024**, *25*, 7152. [CrossRef]
73. Long, Z.; Li, K.; Xue, Y.; Sun, Y.; Li, J.; Su, Z.; Sun, J.; Liu, Q.; Liu, H.; Wei, T. Purification and Biochemical Characterization of a Novel Ene- Reductase from *Kazachstania exigua* HSC6 for Dihydro-β-Ionone from β-Ionone. *Biotechnol. Lett.* **2023**, *45*, 499–508. [CrossRef]
74. Zhang, B.; Zheng, L.; Lin, J.; Wei, D. Characterization of an Ene-Reductase from *Meyerozyma guilliermondii* for Asymmetric Bioreduction of α,β-Unsaturated Compounds. *Biotechnol. Lett.* **2016**, *38*, 1527–1534. [CrossRef]
75. Li, Z.; Wang, Z.; Meng, G.; Lu, H.; Huang, Z.; Chen, F. Identification of an Ene Reductase from Yeast *Kluyveromyces marxianus* and Application in the Asymmetric Synthesis of (*R*)-Profen Esters. *Asian J. Org. Chem.* **2018**, *7*, 763–769. [CrossRef]
76. Zhang, B.; Sun, J.; Zheng, Y.; Mao, X.; Lin, J.; Wei, D. Identification of a Novel Ene Reductase from *Pichia angusta* with Potential Application in (*R*)-Levodione Production. *RSC Adv.* **2022**, *12*, 13924–13931. [CrossRef] [PubMed]
77. Shi, Q.; Wang, H.; Liu, J.; Li, S.; Guo, J.; Li, H.; Jia, X.; Huo, H.; Zheng, Z.; You, S.; et al. Old Yellow Enzymes: Structures and Structure-Guided Engineering for Stereocomplementary Bioreduction. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 8155–8170. [CrossRef] [PubMed]
78. Amato, E.D.; Stewart, J.D. Applications of Protein Engineering to Members of the Old Yellow Enzyme Family. *Biotechnol. Adv.* **2015**, *33*, 624–631. [CrossRef] [PubMed]
79. Klepach, H.M.; Zakalskiy, A.E.; Zakalska, O.M.; Gayda, G.Z.; Smutok, O.V.; Gonchar, M.V. Alcohol Oxidase from the Methylotrophic Yeast *Ogataea polymorpha*: Isolation, Purification, and Bioanalytical Application. In *Flavins and Flavoproteins*; Barile, M., Ed.; Methods in Molecular Biology; Springer: New York, NY, USA, 2021; Volume 2280, pp. 231–248, ISBN 978-1-07-161285-9.
80. Ribeaucourt, D.; Bissaro, B.; Lambert, F.; Lafond, M.; Berrin, J.-G. Biocatalytic Oxidation of Fatty Alcohols into Aldehydes for the Flavors and Fragrances Industry. *Biotechnol. Adv.* **2022**, *56*, 107787. [CrossRef]
81. Schober, L.; Dobiašová, H.; Jurkaš, V.; Parmeggiani, F.; Rudroff, F.; Winkler, M. Enzymatic Reactions towards Aldehydes: An Overview. *Flavour Fragr. J.* **2023**, *38*, 221–242. [CrossRef]
82. Mangkorn, N.; Kanokratana, P.; Roongsawang, N.; Laobuthee, A.; Laosiripojana, N.; Champreda, V. Synthesis and Characterization of *Ogataea thermomethanolica* Alcohol Oxidase Immobilized on Barium Ferrite Magnetic Microparticles. *J. Biosci. Bioeng.* **2019**, *127*, 265–272. [CrossRef]
83. Li, Q.; Wang, H.; Zhang, W.; Wang, W.; Ren, X.; Wu, M.; Shi, G. Structure-Guided Evolution Modulate Alcohol Oxidase to Improve Ethanol Oxidation Performance. *Appl. Biochem. Biotechnol.* **2024**, *196*, 1948–1965. [CrossRef]
84. Cazetta, T.; Moran, P.J.S.; Rodrigues, J.A.R. Highly Enantioselective Deracemization of 1-Phenyl-1,2-Ethanediol and Its Derivatives by Stereoinversion Using *Candida albicans* in a One-Pot Process. *J. Mol. Catal. B Enzym.* **2014**, *109*, 178–183. [CrossRef]
85. Chadha, A.; Venkataraman, S.; Preetha, R.; Padhi, S.K. *Candida parapsilosis*: A Versatile Biocatalyst for Organic Oxidation-Reduction Reactions. *Bioorg. Chem.* **2016**, *68*, 187–213. [CrossRef] [PubMed]
86. Bartsch, S.; Brummund, J.; Köpke, S.; Straatman, H.; Vogel, A.; Schürmann, M. Optimization of Alcohol Dehydrogenase for Industrial Scale Oxidation of Lactols. *Biotechnol. J.* **2020**, *15*, 2000171. [CrossRef] [PubMed]
87. Bordewick, S.; Beier, A.; Balke, K.; Bornscheuer, U.T. Baeyer-Villiger Monooxygenases from *Yarrowia lipolytica* Catalyze Preferentially Sulfoxidations. *Enzyme Microb. Technol.* **2018**, *109*, 31–42. [CrossRef] [PubMed]
88. Dulak, K.; Sordon, S.; Matera, A.; Kozak, B.; Huszcza, E.; Popłoński, J. Novel Flavonoid C-8 Hydroxylase from *Rhodotorula glutinis*: Identification, Characterization and Substrate Scope. *Microb. Cell Factories* **2022**, *21*, 175. [CrossRef]
89. Pollegioni, L.; Molla, G.; Sacchi, S.; Rosini, E.; Verga, R.; Pilone, M.S. Properties and Applications of Microbial D-Amino Acid Oxidases: Current State and Perspectives. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 1–16. [CrossRef]
90. Wang, L.; Tang, H.; Zhu, H.; Xue, Y.; Zheng, Y. Enhancement of the Substrate Specificity of D-amino Acid Oxidase Based on Tunnel-pocket Engineering. *Biotechnol. Bioeng.* **2023**, *120*, 3557–3569. [CrossRef] [PubMed]
91. Wang, L.; Tang, H.; Zhao, J.-Q.; Wang, M.-N.; Xue, Y.-P.; Zheng, Y.-G. Correlation Analysis of Key Residue Sites between Computational-Aided Design Thermostability D -Amino Acid Oxidase and Ancestral Enzymes. *J. Agric. Food Chem.* **2023**, *71*, 20177–20186. [CrossRef]
92. Thuku, R.N.; Brady, D.; Benedik, M.J.; Sewell, B.T. Microbial Nitrilases: Versatile, Spiral Forming, Industrial Enzymes. *J. Appl. Microbiol.* **2009**, *106*, 703–727. [CrossRef] [PubMed]
93. Shen, J.-D.; Cai, X.; Liu, Z.-Q.; Zheng, Y.-G. Nitrilase: A Promising Biocatalyst in Industrial Applications for Green Chemistry. *Crit. Rev. Biotechnol.* **2021**, *41*, 72–93. [CrossRef]
94. Zhou, S.-P.; Xue, Y.-P.; Zheng, Y.-G. Maximizing the Potential of Nitrilase: Unveiling Their Diversity, Catalytic Proficiency, and Versatile Applications. *Biotechnol. Adv.* **2024**, *72*, 108352. [CrossRef]
95. Bhalla, T.C.; Sharma, M.; Sharma, N.N. Nitrile Metabolizing Yeasts. In *Yeast Biotechnology: Diversity and Applications*; Satyanarayana, T., Kunze, G., Eds.; Springer: Dordrecht, The Netherlands, 2009; pp. 715–735, ISBN 978-1-4020-8291-7.
96. Gong, J.-S.; Lu, Z.-M.; Li, H.; Shi, J.-S.; Zhou, Z.-M.; Xu, Z.-H. Nitrilases in Nitrile Biocatalysis: Recent Progress and Forthcoming Research. *Microb. Cell Factories* **2012**, *11*, 142. [CrossRef] [PubMed]

97. Rustler, S.; Stolz, A. Isolation and Characterization of a Nitrile Hydrolysing Acidotolerant Black Yeast—*Exophiala oligosperma* R1. *Appl. Microbiol. Biotechnol.* **2007**, *75*, 899–908. [CrossRef] [PubMed]
98. Prasad, S.; Sharma, D.R.; Bhalla, T.C. Nitrile- and Amide-Hydrolysing Activity in *Kluyveromyces thermotolerans* MGBY 37. *World J. Microbiol. Biotechnol.* **2005**, *21*, 1447–1450. [CrossRef]
99. Dias, J.C.; Rezende, R.P.; Rosa, C.A.; Lachance, M.-A.; Linardi, V.R. Enzymatic Degradation of Nitriles by a *Candida guilliermondii* UFMG-Y65. *Can. J. Microbiol.* **2000**, *46*, 525–531. [CrossRef] [PubMed]
100. Zhang, Q.; Gong, J.-S.; Dong, T.-T.; Liu, T.-T.; Li, H.; Dou, W.-F.; Lu, Z.-M.; Shi, J.-S.; Xu, Z.-H. Nitrile-Hydrolyzing Enzyme from *Meyerozyma guilliermondii* and Its Potential in Biosynthesis of 3-Hydroxypropionic Acid. *Bioprocess Biosyst. Eng.* **2017**, *40*, 901–910. [CrossRef] [PubMed]
101. Serra, I.; Capusoni, C.; Molinari, F.; Musso, L.; Pellegrino, L.; Compagno, C. Marine Microorganisms for Biocatalysis: Selective Hydrolysis of Nitriles with a Salt-Resistant Strain of *Meyerozyma guilliermondii*. *Mar. Biotechnol.* **2019**, *21*, 229–239. [CrossRef]
102. Chandra, P.; Enespa; Singh, R.; Arora, P.K. Microbial Lipases and Their Industrial Applications: A Comprehensive Review. *Microb. Cell Factories* **2020**, *19*, 169. [CrossRef]
103. Soni, S. Trends in Lipase Engineering for Enhanced Biocatalysis. *Biotechnol Appl. Biochem.* **2022**, *69*, 265–272. [CrossRef]
104. Kuncharoen, N.; Techo, S.; Savarajara, A.; Tanasupawat, S. Identification and Lipolytic Activity of Yeasts Isolated from Foods and Wastes. *Mycology* **2020**, *11*, 279–286. [CrossRef]
105. Atalah, J.; Cáceres-Moreno, P.; Espina, G.; Blamey, J.M. Thermophiles and the Applications of Their Enzymes as New Biocatalysts. *Bioresour. Technol.* **2019**, *280*, 478–488. [CrossRef]
106. Chen, M.; Jin, T.; Nian, B.; Cheng, W. Solvent Tolerance Improvement of Lipases Enhanced Their Applications: State of the Art. *Molecules* **2024**, *29*, 2444. [CrossRef] [PubMed]
107. Wang, H.H.; Zhang, Q.; Yu, X.; Liang, J.; Zhang, Y.; Jiang, Y.; Su, W. Application of Lipase B from *Candida antarctica* in the Pharmaceutical Industry. *Ind. Eng. Chem. Res.* **2023**, *62*, 15733–15751. [CrossRef]
108. Wu, Z.; Shi, W.; Jin, M.; Zhou, W. Efficient Enzymatic Synthesis of Chiral 2,3-Dihydro-1,4-Benzodioxane Motif Using Engineered *Candida antarctica* Lipase B. *RSC Adv.* **2023**, *13*, 18953–18959. [CrossRef]
109. Nietz, D.; Bode, R.; Kunze, G.; Rauter, M. A New Lipase (Alip2) with High Potential for Enzymatic Hydrolysis of the Diester Diethyladipate to the Monoester Monoethyladipate. *Enzyme Microb. Technol.* **2022**, *153*, 109898. [CrossRef] [PubMed]
110. Deng, L.; Wang, X.; Nie, K.; Wang, F.; Liu, J.; Wang, P.; Tan, T. Synthesis of Wax Esters by Lipase-catalyzed Esterification with Immobilized Lipase from *Candida* sp. 99–125. *Chin. J. Chem. Eng.* **2011**, *19*, 978–982. [CrossRef]
111. Kuo, C.H.; Ju, H.Y.; Chu, S.W.; Chen, J.H.; Chang, C.M.J.; Liu, Y.C.; Shie, C.J. Optimization of Lipase-Catalyzed Synthesis of Cetyl Octanoate in Supercritical Carbon Dioxide. *J. Am. Oil Chem. Soc.* **2012**, *89*, 103–110. [CrossRef]
112. De Souza, C.E.C.; Ribeiro, B.D.; Coelho, M.A.Z. Characterization and Application of *Yarrowia lipolytica* Lipase Obtained by Solid-State Fermentation in the Synthesis of Different Esters Used in the Food Industry. *Appl. Biochem. Biotechnol.* **2019**, *189*, 933–959. [CrossRef]
113. De Menezes, L.H.S.; Do Espírito Santo, E.L.; Dos Santos, M.M.O.; De Carvalho Tavares, I.M.; Mendes, A.A.; Franco, M.; De Oliveira, J.R. *Candida rugosa* Lipase Immobilized on Hydrophobic Support Accurel MP 1000 in the Synthesis of Emollient Esters. *Biotechnol. Lett.* **2022**, *44*, 89–99. [CrossRef]
114. Wagner, K.; Hummel, A.; Yang, J.; Horino, S.; Kanomata, K.; Akai, S.; Gröger, H. Protein Engineering of Lipase A from *Candida antarctica* to Improve Esterification of Tertiary Alcohols. *ChemBioChem* **2024**, *25*, e202400082. [CrossRef]
115. Zhang, H.; Sang, J.; Zhang, Y.; Sun, T.; Liu, H.; Yue, R.; Zhang, J.; Wang, H.; Dai, Y.; Lu, F.; et al. Rational Design of a *Yarrowia lipolytica* Derived Lipase for Improved Thermostability. *Int. J. Biol. Macromol.* **2019**, *137*, 1190–1198. [CrossRef]
116. Duchiron, S.W.; Pollet, E.; Givry, S.; Avérous, L. Enzymatic Synthesis of Poly(ε-Caprolactone-Co-ε-Thiocaprolactone). *Eur. Polym. J.* **2017**, *87*, 147–158. [CrossRef]
117. Todea, A.; Bîtcan, I.; Aparaschivei, D.; Păușescu, I.; Badea, V.; Péter, F.; Gherman, V.D.; Rusu, G.; Nagy, L.; Kéki, S. Biodegradable Oligoesters of ε-Caprolactone and 5-Hydroxymethyl-2-Furancarboxylic Acid Synthesized by Immobilized Lipases. *Polymers* **2019**, *11*, 1402. [CrossRef] [PubMed]
118. Valotta, A.; Stelzer, D.; Reiter, T.; Kroutil, W.; Gruber-Woelfler, H. A Multistep (Semi)-Continuous Biocatalytic Setup for the Production of Polycaprolactone. *React. Chem. Eng.* **2024**, *9*, 713–727. [CrossRef]
119. Andreu, C.; Del Olmo, M. Potential of Some Yeast Strains in the Stereoselective Synthesis of (R)-(−)-Phenylacetylcarbinol and (S)-(+)-Phenylacetylcarbinol and Their Reduced 1,2-Dialcohol Derivatives. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 5901–5913. [CrossRef]
120. Andreu, C.; ·Lí Del Olmo, M. Biotransformation Using Halotolerant Yeast in Seawater: A Sustainable Strategy to Produce R-(−)-Phenylacetylcarbinol. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 4717–4727. [CrossRef]
121. Yan, W.; Zhang, X.; Qian, X.; Zhou, J.; Dong, W.; Ma, J.; Zhang, W.; Xin, F.; Jiang, M. Comprehensive Investigations of 2-Phenylethanol Production by High 2-Phenylethanol Tolerating *Meyerozyma* sp. Strain YLG18. *Enzyme Microb. Technol.* **2020**, *140*, 109629. [CrossRef]
122. Dai, J.; Li, K.; Song, N.; Yao, W.; Xia, H.; Yang, Q.; Zhang, X.; Li, X.; Wang, Z.; Yao, L.; et al. *Zygosaccharomyces rouxii*, an Aromatic Yeast Isolated From Chili Sauce, Is Able to Biosynthesize 2-Phenylethanol via the Shikimate or Ehrlich Pathways. *Front. Microbiol.* **2020**, *11*, 597454. [CrossRef] [PubMed]

123. Zhou, R.; Song, Q.; Xia, H.; Song, N.; Yang, Q.; Zhang, X.; Yao, L.; Yang, S.; Dai, J.; Chen, X. Isolation and Identification of Non-*Saccharomyces* Yeast Producing 2-Phenylethanol and Study of the Ehrlich Pathway and Shikimate Pathway. *J. Fungi* **2023**, *9*, 878. [CrossRef]
124. Hu, D.; Jia, X.-W.; Lu, J.-L.; Lu, Z.-Y.; Tang, C.-D.; Xue, F.; Huang, C.; Ren, Q.-G.; He, Y.-C. Chemoenzymatic Asymmetric Synthesis of Chiral Triazole Fungicide (*R*)-Tebuconazole in High Optical Purity Mediated by an Epoxide Hydrolase from *Rhodotorula paludigensis*. *J. Agric. Food Chem.* **2024**, *72*, 10428–10438. [CrossRef]
125. Krahe, N.-K.; Berger, R.G.; Ersoy, F. A DyP-Type Peroxidase of Pleurotus Sapidus with Alkene Cleaving Activity. *Molecules* **2020**, *25*, 1536. [CrossRef]
126. Krahe, N.-K.; Berger, R.G.; Witt, M.; Zorn, H.; Omarini, A.B.; Ersoy, F. Monokaryotic Pleurotus Sapidus Strains with Intraspecific Variability of an Alkene Cleaving DyP-Type Peroxidase Activity as a Result of Gene Mutation and Differential Gene Expression. *Int. J. Mol. Sci.* **2021**, *22*, 1363. [CrossRef] [PubMed]
127. Szczepańska, P.; Rychlicka, M.; Moroz, P.; Janek, T.; Gliszczyńska, A.; Lazar, Z. Elevating Phospholipids Production *Yarrowia lipolytica* from Crude Glycerol. *Int. J. Mol. Sci.* **2022**, *23*, 10737. [CrossRef] [PubMed]
128. Wu, Y.; Li, S.; Sun, B.; Guo, J.; Zheng, M.; Li, A. Enhancing Gastrodin Production in *Yarrowia lipolytica* by Metabolic Engineering. *ACS Synth. Biol.* **2024**, *13*, 1332–1342. [CrossRef] [PubMed]
129. Guo, F.; Dai, Z.; Peng, W.; Zhang, S.; Zhou, J.; Ma, J.; Dong, W.; Xin, F.; Zhang, W.; Jiang, M. Metabolic Engineering of *Pichia pastoris* for Malic Acid Production from Methanol. *Biotechnol. Bioeng.* **2021**, *118*, 357–371. [CrossRef]
130. Zhang, J.; Saerens, K.M.J.; Van Bogaert, I.N.A.; Soetaert, W. Vegetable Oil Enhances Sophorolipid Production by *Rhodotorula bogoriensis*. *Biotechnol. Lett.* **2011**, *33*, 2417–2423. [CrossRef] [PubMed]
131. Zhu, L.; Zhou, L.; Huang, N.; Cui, W.; Liu, Z.; Xiao, K.; Zhou, Z. Efficient Preparation of Enantiopure D-Phenylalanine through Asymmetric Resolution Using Immobilized Phenylalanine Ammonia-Lyase from *Rhodotorula glutinis* JN-1 in a Recirculating Packed-Bed Reactor. *PLoS ONE* **2014**, *9*, e108586. [CrossRef]
132. Bettio, G.; Zardo, L.C.; Rosa, C.A.; Záchia Ayub, M.A. Bioconversion of Ferulic Acid into Aroma Compounds by Newly Isolated Yeast Strains of the Latin American Biodiversity. *Biotechnol. Progr.* **2021**, *37*, e3067. [CrossRef]
133. Shrikhande, G.; Mulay, P.; Puskas, J.E. PEGylation of Fluorescein by Enzyme-Catalyzed "Click" Michael Addition. *Macromol. Rapid Commun.* **2020**, *41*, 2000163. [CrossRef]
134. Liang, C.; Duan, X.; Gao, H.; Shahab, M.; Zheng, G. Chemoenzymatic Synthesis of (1R,3R)-3-Hydroxycyclopentanemethanol: An Intermediate of Carbocyclic-ddA. *J. Biosci. Bioeng.* **2024**, *138*, 111–117. [CrossRef]
135. Hualpa-Cutipa, E.; Solórzano Acosta, R.A.; Luis-Alaya, B.; León, J.M. Extremophilic Yeasts and Their Potential in Bioremediation of Polluted Environments. In *Advances in Yeast Biotechnology for Biofuels and Sustainability*; Daverey, A., Dutta, K., Joshi, S., Gea, T., Eds.; Elsevier: Amsterdam, The Netherlands, 2023; pp. 521–542, ISBN 978-0-323-95449-5.
136. Correa, H.T.; Vieira, W.F.; Pinheiro, T.M.A.; Cardoso, V.L.; Silveira, E.; Sette, L.D.; Pessoa, A.; Filho, U.C. L-Asparaginase and Biosurfactants Produced by Extremophile Yeasts from Antarctic Environments. *Ind. Biotechnol.* **2020**, *16*, 107–116. [CrossRef]
137. Bezus, B.; Cavello, I.; Contreras-Esquivel, J.C.; Cavalitto, S. Pectinases Produced by Extremophilic Yeasts. In *Value-Addition in Food Products and Processing Through Enzyme Technology*; Kuddus, M., Aguilar, C.M., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; pp. 437–452, ISBN 978-0-323-89929-1.
138. Vashist, S.; Sharma, R. Why Settle for Mediocre, When Extremophiles Exist? In *Extremophiles in Eurasian Ecosystems: Ecology, Diversity, and Applications*; Egamberdieva, D., Birkeland, N.-K., Panosyan, H., Li, W.-J., Eds.; Microorganisms for Sustainability; Springer: Singapore, 2018; Volume 8, pp. 435–451, ISBN 9789811303289.

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