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Review

Saccharomyces cerevisiae: a potential stereospecific reduction tool for biotransformation of mono- and sesquiterpenoids

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Abstract

Terpenes and terpenoids are among the key impact substances in the food and fragrance industries. Equipped with pharmacological properties and applications as ideal precursors for the biotechnological production of natural aroma chemicals, interests in these compounds have been escalating. Hence, the syntheses of new derivatives that can show improved properties are often called for. Stereoselective biotransformation offers several benefits to increase the rate of production, in terms of both the percentage yield and its enantiomeric excesses. Baker's yeast (*Saccharomyces cerevisiae*) is broadly used as a whole cell stereospecific reduction biocatalyst, due to its capability in reducing carbonyls and carbon–carbon double bonds, which also extends its functionality as a versatile biocatalyst in terpenoid biotransformation. This review provides some insights on the development and prospects in the reductive biotransformation of monoterpenoids and sesquiterpenoids using *S. cerevisiae*, with an overview of strategies to overcome the common challenges in large-scale implementation.

Keywords: monoterpenoids; sesquiterpenoids; biotransformation; *Saccharomyces cerevisiae*; whole cell; stereospecific reduction

Introduction

Terpenes and terpenoids are natural organic substances that are ubiquitous in all classes of living organisms, forming the largest and most widespread family of natural products. These compounds impart a wide variety of pleasant and floral scents that are used extensively for their aromatic qualities in the perfumery and food industries. To date, over 55 000 terpenes have been isolated, doubling the amount each decade [17]. Generally, both mono- and sesquiterpenoids made up the chief constituents of the essential oils, alongside other aromatic and aliphatic constituents that determine the biological properties of the essential oils [8]. Many of these substances not only act as precursors for new flavour derivatives, but are also biologically active compounds that play important role as building blocks for the preparation of new natural product drugs [2].

The biosynthetic pathways of terpenes and terpenoids are well established. All terpenoids are synthesized through the condensation of C5 isomeric precursors dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), which can be derived either from mevalonate (MVA) or non-mevalonate (MEP) pathways in different cellular compartments of higher plants. The important products generated by the action of enzymes known as prenyltransfereases include geranyl, farnesyl, geranylgeranyl pyrophosphates, squalene and phytoene, leading to the diverse formation of terpenoids [56]. While geranyl diphosphate (GPP) plays the role of precursor for monoterpenes as well as of a number of secondary plant metabolites, farnesyl diphosphate (FPP), the

product of FPP synthase, is the precursor to a wide variety of sesquiterpenoids.

In an investigation of mono- and sesquiterpene biosynthesis by baker's yeast [21], the monoterpenes α-terpineol and linalool were found to be the main products afforded by all the *Saccharomyces cerevisiae* wine yeasts tested. Judging by the effect of nitrogen and redox conditions (microaerobic and anaerobic) on terpenes production, it was suggested that the leucine catabolism pathway (MCC pathway), known to occur in the mitochondria of eukaryotic cells, could account for the formation of the monoterpenoids. Conversely, the sesquiterpenoids followed the cytosolic sterol metabolic pathway already described in yeast [21]. In another study [85], both wild-type and farnesyl diphosphate synthase (FPPS)-defective *S. cerevisiae* mutant strains were also discovered to have the ability to produce monoterpene-derived compounds.

Traditionally, terpenoids can be acquired via physical separation from plant or animal sources. Nevertheless, large-scale productions are often inhibited by several shortcomings, such as seasonal variation, and variability in the composition and yield of the final product is difficult to control. Consequently, the quality and price of the given essential oil frequently fluctuates over the year [10]. Currently, advances in chemical processes have enabled these flavour compounds to be produced synthetically in satisfactory yields and high production rates. However, one of the drawbacks of chemical processes is the formation of undesirable racemic mixtures that could only be avoided through the use of chiral building blocks suitable for the total synthesis of the target molecule. However, these building blocks could hardly be obtained from readily available compounds or natural products [108].

In contrast, biotechnological production provides a feasible alternative for the production of flavour compounds. US and European legislations have acknowledged enzymatic or microbial processes as capable of producing enantiospecific compounds labelled as 'natural', which has been advantageous for the production of flavour compounds [28]. Biotechnological production of terpenoids can be either via *de novo* syntheses using simple substrates like sugar and alcohol or through biotransformation of precursor compounds to flavour end-products.

Biotransformation has always been an active participant in the field of biotechnology and, since the number of available cells and enzymes exploitable in performing selective biotransformation into a myriad of desired products continues to expand, bioconversion processes are moving forward at an increasing rate. In particular, the use of enzymes and several wild-type microorganisms can prevent undesirable secondary reactions and side products [19], besides being less hazardous and toxic than the concomitant use of chemical catalysts. Higher yields, minimal generation of by-products, faster reaction rates, milder reaction conditions and easy downstream processing have also prompted the move towards biotransformation and biocatalysis [7,54]. Consequently, several reviews related to the microbial transformation of terpenes and terpenoids have also been published over the past decade [1,3,6,11,12,27,28,34,82,96,101,106]. Studies on the biotransformations of commonly available and relatively inexpensive terpenoids into higher valuable derivatives are favourable due to their significant economical benefits in the food, perfumery and pharmaceutical industries.

This review encompasses the reductive biotransformation studies on some common monoterpenoids and sesquiterpenoids mediated by baker's yeast, spanning over two decades. An overview of strategies to overcome some of the current bottlenecks is also included, providing an insight that could stimulate the enhancement and development of new value-added products through biotransformation processes.

S. cerevisiae as stereospecific bio-reduction tool

Yeasts in general have been employed as useful biocatalysts, since they possess relatively rigid cell walls that enabled their structures to be retained in the presence of various organic compounds and solvents [72]. Perhaps the most widely exploited and commercially significant yeasts are the related species and strains of *S. cerevisiae*, which have been particularly shown to catalyse compounds with carbonyl groups or carbon–carbon bonds. Therefore, they are extensively employed in a variety of formation and dissociation reactions, such as biodehydrogenation, hydrolysis, oxidation and reduction in organic synthesis [24,37,38,94].

The bioreduction of prochiral C=C double bonds and C=O conjugated double bonds is an

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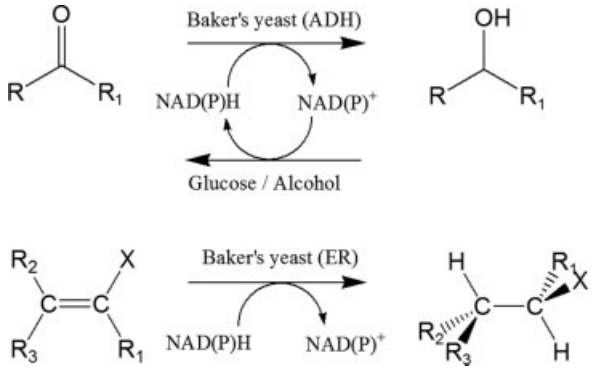

Figure 1. Schematic diagram of co-factor regeneration in baker's yeast reduction process. $NAD(P)^+$, oxidized form; NAD(P)H, reduced form; ADH, alcohol dehydrogenase; ER, enoate reductase; X, electron-withdrawing substituent, e.g. carbonyl-, carboxyl-, imide-, nitro-

important method for preparation of chiral building blocks. Dehydrogenases and reductases have been found to be the key enzymes responsible for catalytic activity in the reduction of carbonyl groups into hydroxyl groups. Alcohol dehydrogenases (ADHs) are responsible for the reduction of carbonyls, while enoate reductases (ERs) account for the reduction of 'activated' carbon–carbon double bonds. The potency of these enzymes is elucidated when they enabled a chiral product to be obtained from a prochiral substrate. However, these enzymes require a nicotinamide co-enzyme, NAD(P)H, from which a hydride is transferred to the substrate carbonyl carbon. The oxidized form of the co-enzyme, $NAD(P)^+$, will be transformed back to its reduced form for the next cycle of the reduction process. For that instance, alcohols and glucoses (co-substrates) serve as great hydrogen sources. Figure 1 illustrates the co-factor regeneration of ADH and ER in biocatalytic reduction process with baker's yeast. The co-factor is regenerated alongside the presence of the co-substrate but, upon depletion, the regeneration process and eventually the reduction process will be compelled to stop. Therefore, the rates of both reactions should be equalized to provide an optimum bioreduction system.

The use of baker's yeast as a stereospecific reduction biocatalyst has been well documented in the literature. Enantioselective reductions of various compounds, including dicarbonyl compounds (especially α- and β-diketones, keto esters and cycloalkanediones) [35,40,58,60,108], oxocarboxylic acids [47], alkyl acetates [23,115] and aromatic ketones [18,67] have been successfully carried out, obtaining products with high enantiomeric excess (ee) in acceptable yields. It is widely used for the asymmetric reduction of prochiral ketones because such reductions with these microorganisms are readily executed and inexpensive. In contrast, studies involving the use of baker's yeasts for the biotransformation of terpenes are less available, which has been partly attributed to the competitiveness of other microorganisms as viable biocatalytic agents, impeding the exploration on the full potential use of this species. To date, published reports have been restricted to only a few common monoterpenoids and sesquiterpenoids that employed baker's yeasts as reductive biocatalysts in the bioconversion processes.

Reductive biotransformation of monoterpenoids using *S. cerevisiae*

Monoterpenes comprising 10 carbon with two isoprene units dominate in most essential hydrocarbons and belong to the most representative molecules, constituting 90% of the essential oils. Monoterpenoids can exist as alcohols, ketones, aldehydes or ethers, in acyclic, monocyclic or bicyclic structures. They possess strong sensory qualities, which instigated their extensive use in the flavour and fragrance industries. Table 1 shows the chemical structures of five monoterpene alcohols commonly used because of their low sensory threshold, including their respective odours and plant origins.

It is well-known that herbs and spices exhibit antimicrobial activity and the principle behind this is often attributed to the essential oil present in these products. A number of studies have found that the monoterpenoid group possesses the most antimicrobial properties [5,33,100]. These substances may be useful in clinical situations where infectious diseases caused by the alarming emergence of a large variety of pathogenic bacteria in humans can be controlled and treated. Tests performed have also revealed that they play a major role as potentially anti-inflammatory agents [86] and provide pharmacological antinociceptive/antihyperalgesic effects (reduction of pain

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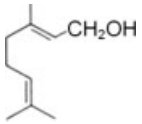
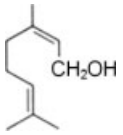
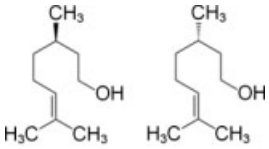
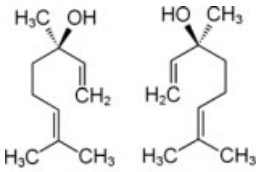
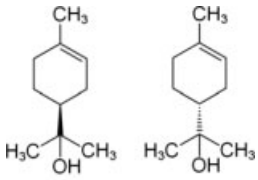
Table 1. Chemical structures, odours and plant origins of various monoterpene alcohols

Monoterpenoid	Chemical structure	Odour[a]	Plant origin[b]
Geraniol		Floral, rose-like	Rose, geranium, citronella, palmarosa
Nerol		Rose-like, fresh green	Rose, palmarosa
R,S-Citronellol	 *R*-enantiomer *S*-enantiomer	Sweet, rose-like, citrus	Rose, geranium, *Rosa rugosa*, ginger, juniper berries
R,S-Linalool	 *R*-enantiomer *S*-enantiomer	Floral, fresh, coriander	Rosewood, freesia, lavender, coriander, basil, citrus leaves and flowers, linaloe wood
R,S-α-Terpineol	 *R*-enantiomer *S*-enantiomer	Floral, lilac	Narcissus, freesia, sage, marjoram, oregano, rosemary, leaf of tea-tree

[a] Adapted from [103].
[b] Adapted from [97].

sensitivity) [87]. Furthermore, monoterpenes and monoterpenoids have been shown to exert antitumour properties, which can effectively prevent the growth of cancer cells and be potentially used in the treatment of cancer [20,50]. In addition, these compounds have long been used by our ancestors as the active ingredients that produce effective natural plant-based insect repellents [9,48], which now find their potential to prevent and control the outbreak of insect-borne diseases.

Mayer and Neuberg (1915) reported the first work dealing with microbial transformation of terpenes in the synthesis of (+)-citronellol from (+)-citronellal mediated by yeast cells [39]. Stereospecific bioreductions usually involved monoterpenoids, since isolated carbon–carbon double bonds without an allylic alcohol, carbonyl or other 'activating' substituent are only rarely reduced by microorganisms [105]. The capability of baker's yeast in reductive biotransformation was elucidated in some reported studies involving optically active monoterpenoids, such as (+/−)-citronellal [22, 118], (+/−)-linalool [73] and (+/−)-camphor [61].

Acyclic monoterpenoids

Stereospecific reductions of both cinnamyl alcohols [52] and aliphatic allylic alcohols [51] using yeasts have been reported. The latter successfully obtained satisfactory yield of (*R*)-(+)-citronellol in enantiomerically pure form reduced from geraniol, using a suspension of resting cells of *S. cerevisiae*.

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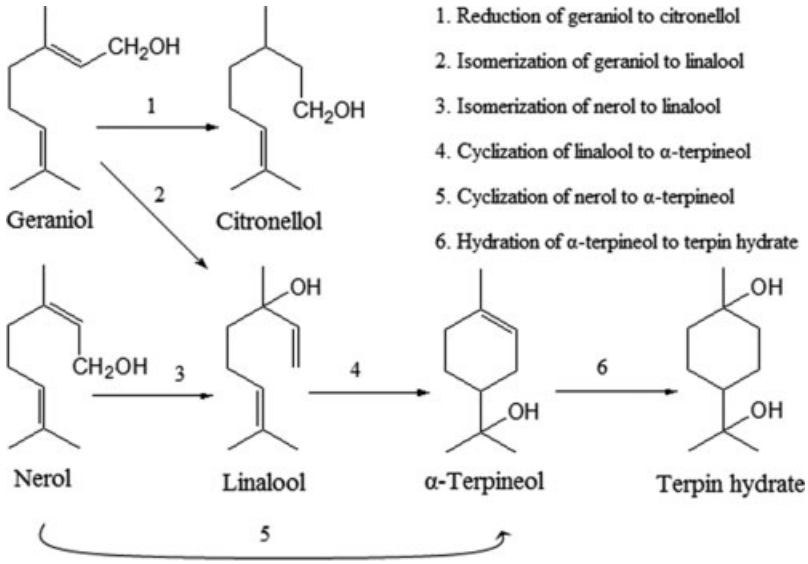

Figure 2. Scheme of monoterpenoid biotransformation reactions catalysed by *S. cerevisiae* [modified from 64]

In contrast, King and Dickinson [64] discovered that the production of linalool and α-terpineol from biotransformation of geraniol and nerol resulted in something akin to a racemic mixture, thus displaying a non-stereospecific production by the yeast. No products were found for the biotransformation of citronellol, while it was deduced that the α-terpineol formed was probably a result of transformation from linalool. In addition, α-terpineol was discovered to undergo spontaneous hydroxylation to form diol *cis*-terpin hydrate. Figure 2 depicts the overall biotransformation processes of various monoterpene alcohols involving *S. cerevisiae* as a biocatalyst.

Baker's yeast has been widely employed as a versatile biocatalyst for organic synthesis in aqueous mixtures. However, due to the hydrophobic nature of the substrates and products, reductive biotransformation of organic compounds such as monoterpenoids often suffers from low yield. Thus, the implementation of biocatalytic processes in organic solvent-containing media provides several advantages over conventional aqueous media, which include improving the solubility of poorly water-soluble compounds, suppressing undesirable side reactions and product inhibition that will enhance the overall performance of the reactions [81]. Investigations of reductive biotransformations using whole cells of *S. cerevisiae* in organic solvents [46] and aqueous–organic solvents [79, 80] have been well documented. This has subsequently prompted the kinetics study of stereoselective reduction of geraniol to citronellol by *S. cerevisiae* in a biphasic system [32]. The influence of system parameters on the reaction rate was investigated and thereafter enabled simple and accurate measurement of the kinetics leading to parameter optimization. Biphasic aqueous-organic systems are pertinent in bioprocesses as this system can be effectively approached for a wide selection of products. Herein, the choice of organic solvents is the decisive factor that affects the overall performance of the system [88]. The reductive biotransformation of geraniol to citronellol in organic solvents utilizing various yeasts (baker's yeast, XST-KO yeast, LP yeast, alcohol yeast, dry yeast, Angel yeast and Meishan yeast) was also studied, with the results displaying the superiority of *N*-hexane over the use of butyl acetate in the conversion process [119].

The production of citronellal using citral (mixture of geranial and neral) as starting material via chemical synthesis usually involves tedious and high-cost methods, which has led to the development of enantioselective reduction in the presence of enoate reductase [95]. In the particular process, the α, β-unsaturated carbon bond to the carbonyl function is reduced into a single bond, whilst the carbonyl function itself is reduced into the alcohol function. In another study, the enantiospecific reduction of citral to produce citronellal was

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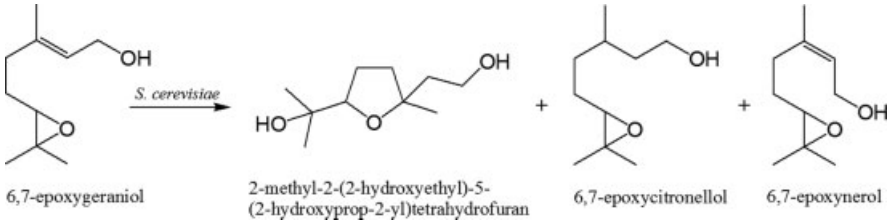

Figure 3. Baker's yeast-mediated biotransformation of 6,7-epoxygeraniol

also investigated, using various strains of yeasts alongside other bacteria and filamentous fungi [75]. The biotransformation reactions performed in an aqueous/organic two-phase system yielded higher selections of microorganisms capable of performing the reduction process in comparison to that of an aqueous system. While the prokaryotic strains (*Zymomonas mobilis* and *Citrobacter freundii*) favour the production of (*S*)-enantiomer, the opposite enantiospecificity was detected for the eukaryotic strains (*Candida rugosa, Saccharomyces bayanus* and *S. cerevisae*), with high ee values observed for both cases. This technique was subsequently patented [102]. In a more recent study, the biotransformation of citral was compared using the free cell method (FCM) and the immobilized cell method (ICM) with *S. cerevisiae*, wherein various parameters and pathway involved were investigated. A mixture of terpenes and terpenoids were produced. Citronellol was acquired as the major component with higher composition found using ICM over FCM [36], probably due to the specific metabolic improvements or products created upon immobilization [121].

Resting cells of *S. cerevisiae* AM464 was employed in the reductive biotransformation of 6,7-epoxygeraniol (a monoterpene epoxide with a rosy-like smell) [4]. The substrate was partly cyclized to 2-methyl-2-(2-hydroxyethyl)-5-(2-hydroxyprop-2-yl)tetrahydrofuran (a furanoid derivative) via an intermediate formed by hydration of the 2,3-double bond with a subsequent closure of the furan ring by oxirane cleavage. Nonetheless, the yield observed (61%) was lower than those obtained from *Yarrowia lipolytica* (99%) and *Botrytis cinerea* (85.4%), due to concomitant isomerization of the substrate to 6,7-epoxynerol and non-stereospecific reduction to 6,7-epoxycitronellol. Figure 3 shows the baker's yeast-mediated biotransformation process and chemical structures of the substrate and respective products. A further probe was conducted on the biotransformation of 6,7-epoxynerol, but neither of the microorganisms tested produced any results.

Koslitz and co-workers [65] successfully compared the enantiomeric purities of *cis*-rose oxide (a chiral monoterpenoid ether) in wines and grape musts by conducting a model fermentation study. Geraniol, citronellol and a mixture of the geraniol-derived diols I and II [(*E*)-3,7-dimethyl octa-2,5-dien-1,7-diol and (*E*)-3,7-dimethyl octa-2,7-dien-1,6-diol] were tested as precursors. By utilizing the deuterium labelling technique, it was shown that two possible NAD(P)H pools exist in baker's yeast, leading to two different pathways for *cis*-rose oxide formation, as illustrated in Figure 4. The stereoselective reduction of 3,7-dimethyl octa-2,5-dien-1,7-diol (geranyl diol I) resulted in labelled 3,7-dimethyl-5-octen-1,7-diol (citronellyl diol I), which gave rise to (+)-*cis*-rose oxide after acid-catalysed cyclization through pathway *b*. While on the other hand, non-selective reduction performed via pathway *a* yielded an almost racemic *cis*-rose oxide. This substantiated the alteration of *cis*-rose oxide enantiomeric ratios in wines, due to the presence of the (+)-enantiomer, compared to the corresponding musts as an ensuing effect of reductive yeast metabolism during fermentation. It was further deduced that nerol-derived diols can also act as direct precursors that yield rose oxide with different enantiomeric ratios.

Cyclic monoterpenoids

Monoterpenoid ketones are cyclic compounds in which a carbonyl group is bonded to two carbon atoms and can exist as monocyclic or bicyclic. Biotransformation of (4*R*)-(−)-carvone and (4*S*)-(+)-carvone were performed using *S. cerevisiae* strain CBS 3093, yielding dihydrocarvone isomers and dihydrocarveols with the stereochemistry at C-2 always *S* for (−)-carvone but the majority

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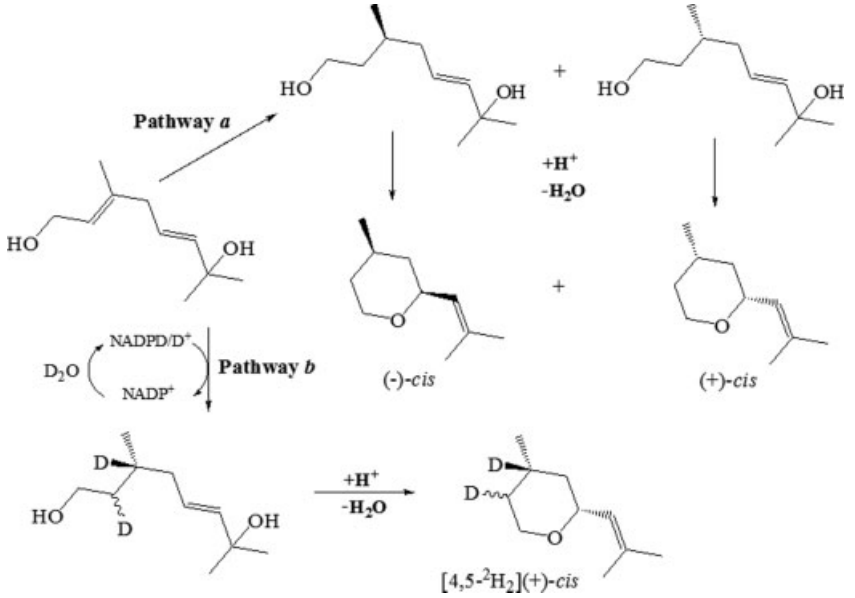

Figure 4. Proposed biosynthetic pathways (a) and (b) for the formation of the labelled and non-labelled rose oxide stereoisomers by yeast fermentation of the model must that contained geranyl diol I as precursor and deuterium oxide as the solvent. Adapted from [65]

of *S* for (+)-carvone [105]. The proposed reaction pathways for the biotransformation of (+)- and (−)-carvone are shown in Figure 5, which are similar to that obtained by *Aspergillus niger* in a previous finding [83]. The reduction processes proceeded firstly through the reductions of the α, β-unsaturated C−C bond of both (*R*,*S*)-carvones, catalysed by enoate reductases to give dihydrocarvones, followed by reductions of the carbonyl group in the dihydrocarvones by alcohol dehydrogenases to form the dihydrocarveols [49].

Reductive biotransformation of (4*S*)-(+)-carvone using freeze-dried *S. cerevisiae* was also investigated [113]. High enantiomeric excess (91%) was observed for the prevalent *S*-enantiomer of neoisodihydrocarveol. It was reported that the use of freeze-dried yeast cells could be considered highly superior to the use of growing and resting ones [49]. Reduction of other monoterpene ketones (menthone and isopiperitenone) to the corresponding alcohols was also reported [113]. The product obtained for menthone is neomenthol, while for isopiperitone, it is isopulegol with isopiperitenol as intermediate. High enantioselectivities (85–89%) were observed for the predominant formation of *S*-enantiomers, showing broad substrate specificities and good yields.

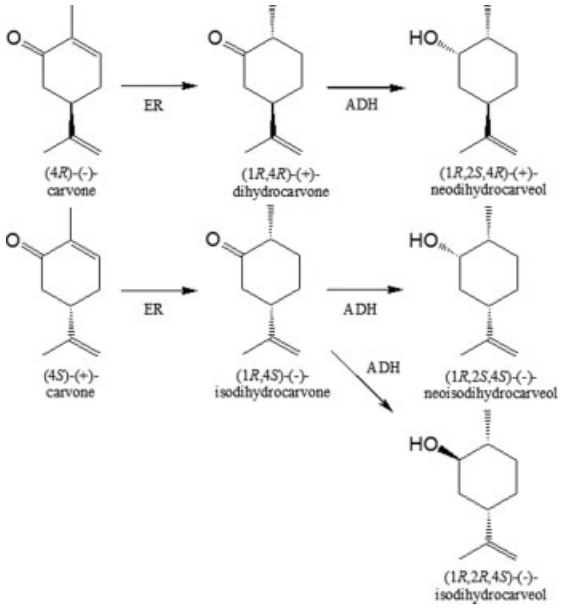

Figure 5. Proposed pathways for reduction of (4*S*)-(+)-carvone and (4*R*)-(−) -carvone to dihydrocarvones and dihydrocarveols. ER, enoate reductase; ADH, alcohol dyhydrogenase

Baker's yeast is capable of reducing the conjugated double bond of carbonyl compounds with high stereo- and regioselectivities, which

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are vital in organic chemistry. The regioselective reduction of the conjugated double bond of perillaldehyde, a monoterpene aldehyde used in the food and perfumery industries, was investigated by means of fermenting baker's yeast in the presence of a non-polar resin [41]. Both (*S*)-(−)-perillaldehyde and (*R*)-(−)-perillaldehyde afforded a mixture of perilyl alcohols and *cis*/*trans*-dihydroperillic alcohols. However, the diastereoselectivities obtained were different and the mechanisms were further elucidated via the deuterium labelling technique and ^{2}H-NMR measurements. The *trans*-isomer of the saturated alcohol was the major diastereoisomer obtained from (*S*)-(−)-perillaldehyde, whereas the reverse was obtained from (*R*)-(−)-perillaldehyde.

Limonene and its derivatives are enantiopure starting materials commonly used as precursors for the synthesis of other useful compounds, due to their inexpensiveness and vast availability. Serra and co-workers [98] synthetically converted both enantiomeric forms of limonene into aldehyde *p*-mentha-1,8-dien-9-al isomers, subsequently reducing the conjugated double bonds into their respective alcohols utilizing baker's yeast. High percentage diastereomeric excesses (96% and 97%) were obtained with good yields, and a new chiral centre formed has high preference for the (*R*) absolute configuration.

Reductive biotransformation of sesquiterpenoids using *S. cerevisiae*

Sesquiterpenoids are terpene derivatives with 15 carbons and consist of three isoprene units. These compounds are found mainly in higher plants and possess biological properties with other desirable characteristics. Apart from being commonly used as flavours and fragrances (except acyclic terpene ketones), it was reported that sesquiterpenes and sesquiterpene lactones displayed antimicrobial activities in several studies conducted [71]. Like their monoterpene counterparts, sesquiterpenes are important constituents in essential oils and have been extensively used as precursors in biotransformation processes, in which the products are usually hydroxylated compounds [15]. However, the flavour value of sesquiterpenes is considered less pronounced than that of monoterpenes [110].

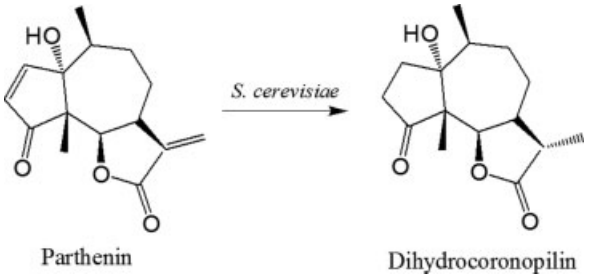

Figure 6. Baker's yeast reduction of parthenin, yielding dihydrocoronopilin

Sesquiterpenoids can exist as acyclic or cyclic (mono-, bi-, tri- and tetra-) in structures.

So far, direct bioconversions of sesquiterpenoids as precursors to target compounds mediated by baker's yeast have seldom been found in the literature. Baker's yeast reduction of parthenin (a sesquiterpene lactone) was investigated for the first time and yielded the naturally occurring dihydrocoronopilin [26]. The reaction proceeded with the reduction of both the double bonds without affecting its carbonyl groups, as shown in Figure 6.

The use of baker's yeast in the reductive bioconversion of several chiral intermediates for the enantioselective synthesis of bisabolane sesquiterpenes was well-established by Fuganti and co-workers [42–44]. The enantioselective preparation of (*S*)-(+)-3-(*p*-tolyl)butan-1-ol by baker's yeast reduction of (*E*)-3-(*p*-tolyl)but-2-en-1-ol was demonstrated [44]. The former compound acted as a useful chiral building block for the synthesis of the most common bisabolane sesquiterpenes bearing a benzylic asymmetric centre of (*S*) configuration (curcumene, turmerone, dehydrocurcumene and nuciferal). 20% isolated yield was obtained with excellent optical purity (ee > 95%) after 3–5 days fermentation at room temperature.

In another study [42], baker's yeast was employed in the bioconversion of unsaturated aldehydes (*R*)- and (*S*)-3-(4-methylcyclohex-3-enyl)but-2-enal into enantiopure saturated alcohol 3-(4-methylcyclohex-3-enyl)butanol, which are also useful chiral building blocks for the synthesis of bisabolane sesquiterpenes, (+)-epijuvabione and (−)-juvabione. The biotransformation process proceeded with high chemical yields and the stereoselectivity of the reduction was strongly influenced by the diastereoisomeric ratio of the substrate, whilst independent of the enantiomeric forms. The

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saturation of the double bond advanced at a slower rate for the *Z* isomer compared to the *E* isomer. In addition, a slow isomerization of the aldehydes was also observed.

The same group also conducted an investigation on the enantioselective synthesis of bisabolane sesquiterpenes (*S*)-(+)-curcuphenol, (*S*)-(+)-xanthorrhizol, (*S*)-(−) -curcuquinone and (*S*)-(+)-curcuhydroquinone [43]. Unsaturated (*E*)-aldehydes were reduced by baker's yeast into saturated alcohols ((3*S*)-3-(2-methoxy-4-methylphenyl)butan-1-ol, (3*S*)-3-(3-methoxy-4-methylphenyl)butan-1-ol, (3*S*)-3-(2,5-dimethoxy-4-methylphenyl)butan-1-ol, with affordable yields (49–56% yield as isolated products) and high optical purity attained (ee > 98%) within 4–6 days of incubation. These compounds will be used as chiral intermediates for the preparation of the phenolic sesquiterpenes.

A large number of sesquiterpenes, possessing the spiro[4.5]decane framework, have been isolated from natural sources [13]. Reductions mediated by baker's yeast were conducted on spiro[4.5]decane-1,4-dione derivatives, in which one of the reduction products was transformed in a few steps to the enantiomerically enriched form of a bicyclic intermediate for the synthesis of the angular triquinane sesquiterpene, pentalenene [120]. The reduction of the diketone provided a single, optically active product in 62% isolated yield, and 23% of was recovered. The yeast-mediated reaction proceeded with high enantioselectivity compared to chemical reduction, despite the poor facial diastereoselectivity.

Challenges and opportunities

Known for their wide applications as ideal precursors for the biotechnological production of natural aroma chemicals and their pharmacological properties, interest in terpenes and terpenoids has been escalating and has impelled the synthesis of new derivatives that can show improved properties. As a supplement to the existing chemists' toolbox in organic chemistry, biotransformation serves as a potential alternative in overcoming the various bottlenecks encountered in chemical processes, thus widening the options in the pursuit of more sustainable methods for the manufacturing of quality compounds. Biotransformation processes have resulted in a vast range of products, including the manufacture of specialty chemicals, such as flavours and fragrances. Although some reported biotransformation studies of terpenes seemed to be the quintessence of viable solutions for high yield and enantiomeric purity, the biotechnological production of these compounds on a large scale remain a challenge [66], despite the escalating demand for naturally produced compounds over synthetic ones. Terpenes and its derivatives are generally both volatile and lipophilic in nature, resulting in various setbacks. Their poor solubility in an aqueous environment often creates an organic layer, due to excess of substrate, and exhibits a rather low chemical stability, causing spontaneous autooxidation processes to take place [39].

While the introduction of organic solvents poses advantages, such as improving the solubility of the substrates, providing the ease of product recovery and preventing unwanted side reactions by water [63], very often organic-solvents raise environmental and health concerns associated with their disposal, in addition to being toxic and affecting the cell viability of biocatalysts, causing deactivation [55]. It has been shown that most organic solvents induce damaging effect towards *S. cerevisiae* strains [45]. This has prompted several studies revolving around the development of more organic solvent-tolerant baker's yeast strains by immobilization with polyurethane [76], entrapment in calcium alginate [91], and repeated use in an organic solvent with occasional reactivation by cultivation [59,63]. Stereoselective reductions carried out revealed an increase in the activities of these immobilized cells and allowed repeated usages for a longer period of time.

In lieu of that, other non-conventional media, such as ionic liquids, have acquired quite a reputation as 'designer solvents', given the fact that they possess favourable characteristics for potential application as reaction media for a diverse range of organic syntheses, including whole cell-mediated reduction processes [16,68]. Howarth and associates [55] pioneered the reductive biotransformation of several ketones to their respective alcohols with immobilized baker's yeast in an ionic liquid–aqueous mixture, with the ee obtained comparable to that of other media. In another study, the investigation of asymmetric reduction of ethyl 2-oxo-4-phenylbutyrate in an ionic liquid–aqueous mixture catalysed by *S. cerevisiae* was carried out, showing improvement in catalytic activity and

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enantioselectivity of the reduction by the addition of ethanol [99]. It was pointed out that ionic liquids can act as substrate reservoirs and *in situ* extracting agents in a multiphase process for the whole cell-catalysed production of fine chemicals [89]. Alternatively, there has been an investigation performed for the reduction of ketones in fluorous media using immobilized *S. cerevisiae* [114]. Perfluorooctane was selected as the medium in the conversion. It was demonstrated that the use of this medium provided a simple approach to obtaining satisfactory yields and enantiomeric excesses, with easy recovery of the solvent without any purification. The resulting effects should prove a great prospect in combining both advantages of baker's yeast cells and non-conventional media, such as ionic liquids and fluorous solvents, in the biotransformation of terpenes.

Most terpenoids are secondary metabolites that cells do not require for growth. Generally, these compounds and/or their precursors result in cytotoxicity, causing the cells to lose membrane integrity and cell lysis [96]. Nevertheless, several strategies have been proposed to prevent the impedance of functionality in the cells during the biotransformation process. This includes employing fungal spores instead of vegetative cells, since the former have more resistance to the toxicity of these compounds [111,112]. The biotransformation of several monoterpenoids, such as geraniol, nerol and citral, using sporulated surface cultures has been well demonstrated [29–31]. Fungal spores maintain prolonged activities and higher substrate concentrations can be applied compared to those with viable cells, without causing toxicity and growth inhibition [29]. On the other hand, biotransformation by bacteria is often perceived to be simpler than those using fungi, attributed to the fact that the former do not form dense mycelia that may hinder the agitation of large-scale reactions when whole-cell (fungal) systems are employed [84]. Nevertheless, the higher metabolic rates of bacteria often result in product degradation instead, in comparison to biotransformation using fungi, which has slower rates, thus leading to desired products in acceptable yields.

Resting cells retain much more enzyme activities than those of growing cells, thus biotransformation using resting cells provides a number of benefits [107]. It diminishes problems related to growing cultures in growth media, such as avoiding overmetabolism, which will suppress the yield of the required products [109], and difficulties in isolating/purifying desired products due to the concurrent presence of other metabolites generated by the microorganisms from components within the medium [92]. Production of terpenoids such as nerol [53], α- and β-ionone [104] has been demonstrated using immobilized cells. Alternatively, the concentration of the compounds in the medium can be kept below a certain amount, so as to prevent the inhibitory effects on microorganisms. In most cases, *in situ* product removal (ISPR) provides a sustainable option [14,69]. The removal of an inhibiting or degrading product from a bioreactor as soon as the product is formed allows more feasible downstream processing and is easier to develop and apply industrially.

Some microorganisms, in particular *S. cerevisiae*, are well known to possess several distinct characteristics, such as process robustness, ability to grow anaerobically, high tolerance to low pH, high sugar and ethanol concentrations that lower the risk of contamination in industrial fermentation [78], in addition to being inexpensive, safe and readily available. However, for many instances, the performance of *S. cerevisiae* as a biocatalyst is often outshone by other organisms. Many of the reductases involved have yet to be fully characterized and are still within the research phase. In addition, low yields and low enantiomeric purities due to the different stereospecificities of the reductases present in a single cell have often limited its usage as a versatile biotransformation tool. Again, studies on the use of immobilized cells have been attempted to overcome this problem [74,116], while others have sought improvement using engineered recombinant strains and process design [40,57]. Combining overexpression and knockout strategies would significantly enhance the levels of stereoselectivity of the baker's yeast [93]. Alternatively, the enantioselectivity of the reduction process can also be controlled using thermal pretreated baker's yeast, which has been successfully carried out in the reduction of 3-oxoalkanoates [62,117], α-diketones [70,77] and 2,4-alkanediones [25]. Addition of poisoning agents that inhibit undesirable enzymatic activities, the use of a two-phase system, substrate modifications and a change of energy source (carbon or growing conditions) have been shown to provide equal usefulness [90,93].

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Existing successful chemical processes and several biocatalyst-associated problems, such as lack of stability and limited operating range, have often thwarted efforts to substitute them with biotransformation processes. Several reported processes attempting to produce a single target compound often led to impediments, such as complicated downstream processing [27]. Furthermore, the availability of enzymes and tailoring them for specific scope of biotransformation reactions are still within conservative amounts, which explains why biotransformation of monoterpenoids using baker's yeast has yet to undergo scale-up implementation. Nevertheless, numerous efforts have been carried out to engineer and characterize new enzymes and more potent microorganism strains. The revolutionary breakthrough in metabolic engineering has opened up possibilities in the manifestations of engineering strategies to develop cells with strain improvements. The combination of high-throughput screening methods and directed evolution, alongside other novel approaches, has opened out the strains and enzymes databases, elucidating the pathway to cater for the needs of 'green chemistry' development. The progress in fermentation technology and fine-tuning of substrate/product integrated bioprocesses will also lead to enhancement for optimized biotransformations.

Microorganisms and their enzymes as superior catalysts have shown remarkably high chemo-, regio- and enantioselectivity, which favour the synthesis of chiral compounds under mild conditions, an additional mode for energy savings. As challenges remain for new technology to make a paradigm shift, bioconversion routes still possess great commercial interest for the food and perfumery industries, since utilizing biotechnological methods for the production of flavour and fragrances enables the products to be labelled as 'natural'. This has subsequently led to extensive studies being imposed on biotransformation of terpenes as well.

Conclusion

The capability of *S. cerevisiae* in carbonyl and carbon–carbon double bonds reductive biotransformation has been well-defined and henceforth, made it possible to perform biotransformation of terpenes and their derivatives. Nevertheless, due to non-optimized reduction conditions and overlapping activities of the cells, biotransformation of monoterpenoids using *S. cerevisiae* has yet to make its way to large-scale implementation. Despite the many drawbacks, baker's yeast remains the most sought-after biocatalytic tool in organic chemistry. Several strategies have been imposed to resolve the major prpblems faced during the biotransformation process, as well as to improve the overall performance of *S. cerevisiae*. This helps not only to achieve a lucid understanding of the biochemical pathways of terpene biotransformation, but also to find alternative synthetic routes towards highly valuable industry-relevant flavour and fragrance compounds from cheap natural precursors.

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