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Rhodotorula genus: vast knowledge, limited commercialization – What's holding us back?

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ABSTRACT

Recently, the oleaginous yeast *Rhodotorula*, belonging to the phylum Basidiomycota, has emerged as a sustainable biotechnology microorganisms due to its unique ability to produce bioactive and functional carotenoids and lipids. This review outlines the possible applications of *Rhodotorula* biomass, carotenoids and lipids in various sectors including food, feed, agriculture, pharmaceuticals, cosmetics and energy. Moreover, this review is highlighting the key barriers limiting the commercialization of *Rhodotorula*-derived products while suggesting potential solutions an such as the utilization of low-cost feedstocks. Further, the methods and approaches to boost the biomass, carotenoids and lipids are also discussed while indicating the main opportunities to explore for sustainable biotechnology and viable commercialization of this yeast.

1. Introduction

Microbial biotechnology, driven by the vast metabolic diversity of microorganisms, is at the forefront of innovation, offering transformative solutions across a wide range of industries, including food production, animal feed, pharmaceuticals, cosmetics, or for the purpose of environmental management. Microbial processes are increasingly employed to produce biomass and metabolites via fermentation, which can be used in the food and feed industries, to develop biotechnological systems for antibiotics and vaccines and in the biodegradation of environmental pollutants for environmental protection (Satyanarayana and Kunze, 2009; You et al., 2021; Fouillaud and Dufossé, 2022; Akki et al., 2024; Santos Beneit, 2024).

Yeasts are notable for their ability to convert chemical inputs into biological forms through various metabolic pathways. Their adaptability enables them to thrive in diverse environments, including extreme conditions. Historically, yeasts have been essential in baking and brewing, with species such as *Saccharomyces*, *Torulaspora delbrueckii*, *Lachancea thermotolerans*, and *Metschnikowia pulcherrima* widely used for bread leavening and flavor enhancement in wine-production (Vejarano and Gil Calderón, 2021). Since the mid-20th century, advancements in yeast biotechnology have expanded significantly. Leveraging their genetic diversity and unique physiological characteristics, yeasts are now cultivated to produce a range of valuable substances, including proteins, vitamins, polyunsaturated fatty acids, carotenoids, and enzymes. Recently, yeasts have emerged as promising living-cell factories for generating biofuels and beneficial biochemicals, with applications spanning the food, feed, pharmaceutical, and cosmetic industries (Nandy and Srivastava, 2018; Karim et al., 2020; Suchová et al., 2022; Godana et al., 2024). Among the various yeast strains, the *Rhodotorula* genus has garnered particular interest due to its unique biological and physiological traits. Research indicates that *Rhodotorula*

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species, such as *R. glutinis*, *R. mucilaginosa*, and *R. toruloides*, can thrive in diverse environments, including polluted waters, and produce bioactive carotenoids, lipids, and enzymes (Mahajan et al., 2024). These characteristics position *Rhodotorula* species as promising candidates for various applications. Specifically, *Rhodotorula* is recognized for synthesizing carotenoids like β-carotene, torulene, and torularhodin, which serve as valuable natural colorants, and antioxidants (Kot et al., 2016; Zhao et al., 2019; Ochoa Viñals et al., 2024).While microalgae strains such as *Dunaliella, Haematococcus,* and *Chlorella,* are currently leading industrial producers of natural carotenoids, their production faces challenges, including specific cultivation requirements, water loss in open cultivation facilities, low productivities, contamination risks, and sensitivity to environmental conditions (Wang et al., 2019; Papadaki and Mantzouridou, 2021; Xie et al., 2022 Razzak, 2024; Duan et al., 2024). Although *Rhodotorula* species generally yield lower amounts of β-carotene compared to microalgae, they can simultaneously produce other valuable carotenoids and lipids. Furthermore, *Rhodotorula* exhibits a higher specific growth rate facilitates rapid biomass production, and its ability to utilize diverse carbon and nitrogen sources, including food wastes, presents a cost-effective strategy for large-scale production (Schneider et al., 2013; Cheng and Yang, 2016; Yen et al., 2019; Kot et al., 2020; Mussagy et al., 2022; Angelicola et al., 2023; Keskin et al., 2023).

Despite these advantages, the commercialization of *Rhodotorula* and its bioproducts remains constrained. A substantial gap persists between intensive academic research and industrial -scale applications. High production costs, technical limitations, food/feed safety concerns, and insufficient awareness among potential industrial users contribute to this disparity (Mussagy et al., 2022). Furthermore, the development of sustainable downstream processing platforms for extracting and purifying target compounds is crucial, given the complex chemical composition of *Rhodotorula* species.

This review aims to present a comprehensive overview of strategies for optimizing cultivation conditions to enhance *Rhodotorula* biomass and valuable metabolites production, focusing on carotenoids and lipids, as well as their applications across various sectors. It will also examine the metabolic flexibility of *Rhodotorula* in adapting to low-cost substrates, including agro-industrial wastes, which are vital for the economic feasibility of processes involving this genus. Finally, the review addresses the factors contributing to the limited commercialization of *Rhodotorula*, identifying key challenges and potential strategies to overcome these limitations. A better understanding of these barriers could help unlock the full industrial potential of this promising yeast genus.

2. *Rhodotorula* genus: taxonomy, morphological and physiological characteristics

Rhodotorula is a genus of basidiomycetous yeasts classified within the phylum Basidiomycota, class Microbotryomycetes, order Sporidiobolales, and family Sporidiobolaceae. Species within this genus typically exhibit spherical or ellipsoidal cell morphology, with diameters ranging from 2 to 10 μm. Colonies display a smooth surface and creamy texture, often presenting a color spectrum from pink to orange due to intracellular lipid droplets enriched with carotenoids (Wang et al., 2015; Kot et al., 2016). These yeasts are unicellular, immobile, saprophytic eukaryotes that demonstrate typical yeast-like physiological traits (Li et al., 2022; Zhao and Li, 2023).

Rhodotorula species are widely distributed in a variety of ecological niches including extreme and polluted environments (Saha and Seal, 2015; Zhao et al., 2019). They have also been identified as endophytes within the roots of plants. Physiologically, *Rhodotorula* species show optimal growth within a temperature range of approximately 20 °C–30 °C and rely on obligatory aerobic conditions for metabolic processes (Hamidi et al., 2020). These yeasts demonstrate notable metabolic flexibility, utilizing a broad spectrum of carbon sources such as glucose, galactose, sucrose, maltose, trehalose, ethanol, and glycerol (Kot et al., 2020). Under nutrient limitation or exposure to abiotic stress, *Rhodotorula* cells enhance pigment synthesis, resulting in increased accumulation of lipids and carotenoids (Lee et al., 2017).

Although most *Rhodotorula* species are non-pathogenic and exhibit low virulence, isolated cases of pathogenicity have been reported, primarily causing dermatomycosis in immunocompromised individuals. In healthy individuals, the risk of infection is exceedingly low, and *Rhodotorula* is generally not considered a significant pathogen outside of hospital environments or settings involving severely immunocompromised patients (Sabaté et al., 2002; Mohd Nor et al., 2015; Wang et al., 2019). Importantly, the biotechnological use of *Rhodotorula* species does not pose a risk to human health since their application could focus on the yeast's metabolic activities, with minimal direct interaction with human systems, further minimizing the risk of infection (Li et al., 2022b).

3. Potential applications of *Rhodotorula* spp

In the pursuit for sustainable solutions, the exploration of natural microbial resources is essential. Within this framework, *Rhodotorula* emerges as a promising organism offering diverse applications spanning industries such as food, agriculture and environment fields (Kot et al., 2018; Mussagy et al., 2022) (Fig. 1).

3.1. Environmental applications

The *Rhodotorula* genus, demonstrates a significant potential as a bioremediation agent particularly due the ability to sequester heavy metals within vacuoles. Recent studies underscore the benefits of employing biological systems such as *Rhodotorula* over physical or chemical methods for pollutant remediation, highlighting its potential for eco-friendly and cost-effective environmental management (Singh and Prasad, 2015; Gavrilescu et al., 2015).

A notable example is *Rhodotorula mucilaginosa*, which has demonstrated effective heavy metal detoxification. The strain *R. mucilaginosa* RO7, noted for its resistance to lead (Pb), has shown remarkable efficiency in Pb removal, purifying Pb concentrations of 31.25 ppm with 95.32 % efficiency. Even at higher Pb concentrations (125 ppm), a biomass of just 0.6 g achieved a removal efficiency of 15.55 mg g^{-1} (Aibeche et al., 2022). Similarly, *R. mucilaginosa* AN5 has been effective in mitigating cadmium (Cd) stress through significant metabolic adjustments, proving its potential for Cd bioremediation (Zhang et al., 2022). Beyond heavy metal detoxification, *Rhodotorula* species show versatility in addressing other pollutants. For example, *R. mucilaginosa* RCL-1has proven effective in remediating copper (Cu II)-contaminated environments while simultaneously producing high levels of carotenoids (Irazusta et al., 2013). The yeast *R. mucilaginosa* also has the ability to remove manganese ions. This highlights the genus's broad applicability in various contamination scenarios. Moreover, *R. mucilaginosa* also contributes to wastewater treatment. It has effectively degraded phenolic compounds and reduced chemical oxygen demand (COD) in olive mill wastewater (Karakaya et al., 2012; Boutafda et al., 2019).

Research indicates that *R. mucilaginosa* can degrade several phenolic compounds, including protocatechuic, vanillic, and p-coumaric acids, as well as tyrosol, gallic acid, and catechol (Jarboui et al., 2012b). When used in combination with *Aspergillus niger, R. mucilaginosa* significantly enhanced wastewater treatment, achieving chemical oxygen demand (COD) removal rates between 56.71 % and 98.02 %, and polyphenolic compound removal ranging from 45 % to 94 % (Jarboui et al., 2013). Although fungal growth kinetics decreased with higher wastewater concentrations, sequential use of these fungi enhanced overall treatment efficiency.

Despite these promising findings, the practical application of *Rhodotorula* in bioremediation still requires further exploration. Future research should focus on optimizing conditions for maximum efficiency and investigating potential limitations, such as the scalability of these processes and their long-term impact on ecosystems. Overcoming these

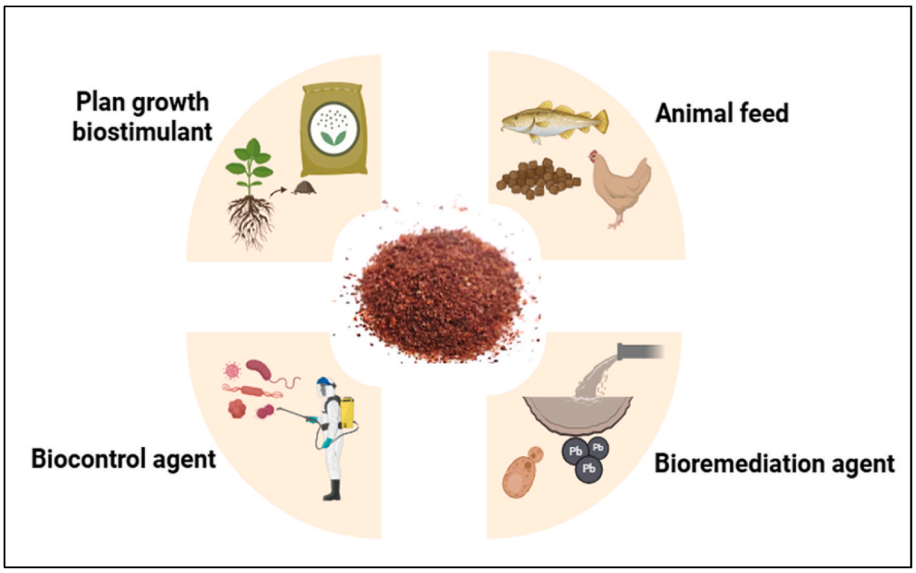

Fig. 1. Applications of biomass from the *Rhodotorula* genus.

barriers will be essential to fully realize the potential of *Rhodotorula* in environmental remediation.

3.2. Agricultural applications

3.2.1. Biological control agent

Rhodotorula species have shown notable potential as biological control agents for managing postharvest pathogens in fruits. Sandberg (2019) reported the antagonistic activity of *R. babjevae* and its extracellular metabolites against a range of pathogens, indicating its promise for practical applications. Similarly, *R. mucilaginosa* has proven effective in controlling *Phytophthora capsici*, pathogen responsible for basal stem rot in black pepper plants (Safitri et al., 2021). Zhang et al. (2014) further investigated the biocontrol properties of *R. mucilaginosa* against common postharvest fungi such as *Rhizopus stolonifer* and *Botrytis cinerea*, that affect strawberries and pears. Setiawan et al. (2020) demonstrated the antagonistic potential of the *Rhodotorula minuta* Dmg 16 BEP strain against the *Alternaria solani* mould causing diseases of tomatoes and peppers highlighting the versatility of the strain.

In addition, *R. glutinis* has demonstrated insecticidal activity against *Trichoplusia* and *Aedes aegypti*, suggesting its potential for integrated pest management. The effectiveness of *Rhodotorula* species is enhanced combination strategies. For instance, *R. mucilaginosa* combined with salicylic acid has shown effectiveness in controlling *Penicillium digitatum* infections in oranges (Ahima et al., 2019). This strain has also proven successful in reducing *P. expansum* contamination and patulin production in apples when applied individually or in combination with compounds like phytic acid (Yang et al., 2015; Li et al., 2019, 2022b; Tang et al., 2019; Qian et al., 2020).

Despite these encouraging findings, the full potential of *Rhodotorula* species in biocontrol applications requires further investigation. While the production of melanin pigments and insecticidal toxins may enhance efficacy, more mechanistic studies are needed to understand how these compounds contribute to pathogen control. Future research should include comparative assessment of *Rhodotorula* strains, investigations into their interactions with other biocontrol agents, and studies on the specific molecular mechanisms of their pathogen management capabilities. These targeted studies will be essential for developing improved and environmentally sustainable biocontrol strategies.

3.2.2. Plant growth biostimulants

The biomass derived from the genus *Rhodotorula* exhibits significant potential for promoting plant growth. For example, *R. mucilaginosa* JGTA-S1, an endophytic strain, acts as both a microbial fertilizer and probiotic by supplying ammonium nitrogen to plants and enhancing nitrogen nutrition through its interaction with the *Pseudomonas aeruginosa* nitrogen reductase gene (Sen et al., 2019). Studies on rice (*Oryza sativa*) treated with *R. mucilaginosa* JGTA-S1 has shown a 2.62-fold increase in relative nitrogen use efficiency compared to controls (Paul et al., 2020). In addition to its role in nitrogen nutrition, *R. mucilaginosa* YP 187, carotenoid-producing yeast isolated from soil, has been investigated for its fermentation conditions. Although the direct effects on plant growth were not explicitly reported, carotenoids are known to function as plant growth regulators and stress mitigators. Further research is needed to elucidate how carotenoid production by *R. mucilaginosa* YP 187 specifically contributes to plant growth and stress tolerance (Manimala and Murugesan, 2014; Silambarasan et al., 2019). Future research should also explore other *Rhodotorula* species and compare their growth-promoting effects with those of other biocontrol agents and fertilizers. Comprehensive mechanistic studies on how carotenoids and other biocontrol factors influence plant physiology will be essential. Bridging these knowledge gaps will advance our understanding and support the development of effective and environmentally sustainable agricultural applications for *Rhodotorula* species.

3.3. Food and feed application

Research findings indicate that *Rhodotorula* strains, such as *R. glutinis* and *R. mucilaginosa*, provide notable benefits as feed additives. Incorporating *Rhodotorula* into animal diets, including those for laying hens and piglets, has been shown to significantly enhance growth performance, antioxidant capacity, and egg quality, while also maintaining a balanced intestinal microbial environment (Xu et al., 2023). Notably, the capacity of *Rhodotorula* to produce carotenoids contributes to improved egg yolk pigmentation and enhanced oxidative stability (Sundararajan and Ramasamy, 2024). These carotenoids are also valuable in aquaculture, where enhance pigmentation of flesh and eggs (Ram et al., 2020). Rekha et al. (2024) investigated the use of carotenogenic marine yeasts as pigment enhancers in the diet of koi carp (*Cyprinus carpio*). Their study demonstrated that incorporating the marine yeast *Rhodotorula paludigena* VA242 into the feed led to more intensely pigmented fishscales compared to those fed a control diet. Similarly, Wang et al. (2015) found that supplementing diets with *Rhodotorula benthica* D30 as a probiotic significantly reduced the cumulative mortality of sea

cucumbers (*Apostichopus japonicus*). This supplementation also enhanced growth performance, digestive enzyme activities, immunity, and disease resistance in *A. japonicus*.

Given the bans on antibiotics and antibiotic growth promoters in animal feed imposed by entities such as the European Union, the United States (since 2006), and China (since 2020), alternative strategies have attracted increasing attention. Incorporating *Rhodotorula* species, including *R. benthica*, *R. mucilaginosa*, and *R. paludigena*, into animal feeds has become a viable strategy for promoting health and optimizing zootechnical growth performance (Thumkasem et al., 2023). For instance, research on *R. mucilaginosa* TZR2014 highlights its potential as a growth promoting probiotic for weaned piglets, enhancing growth, antioxidant capacity, gastrointestinal digestion, and maintaining intestinal microbial balance (Hu et al., 2022). Similarly, Sriphuttha et al. (2023) confirmed the efficacy of *R. mucilaginosa* as a probiotic supplement in shrimp feed, resulting in improved growth performance, enhanced immune responses, and better meat quality.

Despite these promising applications, it is important to note that some *Rhodotorula* species, such as *R. mucilaginosa*, *R. glutinis*, and *R. minuta*, have been identified as emerging pathogenic strains capable of forming biofilms and they are not currently classified as Generally Recognized As Safe (GRAS) (Kot et al., 2018). However, safety studies on *R. gracilis* indicate that acute and sub-acute oral administration of lyophilized whole cells for 14 weeks did not produce toxic effects in rats (Naidu et al., 1999). Additionally, *Rhodotorula*-enriched feed has been tested and found to be safe and non-toxic for animals, including in laying hen diets. Nevertheless, the high cost of production remains a significant barrier to the widespread use of *Rhodotorula* yeasts. The high costs associated with modern technologies and the intensification of fermentation processes for carotenoid production is key obstacles (Kot et al., 2016; Mussagy et al., 2022).

4. Carotenoids from *Rhodotorula*: types, structures, and industrial relevance

4.1. Types, structure and biosynthesis pathways

Carotenoids constitute a structurally diverse class of lipophilic pigments that impart yellow, orange, and red colors to numerous organisms. These pigments absorb light in the 400–500 nm range (Zhao et al., 2019; Mussagy et al., 2021; Mussagy et al., 2025a) (Fig. 2). Chemically, carotenoids are isoprenoid derivatives composed of 40-carbon chains with varying numbers of conjugated double bonds, which underlie their coloration and bioactivity (Hernández Almanza et al., 2014). Within microbial producers, the genus *Rhodotorula* has emerged as a valuable source of high-value carotenoids, particularly in *R. mucilaginosa*, *R. glutinis*, and *R. toruloides*. Among these, β-carotene accounts for nearly 70 % of the total carotenoids (Tang et al., 2019; Li et al., 2022b; Liu et al., 2023). With its 11 conjugated double bonds and two retinyl groups, β-carotene functions as a key natural colorant and dietary supplement, offering benefits such as immune system support, cardiovascular protection, and anti-carcinogenic properties (Schierle et al., 2004; Carocho et al., 2015; Pi et al., 2018; Allahkarami et al., 2021).

Rhodotorula species are also able to synthesize torulene and torularhodin with distinct chemical modifications that increase their antioxidant potential. Torulene is a hydrocarbon polyene and torularhodin possesses oxygenated groups, including keto groups (Tang et al., 2019). Both possess diverse bioactivities. Indeed, torulene acts as a photoprotective compounds against UV-B radiation and exhibit a strong antimicrobial and anticancer effects, while torularhodin demonstrates a strong neuroprotective property (Du et al., 2017; Li et al., 2019a; Mussagy et al., 2025a,b). Further, species such as *R. toruloides*, *R. paludigena*, and *R. mucilaginosa* have been shown to synthetize astaxanthin (Huang et al., 2018; Tran et al., 2019; Phuengjayaem et al., 2023; Kingkaew et al., 2023; Hoondee et al., 2024). Astaxanthin is a red xanthophyll carotenoid with a conjugated polyene backbone and terminal β-ionone rings that are substituted by hydroxyl and keto groups (Stachowiak and Szulc, 2021). Its strong antioxidant property allows for diverse applications in foods, nutrition, cosmetics, and pharmaceuticals sectors, where it supports metabolic regulation, reduce oxidative stress and UV-induced damage, and delivers anticancer properties (Sun et al., 2025; Zhu et al., 2025).

Carotenoid biosynthesis in *Rhodotorula* follows the mevalonate pathway. Here, acetyl-CoA is first converted to HMG-CoA by HMG-CoA synthase, then to mevalonic acid (MVA), and subsequently to isopentenyl pyrophosphate (IPP), the universal isoprenoid precursor (Ochoa-Viñals et al., 2024; Simpson et al., 1964; Goodwin, 1961). IPP and its isomer DMAPP condense to form geranyl pyrophosphate (GGPP), which serves as the branching point toward carotenoid biosynthesis (Kot et al., 2016). Two GGPP molecules form phytoene, which undergoes desaturation to lycopene. Lycopene can then be cyclized into γ-carotene and β-carotene by lycopene cyclase/phytoene synthase, while its derivative 3,4-didehydrolycopene gives rise to torulene and, through hydroxylation and oxidation, to torularhodin (Hernández Almanza et al., 2014).

Despite advances, the biosynthetic pathway including, the metabolic route and regulatory mechanisms, for astaxanthin production in *Rhodotorula* strains is still insufficiently elucidated. This lack of genomic data restricts therefore the genetic targets for strain improvement.

Only a few numbers of studies manipulating both whole-genome

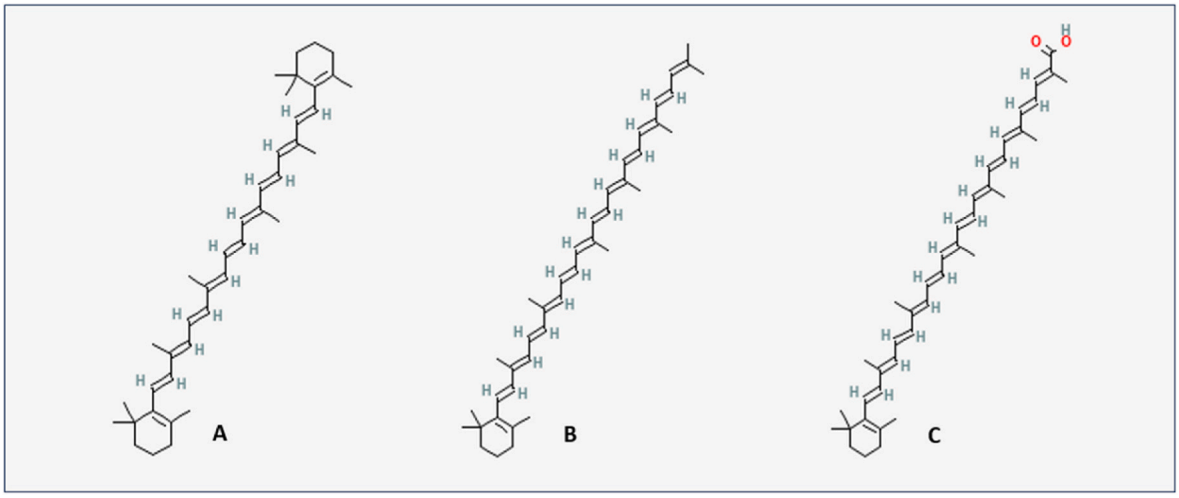

Fig. 2. Structures of β-carotene (A), torulene (B), and torularhodin (C).

sequencing and gene-level analyses have explored astaxanthin production in *Rhodotorula* (Tran et al., 2019; Phuengjayaem et al., 2023; Kingkaew et al., 2023). Previous research suggested the pathway involves intermediates such as lycopene, β-carotene, and echinenone, with a distinct route from β-zeacarotene to trans-astaxanthin (Andrewes et al., 1976). Recently, Hoondee et al. (2025) have identified key genes, including CrtE, CrtYB, CrtI, CrtS, and CrtR, as crucial to astaxanthin biosynthesis in *R. mucilaginosa* strain HL26-1 and *R. paludigena* strain LL69-1. The findings of this study highlight their essential role in pigment formation and establish promising targets for future metabolic engineering strategies.

4.2. Potential applications of carotenoids produced by Rhodotorula

Carotenoids produced by *Rhodotorula* strains have attracted significant attention due to their natural origin, health-promoting properties, and wide-ranging applications across food, pharmaceutical, and cosmetic sectors (Fig. 3). Their role as antioxidant and functional agent makes them promising alternatives to synthetic additives, aligning with the demand for safe, sustainable, and clean-label ingredients.

In the food and nutrition sectors, carotenoids, specifically β-carotene, play a vital role as precursors of vitamin A, which supports vision, immune response, and cellular differentiation (Blomme et al., 2020; Watkins and Pogson, 2020). Supplementing food and diets with provitamin is particularly beneficial for populations at risk of deficiency (Mayo-Wilson et al., 2011; Kot et al., 2018). Moreover, *Rhodotorula*-derived carotenoids can also be employed as natural food colorants, conferring appealing red, orange, or yellow colors to dairy products, confectionery, and beverages (Yoo et al., 2016). Despite all these advantages, *Rhodotorula* strains used for carotenoid production still lack GRAS status, and further research is needed to confirm their safety and acceptable intake levels (Kot et al., 2018; Sen et al., 2019; Jimenez Lopez et al., 2020). In the pharmaceutical sector, carotenoids produced *Rhodotorula* are attracting attention for their potential as bioactive molecules in both disease prevention and therapy.

β-carotene has been reported to trigger apoptosis in cancer cells by activating caspase-3 (Park et al., 2005). Other carotenoids like torulene and torularhodin have demonstrated tumor-suppressing activity, including the ability to slow prostate cancer progression in animal models (Du et al., 2017; Li et al., 2019a). Torularhodin has also appears to offer protection against disorders linked to oxidative stress such as alcoholic liver disease and Alzheimer's-related neuroinflammation (Li et al., 2019b; Zhang et al., 2020). In cosmetics, carotenoids are valued not only for their skin protective properties but also for improving the stability of formulations. β-carotene, torulene, and torularhodin have the potential to act like a natural shield against UV-induced oxidative stress, helping to delay premature aging and maintain skin health. Formulations enriched with carotenoids provide further defense and cutaneous protection by reducing ROS-mediated damage from sunlight exposure. Meanwhile, astaxanthin-based soaps and other antioxidant-enriched products have been already developed and tested, proving to be safe and non-irritating in therapeutic and skincare applications (Anunciato and da Rocha Filho, 2012; Mussagy et al., 2025b; Adamantidi et al., 2025).

5. Lipids from *Rhodotorula*

Strains from *Rhodotorula* genus are well-known for their high ability to accumulate large contents of lipids and fatty acids. Indeed, these yeast cells can accumulate more than 60 % of their dry weight as lipids, with the main fatty acids being palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) (Sundaramahalingam et al., 2022; Robles-Iglesias et al., 2023). Owing to this ability, *Rhodotorula* species reinforcing their position as a potent candidate for sustainable industrial lipid production. In this context, they offer numerous advantages such as relatively low production costs, high lipid yields, the ability to achieve high-density-cell in basic bioreactors, and the availability of genetic engineering tools to improve performance (Mussagy et al., 2022; Liu et al., 2023). *Rhodotorula* yeast can synthetize lipids via two main pathways: the de novo pathway, which starts from acetyl-CoA and malonyl-CoA, and the ex-novo pathway, which uses hydrophobic molecules. Further, lipid

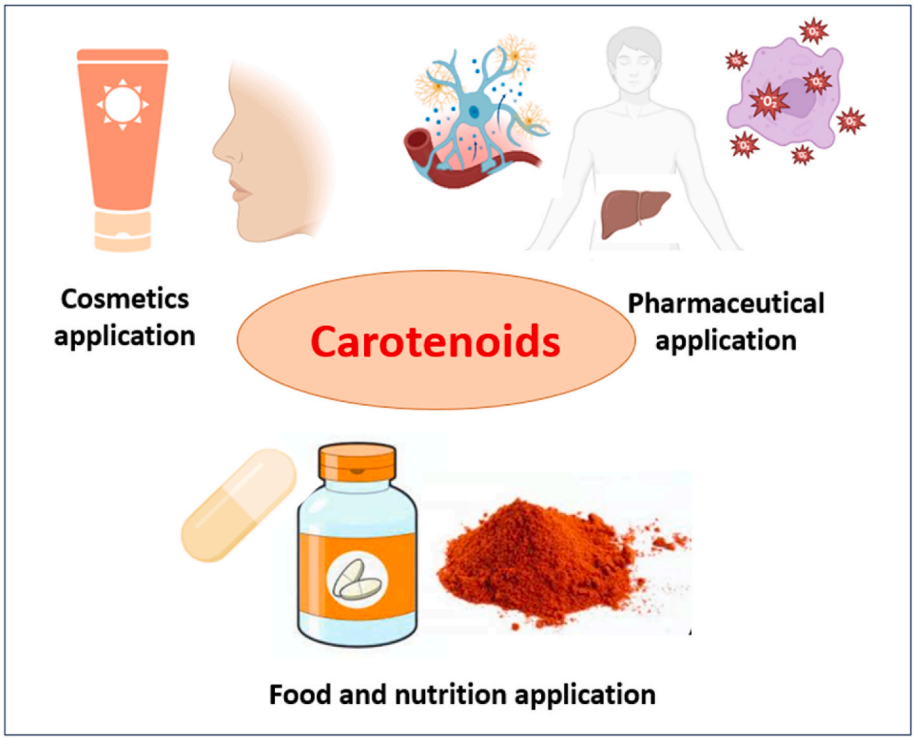

Fig. 3. Potential industrial applications of carotenoids produced by *Rhodotorula* genus.

overproduction is commonly triggered under nitrogen limitation. This condition activates AMP deaminase, interrupts the Krebs cycle, and promotes the export of citrate into the cytoplasm. There, ATP-citrate lyase converts citrate into acetyl-CoA, which promote the fatty acids biosynthesis. The fatty acid synthase complex then drives the triglycerides formation (Wu et al., 2021). Moreover, the *Rhodotorula* species represent also a versatile platform for producing various of oleochemicals with applications in fuels, industrial chemicals, and value-added feedstocks. Fatty acid ethyl esters can reach levels close to 10 g L^{-1} in fed-batch fermentation mode, with most of the product secreted extracellularly, helping to a reduce the purification and downstream processing costs (Zhang et al., 2021). Engineered strains, such as *Rhodotorula toruloides* IFO 0880 have been developed to produce various oily compounds including very long-chain fatty acids (i.e. erucic and nervonic acids), polyunsaturated fatty acids like linoleic acid, fatty alcohols, polyol esters, and oleogels (Fillet et al., 2017; Wu et al., 2021; Liu et al., 2020; Boviatsi et al., 2020; Papadaki et al., 2019). The accumulated lipids can be further processed into biofuel, including so-called "drop-in oils,". These are oxygen-free that can be used directly in conventional engines without blending, avoiding common issues encountred with bioethanol and biodiesel, such as combustion inefficiency and storage instability (Saini et al., 2020). Additionally, *Rhodotorula toruloides* can also synthesize sesquiterpenes which are important sustainable precursors for next-generation fuel production (Yaegashi et al., 2017; Geiselman et al., 2020). Beyond the energy sector, lipids from *Rhodotorula* possess wide-ranging applications. They provide fatty acids employed in feed, cosmetics, bioplastics, lubricants, and nylon (Park et al., 2018; Vasconcelos et al., 2019; Wu et al., 2021; Xue et al., 2018). Recent study points to potential cosmetic use. Kim et al. (2023) reported that total lipid extracted from *R. toruloides*, improved skin barrier function by boosting the filaggrin expression and suppressed heat-induced Matrix metalloproteinase 1 in keratinocytes, marking them promising candidates for skincare ingredients. Finally, the strain *R. mucilaginosa* can produce essential polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA, C20:5) and eicosadienoic acid (~19 %), which hold significant potential for use in nutritional supplements, functional foods and feed, and other specialized lipid-based products (Abaza et al., 2024).

6. Low-cost substrates for sustainable production of biomass and metabolites

One of the key barriers to the industrialization of *Rhodotorula* is the high expense of conventional production media, which significantly reduces economic feasibility. To overcome this, numerous studies have investigated the use of low-cost and renewable feedstocks, including lignocellulosic residues, agro-industrial by-products, and waste oils or effluents, as alternative carbon and nutrient sources. These strategies also align with the principles of a circular bioeconomy by transforming waste materials into valuable inputs (Cheng and Yang, 2016; Machado et al., 2022). Several studies have shown that such unconventional substrates can successfully support the growth of *Rhodotorula* for the production of biomass, lipids and carotenoids (Table 1). In fact, biomass yields obtained from agro-industrial wastes are comparable to or in some case higher than those obtained with refined substrates. For example, *Rhodotorula* strains grows well on lignocellulosic hydrolysates, starchy residues (Mast et al., 2014; Liu et al., 2015), and inulin from tubers of Jerusalem artichoke (whether in raw or hydrolyzed forms) (Zhao et al., 2010, 2011; Wang et al., 2014, 2019) often reaching biomass concentrations above 10 g L^{-1} and exceeding 700 μg g^{-1} in carotenoids (Kot et al., 2017; Sharma and Ghoshal, 2020). Likewise, when cultivated on oily substrate, lipids yields are comparable to those obtained on pure carbon source (Saenge et al., 2011; Sineli et al., 2022; Hong-Wei et al., 2019; Yen et al., 2019). A more detailed discussion of high- and low-yield processes with these substrates is provided in the following sections of this review.

7. Optimization strategies for biomass, carotenoids and lipids production in *Rhodotorula*

Enhancing the industrial feasibility of *Rhodotorula* requires the optimization of biomass, carotenoids, and lipids production. These yields are significantly influenced by factors such as nutrient composition, environmental conditions, and genetic modifications. This section explores key strategies to maximize productivity and improve cost-effectiveness for large-scale applications.

7.1. Biomass production

The *Rhodotorula* genus exhibits significant industrial potential due to its rapid growth and metabolic versatility, especially in lipid and carotenoid production (Mussagy et al., 2020). While carotenoid production has been a focal point of research, the optimization of productivity remains critical to enhancing overall yields. This involves not only increasing biomass but also enhancing the content of valuable compounds such as lipids and carotenoids within the biomass, as higher intracellular content reduces the need to process large volumes during extraction. Efficient biomass production with maximized compound content not only improves economic viability by reducing processing costs and enhancing profitability, but also ensures consistent product quality, which is essential for marketability and functional efficacy. Furthermore, this approach supports sustainability by reducing resource consumption and waste generation, driving innovation in biotechnological applications. To optimize biomass production, various strategies have been employed. One approach involves selecting the most efficient strains with naturally higher growth rates and biomass yields (Grigore et al., 2023). Additionally, optimizing cultivation modes and conditions is vital for large-scale production. Mixed cultures, where *Rhodotorula* is co-cultivated with bacteria or microalgae have also shown promise (Table 2). Recent developments in cultivation techniques have prioritized both maximizing biomass output and refining the biochemical composition of *Rhodotorula* species (Gong et al., 2020). *Rhodotorula* strains are predominantly cultivated through batch, fed-batch, and continuous cultivation approaches. Batch fermentation, though simple, has limitations in controlling critical culture parameters like pH and aeration. In contrast, bioreactor-based cultivation offers precise control over environmental factors, albeit at higher operational costs (Malisorn and Suntornsuk, 2009; Braunwald et al., 2013; Mussagy et al., 2020). Fed-batch cultivation enables higher cell densities by supplementing the culture with concentrated carbon or nitrogen sources, though it requires careful monitoring and adjustment to maintain optimal nutrient concentrations (Karamerou et al., 2019). Continuous culture, while offering continuous growth and elevated productivity, poses challenges such as contamination risks and the need for precise control over operating parameters (Malisorn and Suntornsuk, 2009). In addition to cultivation modes, it is important to take into consideration the optimization of the cultivation process, as several factors, such as the choice of carbon and nitrogen sources, C/N ratio, temperature, pH, inoculum size, and agitation speed, significantly influence *Rhodotorula* biomass production. Table 3 presents an overview of the main results reported in the literature. Carbon sources such as glucose, fructose, and glycerol have been found to lead to higher biomass yields, though the choice of carbon source can vary depending on the strain (Gong et al., 2019a, 2019b; Maza et al., 2020). Nitrogen sources, both organic (yeast extract, peptone, urea) and inorganic (ammonium sulphate), strongly affect biomass synthesis, with studies showing that a combination of these sources can enhance growth (Byrtusová et al., 2021; Mussagy et al., 2019; Mihalcea et al., 2011). The C/N ratio is crucial, with different strains responding differently to varying ratios, and literature suggests that lower C/N ratios are particularly effective in boosting growth, underscoring the need for tailored cultivation strategies (Liu et al., 2015; Tkáčová et al., 2017; Zhang et al., 2019a; Holub et al., 2023). Temperature and pH are key environmental factors that affect *Rhodotorula*

Table 1
Rhodotorula cultivation on low cost substrates.

Strain	Substrates	Cultivation medium/Cultivation conditions	Biomass	Carotenoids	Lipids	References
Rhodotorula glutinis						
CGMCC 2.703	Undetoxified corncob hydrolysate	**Batch fermentation.** Medium: Glucose (42.4 g L^{-1}), $(NH_4)_2SO_4$ (4 g L^{-1}), KH_2PO_4 (6 g L^{-1}), Na_2HPO_4 (2 g L^{-1}), $MgSO_4$ (2 g L^{-1}), $CaCl_2$ (0.1 g L^{-1}), $FeCl_3$ (0.12 g L^{-1}). pH 6.0, Temperature 30 °C, 65 h, 180 rpm, C/N ratio 75, inoculum size 10 % (v/v	15.1 gL^{-1}	Not reported	36.4 %	Liu et al. (2015)
CBS 20	Hydrolysates of Wheat Straw	Batch fermentation. Medium: Hydrolysates of wheat straw (18.6 g L^{-1} total sugars) supplemented with starch (0.07 g L^{-1}), yeast extract (0.07 g L^{-1}), casamino acids (0.01 g L^{-1}), gelatin (0.07 g L^{-1}), K_2HPO_4 (1 g L^{-1}), NaH_2PO_4 (1.5 g L^{-1}), trace mineral supplement (5 mL/L), and NH_4–N (1 g L^{-1}). pH 6.0, Temperature 30 °C, 168 h.	≈11.8gL^{-1}	Not reported	12.0 %	Mast et al. (2014)
Y54	Dephenolised olive Alperujo	phenolic compounds (1.51 ± 0.03 g L^{-1}), pH 5.5. T° 30 °C, 150 rpm.	–	43.0 ± 3.1 mg g^{-1} β-carotene: 2.2 mg g^{-1} Torulene: 25.6 mg g^{-1} Torularhodine: 9.3 mg g^{-1}	Not reported	(Ghilardi et al., 2022)
KCTC	Sugarcane molasses	Medium: Sugar cane molasses (10 g L^{-1}), urea (5 g L^{-1}), KH_2PO_4 (3 g L^{-1}), NaCl (0 g L^{-1}). pH 7.0, 20 °C, 150 rpm, 120 h (5 days).	2.6 g L^{-1}	0.393 mg g^{-1}	Not reported	Park et al.. (2005)
R glutinis	Whey lactose	Medium: Lactose (13.2 g L^{-1}), yeast extract (2.5 g L^{-1}), malt extract (2 g L^{-1}), $(NH_4)_2SO_4$ (1 g L^{-1}), KH_2PO_4 (1 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.25 g L^{-1}). pH 6.0, 30 °C, 100 rpm, 240 h (10 days).	1.6 g L^{-1}	35.5 mg g^{-1}	Not reported	Aksu and Eren (2007)
MT-5	Waste chicken feathers	Medium: Chicken feather peptone (8 g L^{-1}), Glucose (40 g L^{-1}), yeast extract (4 g L^{-1}), pH 6.0, T° 30 °C, 200 rpm	14.2 g L^{-1}	6.47 mg g^{-1}	Not reported	Taskin et al. (2016)
BIM Y- 253	Beer wort	Sugar concentration 6° Balling. 26 °C. 200 rpm. 4 days of fermentation	10.0 g L^{-1}	0.150 mg g^{-1}	Not reported	Besarab et al. (2018)
R4	Residual glycerol from the biodiesel industry	GMY medium: 40 g/L (81.6 % glycerol). KH_2PO_4 8 g L^{-1}; $MgSO_4.7H_2O$ 0.5 g L^{-1}; yeast extract 3 g L^{-1}; pH 5.5. Stirring: 250 rpm. T°C: 25 °C for 120h Inoculum size 10 % v/v	12.21 g L^{-1}	Not reported	Not reported	Sineli et al. (2022)
R. glutinis	Olive mill wasterwater	Batch fermentation in shake flasks 25 % olive mill wastewater. 1 g L^{-1} yeast extract. 30 °C. 150 rpmfor 208h.	≈1.7 g L^{-1}	Not reported	Not reported	Karakaya et al. (2012)
LOCKR1	potato wastewater + glycerol	Batch fermentation in shake flasks. Medium: potato wastewater+ 5 % (w/v) glycerol. C/N 4.2/1. The inoculum size was 10 % (v/v). pH 7. 200 rpm at 28 °C for 72 h	17.2 gL^{-1}	0.202 mg g^{-1}	12.7 %	Kot et al. (2017)
R. glutinis	Brewery effluents	Batch fermentation in shake flasks Brewery effluents+ 9.38 g L^{-1} glucose pH 5.5. 115 rpm at 25 °C for 168 h. C/N 33:1	7.82 g L^{-1}	0.12 mg g^{-1}	≈0.5 %	Schneider et al. (2013)
Rhodotorula mucilaginosa						
URM 7409	Cassava wastewater and sugar cane molasses	Cassava wastewater: 35 g L^{-1} sugar cane molasses: 55 g L^{-1} pH 6.0, T° 25 °C, 130 rpm for 168 h	10.55 g L^{-1}	0.192 mg g^{-1}	Not reported	Machado et al. (2022)
CCT 3892	Sisal bagasse hydrolyzate	Medium: KH_2PO_4 (6 g L^{-1}). $MgSO_4$(0.8 g L^{-1}). Yeast extract(7 g L^{-1}). pH 7.0.T. $CaCl_2$ (0.2 g L^{-1}). $(NH_4)SO_4$(3.0 g L^{-1}). NaCl (0.1 g L^{-1}) T° 22 °C, 200 rpm for 96 h	4.69 g L^{-1}	0.179 mg g^{-1}	Not reported	(Silva et al. 2020)
LBP4	Brewer's spent grain	2.5 g.L^{-1} of sugar, T°C 30 °C, 120 rpm, and pH 4	–	Not reported	–	(Azevedo et al., 2024)
R.mucilaginosa	Onion peels Potato skin Mung bean husk Pea pods	Batch fermentation in shake flasks mung bean husk and pea pods as nitrogen sources. onion peels and potato skin as a carbone source. pH 6.1 at 25.8 °C with agitation of 119.6 rpm for 84 h	7.33 g L^{-1}	0.717 mg g^{-1}	Not reported	Sharma and Ghoshal (2020)
R. mucilaginosa	Food industry effluent	Food industry effluent + 40 g L^{-1}glucose. peptone. inoculum size 5 %(v/v). C/N 40. pH 6.6 at 30 °C for 68h.	≈4.8 g L^{-1}	Not reported	≈3.2 %	Sundaramahalingam and Sivashanmugam (2023)
R. mucilaginosa TJY15a	Inulin	Inulin 2.0 %, yeast extract 0.5 % and (NH4)2SO4 0.1 % or extract of Jerusalem artichoke tubers containing 0.1 % (NH4)2SO4, 28 C and 170 rpm for 48 h	12.6 g L^{-1}	Not reported	53.2 %	Zhao et al. (2010)
R. mucilaginosa TJY15a	Extract of tubers of Jerusalem artichoke rich in inulin	Jerusalem artichoke extract (total sugar content adjusted to 10 g L^{-1}) + 0.1 % $(NH_4)_2SO_4$., 2 L fermentor, 28 C and 170 rpm for 48 h.	19.6 g L^{-1}	Not reported	56.6 %	Zhao et al. (2010)
Rhodotorula alborubescrns						

(*continued on next page*)

Table 1 (*continued*)

Strain	Substrates	Cultivation medium/Cultivation conditions	Biomass	Carotenoids	Lipids	References
R. alborubescrns	Cantaloup juice	Batch fermentation in shake flasks. Medium composition: cantaloup juice. the inoculum size was 10 %(v/v). pH 5.15 ± 0.11 0.180 rpm 25 °C.for 72 h.	8.82 ± 0.10 log CFUmL^{-1}	29.78 mg g^{-1}	–	Hou et al. (2024)
Rhodotorula kratochvilovae						
EXF3471	Lignocellulosic effluents	Batch fermentation in shake flasks. phenolic waste stream. 0.1 g L^{-1}NH$_4$Cl. 0.5 μgL^{-1} thiamine. for 136.5 ± 1.5 h at 150 rpm and 30 °C. pH 6.0. C:N 216.7.	3.4 g L^{-1}	Not reported	0.40 %	(Broos et al., 2024).
Rhodotorula toruloides						
R. toruloides	Palm oil mill effluent	Batch fermentation in shake flasks Palm oil mill effluent. pH 6.0 inoculum size was 10 %(v/v).for 36 h at 30 °C and 180 rpm	152.0 mg L^{-1}	Not reported	4.51 %	Justine et al. (2023)

Table 2
Cultivation modes of *Rhodotorula* yeast.

Yeast species	Culture Mode	Biomass	Carotenoids	Lipids	Reference
Rhodotorula glutinis					
CGMCC 2.703	Fed-batch (Two-stage nitrogen fed-batch)	71.00 g L^{-1}	Not reported	33.5 g L^{-1}	Liu et al. (2015)
	Constant nitrogen fed-batch culture	75.4 g L^{-1}	Not reported	30.6 g L^{-1}	
	Batch culture by shake flask	11.7 g L^{-1}	Not reported	3.82 g L^{-1}	
	Batch culture using fermentor	15.1 g L^{-1}	Not reported	5.5 g L^{-1}	
DSM 70398	Batch culture by shake flask	1.77 g L^{-1}	Not reported	Not reported	Karakaya et al. (2012)
CGMCC No. 2258	Batch culture by shake flask	5.89 g L^{-1}	0.167 mg L^{-1}	Not reported	Gong et al. (2020)
ZHK	Batch culture by shake flask	Not reported	0.0493 mg L^{-1}	Not reported	(Zhao & Li. 2023)
CTT 2182	Batch culture by shake flask	10.28 g L^{-1}	0.98 mg L^{-1}	1.34 g L^{-1}	Ribeiro et al. (2019)
CGMCC No. 2258	Batch culture in 5 L fermentor	15.9 g L^{-1}	1.2 mg L^{-1}	Not reported	Zhang et al. (2014)
CICC 31596	Batch culture by 2-L bioreactor	5.3 g L^{-1}	Not reported	1.68 g L^{-1}	(Karamerou et al., 2016)
Rh-00301	Two-stage fed-batch process	106 g L^{-1}	Not reported	0.18 g L^{-1}	Lorenz et al. (2017)
BCRC 21418	Fed batch - 50-L airlift bioreactor	39.2 g L^{-1}	Not reported	Not reported	(Yen et al., 2019)
TISTR 5159	Fed batch in 2L-bioreactor	10.05 g L^{-1}	Not reported	Not reported	(Saenge et Cheirsilp2011)
Rhodotorula mucilaginosa					
IIPL32	In a split column inner loop airlift reactor (two stages culture, batch then fed-batch)	11.56 g L^{-1}	Not reported	8.6 g L^{-1}	(Dasgupta et al., 2017)

growth. Studies indicate that temperatures between 20 °C and 30 °C are generally optimal for most *Rhodotorula* strains (Bhosale and Gadre, 2002; Taskin et al., 2016; Zhang et al., 2019). Similarly, pH values around 5.0 to 6.0 are conducive to robust biomass production, though some strains may exhibit optimal growth at slightly different pH levels (Kot et al., 2017; Zhao et al., 2019; Mussagy et al., 2020). Inoculum size and agitation speed also play significant roles in determining biomass yield. Larger inoculum sizes tend to promote more robust growth (Heidari et al., 2016; Juanssilfero et al., 2018), while optimal agitation speeds ensure adequate oxygenation and nutrient availability (Tinoi et al., 2005; Braunwald et al., 2013; Maza et al., 2020). Another promising approach to enhance *Rhodotorula* cultivation is co-cultivation with other microorganisms. For instance, co-culturing *Rhodotorula* with microalgae can create a synergistic system where yeast-produced CO_2 supports microalgal growth, and microalgae, in turn, produce O_2 and release organic carbon or secondary metabolites that promote yeast growth and lipid accumulation. However, the optimization of co-culture conditions remains complex, requiring careful consideration of factors like pH, temperature, and growth rates (Zhang et al., 2014). Understanding these diverse cultivation strategies is essential for developing efficient processes aimed at maximizing both biomass yields and intracellular product accumulation in *Rhodotorula* cultures.

7.2. Carotenoids enhancement

Despite their potential, large-scale production of carotenoids faces challenges due to the need for optimized cultivation conditions. To overcome these limitations, strategic cultivation approaches are essential, considering factors such as carbon and nitrogen sources, C/N ratio, temperature, pH, light exposure, agitation speed, and the presence of minerals, salts, and nanoparticles, all of which significantly impact carotenoid yields in *Rhodotorula* (Table 4). For instance, carbon sources like glucose, fructose, and sucrose yield substantial carotenoid quantities, while non-fermentable sources like xylose are even more effective (Gong et al., 2022). Nitrogen availability also plays a crucial role, where moderate and limited nitrogen concentrations often enhancing carotenoid production (Bellou et al., 2016; Lorenz et al., 2017). Accordingly, the C/N ratio is another key factor, with varying optimal ratios reported for different *Rhodotorula* strains. Relatively higher C/N ratio can reduce competition for carbon, which is crucial for carotenoid synthesis, as proteins require nitrogen while carotenoids do not. Consequently, nitrogen-deficient conditions often lead to increased carotenoid production (Elfeky et al., 2019). Temperature and pH further regulate carotenoid synthesis by influencing enzyme activity. Optimal temperatures for carotenoid production in *Rhodotorula* species generally range from 20 to 30 °C, with specific enzymes showing varying activity at different temperatures (Zhao et al., 2019; Allahkarami et al., 2021). Similarly, pH values between 5.0 and 7.0 have been identified as ideal for maximizing carotenoid yields (Mihalcea et al., 2011; Kot et al., 2017; Allahkarami et al., 2021). Light exposure and agitation speed also play vital roles, with light enhancing carotenoid yields and agitation ensuring

Table 3
Key cultivation parameters affecting *Rhodotorula* biomass production.

Factor	Strain	Source	Concentration	Cultivation medium and conditions	Biomass	References
Carbon	*R. glutinis* CGMCC No. 2258	Fermentable carbon sources Glucose Fructose sucrose	40 g L^{-1}	Batch culture in shake flasks, medium composition: 2 g L^{-1} $(NH_4)_2SO_4$,1.5 g L^{-1} yeast extract, pH 6, T: 30 °C	11.5 g L^{-1} 13 g L^{-1} 14 g L^{-1}	(Gong et al., 2019a, 2019b)
		Non-fermentable carbon sources Xylose Arabinose Glycerol	40 g L^{-1}	Glucose + xylose after 264 h, Glucose after 168 h, Fructose after 192 h, Xylose after 264 h, Arabinose after 240 h, Glycerol after 240 h	11 g L^{-1} 9 g L^{-1} 8 g L^{-1}	
		Mixed carbon source: Glucose + Xylose	Glucose:40 g L^{-1} Xylose: 20 g L^{-1}		15.73 g L^{-1}	
	R. glutinis Rh-00301	Glucose Glycerol Sucrose	20 g L^{-1}	Batch culture in bioreactor WMIX medium (WM, white molasses) containing (g L^{-1}): 20 of carbone source; 2.8 NH_4Cl; T: 30 °C, pH 5.5	10.64 g L^{-1} 3.48 g L^{-1} 13.80 g L^{-1}	Lorenz et al. (2017)
	R. glutinis CGMCC No. 2258	Sodium Acetate	20 g L^{-1}	Batch culture, $(NH_4)_2SO_4$ (2g L^{-1}), yeast extract (1.5 g L^{-1}), pH 6, T = 30 °C.	3.87 g L^{-1}	(Gong et al., 2020)
	R. glutinis R4	Sugar cane molasses	40 g L^{-1} reducing sugar	GMY media: yeast extract 3g L^{-1}; pH 5.5 Shaking: 250 rpm, T: 25 °C for 120 h Inoculum size 10 % v/v	21.33 g L^{-1}	Sineli et al. (2022)
	R. glutinis DBVPG 4620	Glucose Xylose Galactose Mannose Cellobiose Glycérol	50 g L^{-1}	Batch fermentation in shake flask. Medium component (g L^{-1}): $(NH_4)_2SO_4$ 1 of yeast extract 1. pH 6, agitation 200 rpm, 30 °C.	16.09 g L^{-1} (glucose) 10.78 g L^{-1} (Xylose)	Galafassi et al. (2012)
Nitrogen	*R. graminis* CCT 2186	Ammonium nitrate (NH_4NO_3)	6g L^{-1}	Batch culture in shake flasks, Medium composition: Glucose 5g L^{-1}, pH 4, T 30 °C,170 rpm for 72h.	6.06 g L^{-1}	(Mussagyet al. 2021)
		Asparagine	6g L^{-1}	Batch culture in shake flasks, medium composition: Glucose: 5g L^{-1}, pH 7, T 30 °C, 170 rpm for 72h.	4.95 g L^{-1}	
		Ammonium nitrate + Asparagine	Asparagine:20.6 g L^{-1} NH_4NO_3: 4 g L^{-1}	Batch culture in shake flasks, medium composition: Glucose: 10 g L^{-1}, pH 5, T: 30 °C, 170 rpm for 72h	5.67 g L^{-1}	
	R. graminis MT-5	chicken feather peptone tryptone peptone fish peptone	8 g L^{-1}	Batch culture in shake flasks, Glucose (40 g L^{-1}) and yeast extract (4 g L^{-1}). pH 6.0 at 30 °C and 200 rpm.	14.2 g L^{-1} 13.5 g L^{-1} 12.5 g L^{-1}	(Taskin et al., 2016)
	R. Rubra ICCF 20	Mineral nitrogen source: NH_4N0_3, $NH_4H_2PO_4$, $(NH_4)_2SO_4$, NH_4Cl	5 6g L^{-1}	Batch culture in conical flasks 40 g L^{-1} glucose, 1.5 g L^{-1} yeast extract, 5 g L^{-1} $NH_4H_2PO_4$, at 250 rpm, during 144h, 28 °C	better biomass with $NH_4H_2PO_4$ (better absorbance at 600 nm)	(Mihalcea et al., 2011)
		Supplementation of the yeast extract with organic nitrogen source NH_4N0_3 +0,1 % of one of the following amino acids (Tryptophan, threonine, alanine, cysteine, acid glutamic, tyrosine, proline, leucine, valine)		Batch culture in conical flasks 40 g L^{-1} glucose, 1.5 g L^{-1} yeast extract, 5 g L^{-1} NH_4N0_3 +0,1 % alanine at 250 rpm, during 144h, 28 °C.	better biomass with 5 g L^{-1} NH_4N0_3 +0,1 % alanine (better absorbance at 600 nm)	
	R. glutinis	Casein hydrolysate	5 g L^{-1}	Batch culture in shake flasks Glucose (25 g L^{-1}), yeast extract (1 g L^{-1}), C/N 10, pH of 6.2 ± 0.1, T 30 °C, inoculum size of 1 %, 100 rpm and incubation time of 4 days.	9.29 g L^{-1}	(El Banna et al., 2012)
C/N ratio	*R. glutinis* JMT 21978	Glucose/ yeast extract	(20:1, 50:1,70:1100:1) Optimum C/N 70:1	Batch culture in Erlenmeyer Flasks, 0.5 % yeast extract, 7 % glucose for C/N 70:1, T 28 °C for 192h, 180 rpm	14.5 g L^{-1}	(Tkáčová et al., 2017)
	R. glutinis CCY 20-2-26	Glucose/ yeast extract	20:1 50:1 70:1 100:1 Optimum C/N 70:1	Batch culture in Erlenmeyer Flasks yeast extract (5 g L^{-1}), Glucose (70 g L^{-1}), C/N 70:1, 192h, 28 °C, 180 rpm.	18.6 g L^{-1}	(Tkáčová et al., 2018)
	R. glutinis ATCC 15125	Glucose/ Ammonium sulphate	20:1 70:1	Batch culture in Erlenmeyer Flasks, medium composition:	11.4 g L^{-1}	(Braunwald et al., 2013)

(*continued on next page*)

Table 3 (*continued*)

Factor	Strain	Source	Concentration	Cultivation medium and conditions	Biomass	References
			120:1 Optimum C/N 20:1	57.0 g L^{-1} glucose, 1.0 g L^{-1} yeast extract, 4.885 g L^{-1} $(NH_4)_2SO_4$ for 216 h at 130 rpm and 30 °C.		
	R. glutinis CTT 2182	Reducing sugars of Cassava wastewaters (CW)/ Ammonium sulphate	C/N (18.2, 26.5, 18.2, 31.6, 38.0 44.1, 31.6 31.6, 15.0 9.8) Optimum C/N 18.2	Batch fermentation in Erlenmeyer flasks 5g L-1 $(NH_4)_2SO_4$, CW (30g of RS/ l), C/N 18.2, 30 °C and shaken at 200 rpm for 120 h.	12.78 g L^{-1}	Ribeiro et al. (2019)
	R. glutinis TR29	Sugar beet molasses/Ammunium sulphate	C/N (23.53, 20.61, 18.32, 16.45, 14.94, 13.64, 12.23) Optimum C/N 12.23	Batch culture in shake flasks molasses concentration = 20 % (94.4 g L^{-1} total sugar, $(NH_4)_2SO_4$: 6g L^{-1}, pH 5.0, T 25 °C, agitation 200 rpm, for 96 h	17.6 g L^{-1}	(Taskin et al., 2016)
Temperature	*R. glutinis* CGMCC No. 2258	Glucose, yeast extract, $(NH_4)_2SO_4$	24 °C–30 °C Optimum: 30 °C	Batch culture, medium components (w/v): glucose 4 %; yeast extract 0.15 %; $(NH_4)_2SO_4$ 0.2; pH 5.5, shaking: 180 rpm, inoculum size 10 % (v/v), time: 45 h	17.9 g L^{-1}	Zhang et al. (2014)
	R. glutinis NCIM 3353	Glucose, yeast extract	10–40 °C Optimum: 25 °C and 30 °C	Batch fermentation in 1 L stirred fermenter, Glucose (46 g L^{-1}), yeast extract (11.74 g L^{-1}), threonine (18 g L^{-1}),pH 7.0, a shaker (250 rev/ min)	26 g L^{-1}	Bhosale and Gadre (2002)
	R. glutinis TR29	Molasses, $(NH_4)_2SO_4$	10–40 °C Optimum 25 °C	Batch fermentation in shake flasks, molasses 20 % (94.4 g L^{-1} total sugar), $(NH_4)_2SO_4$: 3g L^{-1}, pH = 5.0, T 25 °C, at 200 rpm for 96 h	14.8 g L^{-1}	Taskin et al. (2016)
	R. mucilaginosa CBMAI 1528	Sabouraud dextrose medium	10–35 °C Optimum 20–30 °C	Batch fermentation in shake flasks Fresh Sabouraud dextrose medium, at 250 rpm, 72h.	7.5 g L^{-1}	Lario et al. (2020)
pH	*R. mucilaginosa* CCT 2186	Glucose, asparagine	2.2 to 7.8 Optimum 5.0	Batch fermentation in shake flasks, Glucose (10 g L^{-1}), NH_4NO_3 (4 g L^{-1}) + asparagine (20.6 g L^{-1}), temperature 30 °C, stirring 170 rpm, 72h.	5.67 g L^{-1}	(Mussagy et al., 2020)
	R. mucilaginosa TR29	Molasses, $(NH_4)_2SO_4$	3–7 Optimum 5.5	Batch fermentation in shake flasks, molasses 20 % (94.4 g L^{-1} total sugar), $(NH_4)_2SO_4$: 3g L^{-1}, initial pH = 5.5, T 30 °C, 200 rpm and incubation time = 96 h.	14,9 g L^{-1}	Taskin et al. (2016)
	R. Rubra ICCF 20	Glucose, yeast extract	3–8 Optimum 6	Batch culture in conical flasks, medium components: 40 g L^{-1} glucose, 1.5 g L^{-1}yeast extract, 5g L^{-1} NH_4NO_3, 250 rpm at 28 °C	Maximum yeast growth (maximum absorption at 600 nm)	(Mihalcea et al., 2011)
	R. mucilaginosa M23	YPG	pH 4–8 Optimum pH 6	Batch fermentation in shake flasks Medium: YPG (20 g L^{-1} peptone, 10 g L^{-1} yeast extract, 20 mL/L glycerol), pH 6.0, 150 rpm for 28 °C, 120 h.	23.67 g L^{-1}	Allahkarami et al. (2021)
Agitation	*R. glutinis*	Glycerol, Fresh palm oil, yeast extract	130 rpm	Batch culture, Glycerol: 60 g L^{-1}, Fresh palm oil: 30 g L^{-1}, yeast extract: 20 g L^{-1}, $(NH_4)_2SO_4$ 2g L^{-1}, pH 5.5, T: 24 °C	11.4 g L^{-1}	(Braunwald et al., 2013)
	R. glutinis BCRC 22360	Glucose, molasses, glycerol	(60, 150, 210 rpm) Optimum 210 rpm	Batch culture in Flasks, glucose,30 g L^{-1}, molasses 30 g L^{-1}, glycerol 30g L^{-1}at 24 °C, 50 ml working volume.	10 g L^{-1}	(Yen and Zhang 0.2011)
Inoculum size	*R. Rubra* ICCF 20	Glucose, yeast extract	(0,5 %–5 %) Optimum 5 %	Batch culture in conical Flasks, medium components: 40 g L^{-1} glucose, 1.5 g L^{-1} yeast extract, 5 g L^{-1} NH_4NO_3, 250 rpm, 5–6 days at 28 °C.	Maximum yeast growth (maximum absorption at 600 nm)	Mihalcea et al. (2011)
	R. glutinis	GYM	5 %	Batch culture in Flasks GYM medium (60 g L^{-1} Glucose 0,2 g L^{-1} yeast extract,12 g L^{-1} $(NH_4)_2SO_4$, pH 6, T: 30 °C, 120 rpm for 96 h.	6.25 g L^{-1}	Lakshmidevi et al. (2021)
	R. glutinis	Glucose, Xylose, $(NH_4)_2SO_4$, yeast extract.	10 %	Batch fermentation in shake flasks,40 g L^{-1} (Glucose), 20 g L^{-1} (Xylose), 2g L^{-1} $(NH_4)_2SO_4$, 1.5 g	15.73 g L^{-1}	(Gong et al., 2019a, 2019b)

(*continued on next page*)

Table 3 (*continued*)

Factor	Strain	Source	Concentration	Cultivation medium and conditions	Biomass	References
				L^{-1} yeast extract, pH 6, for 264h, 30 °C.		

even nutrient distribution and better aeration, which are crucial for carotenoid synthesis (Kong et al., 2019; Sharma and Ghoshal, 2020). The addition of minerals, salts, and nanoparticles can either enhance or inhibit carotenoid production, depending on the specific compound and concentration. For example, zinc oxide nanoparticles and zinc sulphate have been shown to significantly increase carotenoid production in *Rhodotorula* species, while other metals like cadmium and lead also have positive effects at specific concentrations. However, the impact of these additives is complex, with different ions promoting or inhibiting various carotenoids (Buzzini and Martini, 2000; Li et al., 2022a).

Genetic modification has emerged as one of the most effective strategies to enhance and boost carotenoid production in *Rhodotorula* species, mainly by modeling their metabolic pathways. One effective method involves reengineering these pathways to optimize the production of key intermediates while reducing unwanted byproducts. To direct pathway engineering, classical methods such as physical and chemical mutagenesis techniques such as ultraviolet (UV) and gamma irradiation have been widely applied to possibly generate mutation that improve carotenoids yields, although these methods can sometimes result in unstable mutant strains (Watabe and Takahashi, 2023). To overcome the limitations related to random mutagenesis, more refined approaches have been developed. Indeed, physical mutagenesis techniques like atmospheric and room temperature plasma (ARTP) with chemical mutagenesis using N-methyl-N′-nitro-N-nitrosoguanidine (NTG) have successfully increased carotenoid production in *R. toruloides* NP11 (Zhang et al., 2014). Another relevant method involves key gene overexpression Furthermore, such as HMG-CoA, while simultaneously applying ergosterol synthesis inhibitors linke ketoconazole, has been shown to substantial enhance carotenoid concentrations. In *R. mucilaginosa* K4, repeated cycles of mutagenesis combined with heterologous expression of the HMG-CoA gene resulted in a 121 % increase in carotenoid production (Wang et al., 2019). Similarly, introducing truncated HMG1 (tHMG1) from *Kluyveromyces marxianus* in *R. glutinis*, significantly improved β-carotene production (Pi et al., 2018).

More advanced transformation methods have also proven valuable. *R. toruloides,* for example, has been genetically engineered using *Agrobacterium*-mediated transformation (AMT), which allows multiple genes to be integrated directly into the yeast chromosome. When combined with strong promoters, plasmid vectors, and selectable markers such as antibiotic resistance genes, this technique has driven significant increase in carotenoid production, including torularhodin. Furthermore, the developement of modern assembly systems like the Golden Gate DNA toolkit (RtGGA), has provided further carotenoids improvements, with notable success reported in *R. toruloides* CCT 0783 (Bonturi et al., 2022). Although these advances demonstrate the potential of genetic strategies, creating stable engineered strain that maintain performance over time remains significant challenge. Nonetheless, targeted metabolic engineering continues to play a key role in advancing carotenoid production in *Rhodotorula* species.

7.3. Lipids accumulation

As explained before, *Rhodotorula* yeasts are considered as oleaginous due to their strong ability to accumulation large amount of lipids. This accumulation usually occurs under the variation of cultivation factors such as the sources and concentrations of carbon and nitrogen, the C/N ratio, as well as physical conditions including agitation, pH, temperature, and inoculum size. All of them influence the balance between biomass production and lipid biosynthesis, thereby determining both yield and fatty acids composition (Ratledge and Wynn, 2002; Papanikolaou and Aggelis, 2011; Jiru et al., 2017). The Table 5 provides an overview of the major factors affecting lipids accumulation in *Rhodotorula* biomass. Carbon source availability plays a vital role in lipid synthesis. Numerous carbon substrates have been studied, resulting in different lipid yields: glucose resulted in a lipid content of 47.24 % (Maza et al., 2020), while sucrose and fructose yielded 28 and 27.5 %, respectively (Gong et al., 2019a, 2019b). Less conventional substrates have also been successful. Indeed, crude glycerol resulted in 35.22 % lipid content, and interestingly, when supplemented with used cooking oil, the lipid content increased to 46 % (Yen et al., 2019). Similarly, substrates such as sugarcane molasses and residual glycerol from the biodiesel industry, have shown lipid yields ranging from 40.66 % to 46.86 % (Lakshmidevi et al., 2021; Sineli et al., 2022). The nitrogen source is equally important in steering metabolism. When nitrogen is depleted, *R. glutinis* reduces cellular growth and redirects carbon towards lipid synthesis in TGA form. High lipid yields have been achieved using asparagine as a nitrogen source, with biomass and lipid yields of 61.60 and 48.99 %, respectively (Mussagy et al., 2021). Ammonium nitrate, particularly when combined with asparagine, yielded a lipid content of 44.08 %. Other waste-based nitrogen inputs like vinasse from rice wine production, provided a lipid content of 36.5 % (Yen et al., 2019). In contrast, yeast extract and ammonium sulphate resulted in comparatively lower lipid production, at 6.80 % and 14.6 %, respectively (Liu et al., 2015; Maza et al., 2020). The carbon-to-nitrogen (C/N) ratio itself is another factor affecting lipid production. Higher C/N ratios generally correlate with increased lipid yields, although lower ratios can also produce significant results. In their study, Saenge et al. (2011) explored the combined effect of glycerol and ammonium sulphate at different C/N ratios on lipids production in *R. glutinis*. The maximum lipid content, 60.7 %, (equivalent to 6.10 g L^{-1}) was obtained at a C/N ratio of 85 with 9.5 % glycerol after 72 h of cultivation in a bioreactor.

Agitation, by enhancing contact between cells and medium, improves nutrient transer and influences the lipids production. In different *R. glutinis* strains, lipids contents varied wirht agitations speeds, recording 47.24 % at 250 rpm, 61.60 % at 170 rpm, and 46 % at speeds between 200 and 500 rpm (Yen et al., 2019; Maza et al., 2020; Mussagy et al., 2021). The pH of the cultivation medium is another sensitive factor. Lipid yields in different strains of *R. glutinis* have been observed at different pH levels, with 44.08 % at pH 3, 41.2 % at pH 4, 52.6 % at pH 5, and 58 % at pH 5.45 (Taskin et al., 2016; Mussagy et al., 2021).

Furthermore, temperature also effects both growth and lipid biosynthesis. In some studies, on *R. glutinis*, lower temperature (24 °C) supported higher lipids levels than 30 °C, while others found optimal yields at 35 °C or even at 10 °C, depending on the specific culture conditions (Zhang et al., 2014b; Taskin et al., 2016). Inoculum size is also an important factor, as increasing the inoculum size can promote microbial growth and enhance lipid production. Studies have demonstrated lipid contents of 42.80 % with a 5 % v/v inoculum size, 32.6 % with 10 % v/v, and 29.7 % with a 10 % v/v inoculum size (Zhang et al., 2014; Liu et al., 2015; Lakshmidevi et al., 2021).

Beyond cultivation, mutagenesis has been employed to generate improved lipid-producing strains. Yamada et al. (2017) combined UV irradiation with stress selection (ethanol, H_2O_2, and cerulenin) to successfully isolate mutant strains of *R. tularoide* with higher palmitic acid levels. Similarly, Guo et al. (2019) used UV mutagenesis and stress selection to obtain strains that produced lipids richer in saturated fatty acid content. Other mutagenesis techniques, such as atmospheric and room temperature plasma (ARTP) and nitrosoguanidine mutagenesis

Table 4
Key cultivation parameters affecting carotenoids accumulation in *Rhodotorula* biomass.

Factors	Yeast	Substrates	Concentration/ Value/ intensity	Conditions	Carotenoids	References
Carbone source	*R. glutinis* CGMCC No. 2258	Fermentable carbon sources: glucose	40 g L^{-1}	Batch culture in shake flasks, medium composition:	≈125 μg g^{-1}	(Gong et al., 2019a, 2019b)
		fructose		Yeast extract 1.5 g L^{-1},	≈150 μg g^{-1}	
		sucrose		$(NH_4)_2SO_4$. 2 g L^{-1}, pH 6.0, at	≈175 μg g^{-1}	
		Non-fermentable carbon sources: xylose	40 g L^{-1}	180 rpm,	≈200 μg g^{-1}	
		Arabinose			≈250 μg g^{-1}	
		glycerol			≈300 μg g^{-1}	
		Mixed carbon source: Glucose + Xylose	40 g L^{-1} (glucose) + 20 g L^{-1}(Xylose)		≈210 μg g^{-1}	
	R. glutinis	Glucose	15 g L^{-1}	Batch culture in shake flasks, medium composition: (2.5 g L^{-1}) yeast extract, (2 g L^{-1}) malt extract, (1 g L^{-1}) $(NH_4)_2SO_4$, pH 6.0, at 30 °C, 100 rpm for 10 days.	≈65 μg g^{-1}	(Aksu and Eren, 2007)
		whey lactose			≈55 μg g^{-1}	
		Molasses sucrose		Batch culture in shake flasks, medium composition, Molasses sucrose pH 6.0, 30 °C, 100 rpm for 10 days	≈110 μg g^{-1}	
	R. glutinis CGMCC No. 2258	Glucose	20 g L^{-1}	Batch culture, $(NH_4)_2SO_4$(2 g L^{-1}),yeast extract (1.5 g L^{-1}) pH 6.0, 200 rpm, T 30 °C	106,25 μg g^{-1}	Gong et al. (2020)
	R. glutinis BIM Y- 253	Beer wort	Sugar concentration 6° Balling	Batch culture, 26 °C, 200 rpm, 4 days of fermentation	150.9 μg g^{-1}	Besarab et al. (2018)
	R. glutinis	Whey lactose	Lactose concentration: 13.2 g L^{-1}	Batch culture 2.5 g L^{-1} yeast extract, 2 g L^{-1} malt extract, 1 g L^{-1} $(NH_4)_2SO_4$, pH 6.0, at 30 °C on a rotary shaker (100 rpm) for 10 days.	35.5 mg g^{-1}	(Aksu and Eren2007)
	R. glutinis	glucose, sucrose, glucose syrup, high fructose corn syrup (HFCS)	concentration of each carbon source was adjusted to give C/N ratio of 10	Batch culture yeast extract (1 g L^{-1}), pH of 6.2 ± 0.1, T 30 °C, 100 rpm and incubation time of 3 days	367 μg g^{-1} (sucrose) 182 μg g^{-1}	(El-Banna et al., 2012)
Nitrogen source	*R. glutinis* CCT-2186	Asparagine	2.0 g L^{-1}	Batch culture Glucose 5.0 g L^{-1}, pH 4.0, T 30 °C, shaking at 170 rpm for 72 h.	β-carotene- 54 μg g^{-1} Torularhodine 171.74 μg g^{-1} Torulene 19.14 μg g^{-1}	(Mussagy al.2021a)
		Ammonium nitrate			β-carotene 157.05 μg g^{-1} Torularhodine 187.07 μg g^{-1} Torulene 15.60 μg g^{-1}	
	R. Rubra ICCF 20	Mineral nitrogen source: NH_4N0_3, $NH_4H_2PO_4$, $(NH_4)_2SO_4$, NH_4Cl	5 g L^{-1}	Batch culture in conical flasks 40 g L^{-1} glucose, 1.5 g L^{-1} yeast extract, 5 g L^{-1} $NH_4H_2PO_4$, at 250 rpm, during 144h, 28 °C	≈650 μg g^{-1}	Mihalcea et al. (2011)
		Supplementation of the yeast extract with organic nitrogen source NH_4N0_3 +0,1 % of one of the following amino acids (Tryptophan, threonine, alanine, cysteine, acid glutamic, tyrosine, proline, leucine, valine)		40 g L^{-1} glucose, 1.5 g L^{-1} yeast extract, 5 g L^{-1} NH_4N0_3 +0,1 % alanine, 250 rpm, during 144h, 28 °C	≈800 μg g^{-1}	
	R. glutinis	Casein	10 g L^{-1}	Batch culture in erlenmeyer fasks in a medium containing: 25 g L^{-1} glucose, 10 g L^{-1} nitrogen source,at pH 6.0, T: 28 °C, 240 rpm.	11.7 μg g^{-1}	(Bhosale and Cadre, 2001)
		Hydrolysate Cotton seed meal			20.2 μg g^{-1}	
		Peptone			23.0 μg g^{-1}	
		Soya Peptone			25.0 μg g^{-1}	
		Soybean meal			16.0 μg g^{-1}	
		Tryptone			26.0 μg g^{-1}	
		Urea			17.0 μg g^{-1}	
		Yeast extract			33.0 μg g^{-1}	
		Glycine			20.6 μg g^{-1}	

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Table 4 (*continued*)

Factors	Yeast	Substrates	Concentration/ Value/ intensity	Conditions	Carotenoids	References
C/N ratio	*R. glutinis* CTT 2182	reducing sugars(RS) of Cassava wastewaters(CW)/ Ammonium sulphate	C/N 9.8–44.1 Optimum: C/N 9.8	Batch fermentation in Erlenmeyer flasks 5g L^{-1} $(NH_4)_2SO_4$, CW (10g of RS/l), 30 °C and shaken at 200 rpm for 120 h in darkness.	167.23 μg g^{-1}	(Santos Beneit, 2024)
	R. glutinis CCY 20-2-26	Glucose/ yeast extract	C/N: (20:1–100:1) Optimum C/N: 20:1	Batch fermentation in Erlenmeyer flasks, C/N 20/1, 5 g L^{-1} yeast extract et 20 g L^{-1} Glucose,180 rpm, 28 °C.	1268 μg g^{-1}	Tkáčová et al. (2018)
	R. glutinis var. *glutinis*	Glucose/ yeast extract	C/N (2.5, 5, 10, 15, 20) Optimum C/N 10	Batch fermentation,medium component: glucose 25 g L^{-1} $(NH_4)_2SO_4$ 5 g L^{-1}, , yeast extract 1 g L^{-1}, pH 6.2, 30 °C, 100 rpm and incubation time of 4 days.	2.44 μg g^{-1}	(El Banna et al., 2012)
	R. glutinis CGMCC No. 2258	Glucose/ yeast extract	C/N: 30 to 150 Optimum C/N: 90	Batch fermentation in Erlenmeyer flasks, Glucose, Yeast extract, $(NH_4)_2SO_4$ 0.2 % pH = 5.5, T = 30 °C, 180 rpm.	1.9 μg g^{-1}	Zhang et al. (2014)
	R. glutinis ATCC 15125	Glucose/Ammonium sulphate	20:1 20:1 70 120:1 120:1 Optimum C/N 120:1c	Batch culture in erlenmeyer Flasks, medium composition: 97.4 g L^{-1} glucose, 1.0 g L^{-1} yeast extract, 4.885 g L^{-1} $(NH_4)_2SO_4$, for 216 h in a rotary incubator at 130 rpm and 30 °C.	1247 μg g^{-1}	(Braunwald et al., 2013)
	R. mucilaginosa URM 7409	Molasses and Cassava wastewater	C/N: 61.09–75.89 Optimum C/N 73.45	Batch culture in shaken Flasks, Molasses: 55 g L^{-1}, Cassava wastewater: 35 g L^{-1}. pH 6.0, at 25 °C, 130 rpm and 168 h.	192.21 μg g^{-1} 2,02709 μg g^{-1}	Machado et al. (2022)
Temperature	*R.glutinis* ZHK	YPD medium: (yeast extract: 10 g L^{-1}, peptone: 20 g L^{-1}, dextrose: 20 g L^{-1})	16 °C 25 °C 32 °C	Batch culture YPD medium: (yeast extract: 10 g L^{-1}, peptone: 20 g L^{-1}, dextrose: 20 g L^{-1}) pH 6.5; in rotary shaker at 180 rpm, for 5 days	Low production of carotenoids Max of total Carotenoids: 49.3 μg g^{-1} Max of Torulene: 11.48 μg g^{-1} And Torularhodine: 23.79 μg g^{-1}	(Zhao and Li, 2023)
	R.glutinis CGMCC No. 2258	Glucose, 4 %; yeast extract 0,15 %; $(NH_4)_2SO_4$ 0,2 %; KH_2PO_4 0,7 %; $MgSO_4$ 0,1 %; Na_2SO_4 0.2 %,	24 °C et 30 °C Optimum: 30 °C	Batch culture Glucose, 4 %; yeast extract 0,15 %; $(NH_4)_2SO_4$ 0,2 %; KH_2PO_4 0,7 %; $MgSO_4$ 0,1 %; Na_2SO_4 0.2 %, pH = 5.5, 180 rpm 45h.	1–2 μg g^{-1}	Zhang et al. (2014)
	R.glutinis NCIM 3353	Glucose (46 g L^{-1}), yeast extract (11.74 g L^{-1}), threonine (18 g L^{-1})	10 °C–40 °C Optimum 20 °C	Batch fermentation in 1 L stirred fermenter, Glucose (46 g L^{-1}), yeast extract (11.74 g L^{-1}), threonine (18 g L^{-1}), pH = 7.0, a shaker 250 rpm	7 μg g^{-1}	Bhosale and Gadre (2002)
	R. glutinis	(20 g L^{-1}) Glucose, (2.5 g L^{-1}) yeast extract, (2 g L-1) malt extract, (1 g L^{-1}) $(NH_4)_2SO_4$,	25 °C,30 °C, 35 °C Optimum 30 °C	Batch culture in Erlenmayer flasks (20 g L^{-1}) Glucose, (2.5 g L^{-1}) yeast extract, (2 g L^{-1}) malt extract, (1 g L^{-1}) $(NH_4)_2SO_4$, pH 6.0, on rotary shaker (100 rpm) for 10 days.	63.4 μg g^{-1}	Aksu and Eren (2007)
	R. rubra ICCF 20	40 g L^{-1} glucose, 1.5 g L^{-1} yeast extract, 5 g L^{-1} NH_4NO_3	28 °C, 30 °C Optimum 28 °C	Batch culture in conical flasks, medium components:40 g L^{-1} glucose, 1.5 g L^{-1} yeast extract, 5 g L^{-1} NH_4NO_3, 250 rpm.	≈400 μg g^{-1}	Mihalcea et al. (2011)
	R. mucilaginosa	YPG (20 g L^{-1} peptone, 10 g L^{-1} yeast extract, 20 mL/L glycerol)	22 °C–37 °C Optimum 28 °C	Batch fermentation in shake flasks Medium: YPG (20 g L^{-1} peptone, 10 g L^{-1} yeast extract, 20 mL/L glycerol), pH 6.0, 150 rpm, 120 h.	≈145 μg g^{-1}	(Allahkarami et al., 2021)
	R. mucilaginosa F-1	YM medium containing (10 g L^{-1} glucose, 5 g L^{-1} peptone, 3 g L^{-1} yeast extract and 3 g L^{-1} malt extract)	22 °C–31 °C Optimum 25 °C	Batch fermentation in shake flasks YM medium containing (10 g L^{-1} glucose, 5 g L^{-1} peptone, 3 g L^{-1} yeast extract and 3 g L^{-1} malt extract) 120 tr/min pendant 216	322.0 μg g^{-1} Max β- carotene At 22 °C Max torulene and torularhodine at 31 °C.	Cheng and Yang (2016)

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Table 4 (*continued*)

Factors	Yeast	Substrates	Concentration/ Value/ intensity	Conditions	Carotenoids	References
pH	*R. mucilaginosa* F-1	YM medium containing (10 g L^{-1} glucose, 5 g L^{-1} peptone, 3 g L^{-1} yeast extract and 3 g L^{-1} malt extract)	pH (4–7) Optimum for total carotenoids pH 5	Batch fermentation in shake flasks YM medium containing (10 g L^{-1} glucose, 5 g L^{-1} peptone, 3 g L^{-1} yeast extract and 3 g L^{-1} malt extract) 120 rpm for 216h.	Max total carotenoids: 317.6 μg g^{-1} (Max β-carotene at pH 4) (Max of torulene and torularhodine pH 7)	Cheng and Yang (2016)
	R. mucilaginosa	YPG (20 g L^{-1} peptone, 10 g L^{-1} yeast extract, 20 mL/L glycerol),	pH 4–8 Optimum pH 6	Batch fermentation in shake flasks Medium: YPG (20 g L^{-1} peptone, 10 g L^{-1} yeast extract, 20 mL/L glycerol), pH 6.0, 150 rpm for 28 °C, 120 h.	≈165 μg g^{-1} Of carotenoids	(Allahkarami et al., 2021)
	R. glutinis LOCKR1	Glycerol + potato wastewater	4.0–7.0 Optimum 7.0	Batch fermentation in shake flasks. Medium composition: potato wastewater+ 5 % (w/v) glycerol, C/N 4.2/1, 200 rpm at 28 °C for 72 h.	Max total carotenoids: 202,9 μg g^{-1} 3.5 μg g^{-1}	Kot et al. (2017)
	R. rubra ICCF 209	Glucose- yeast extract	pH 3–8 Optimum pH 6–7	Batch culture in conical flasks, medium components: 40 g L^{-1} glucose, 1.5 g L^{-1} yeast extract, 5 g L^{-1} NH_4NO_3. 250 rpm.	650–750 μg g^{-1} (β-carotene)	Mihalcea et al. (2011)
	R.glutinis CCT 2186	Glucose (10 g L^{-1}), NH_4NO_3 (4 g L^{-1}) + asparagine (10 g L^{-1}),	2.2 to 7.8 Optimum 5.0	Batch fermentation in shake flasks Glucose (10 g L^{-1}), NH_4NO_3 (4 g L^{-1}) + asparagine (10 g L^{-1}), temperature 30 °C, stirring 170 rpm, 72h.	β-carotene: 220.93 μg g^{-1} torularhodin: 253.33 μg g^{-1} torulene: 28.26 μg g^{-1}	(Mussagy et al., 2020)
Agitation	*R. glutinis* TISTR	Mung bean waste flour (23.62 g L^{-1}) glucose in sweet potato extract (51.78 g L^{-1}),	258 rpm	Batch fermentation Mung bean waste flour (23.62 g L^{-1}) glucose in sweet potato extract (51.78 g L^{-1}), pH 5.91, under 30.3 °C shaking at 258 rpm in 257.84 h	3,48 μg g^{-1}	Tinoi et al. (2005)
	R. glutinis	(20 g L^{-1}) Glucose (2.5 g L^{-1}) yeast extract, (2 g L^{-1}) malt extract, (1 g L^{-1}) $(NH_4)_2SO_4$,	100 rpm	Batch fermentation (20 g L^{-1}) Glucose (2.5 g L^{-1}) yeast extract, (2 g L^{-1}) malt extract, (1 g L^{-1}) $(NH_4)_2SO_4$, pH 6.0, under T 30 °C rotary shaker for 10 days.	63.4 μg g^{-1}	Aksu and Eren (2007)
	R. glutinis ATCC 4054	Rice bran (5.5 g), sucrose (18.6 g L^{-1}),	150 rpm	Batch fermentation in shake flasks Rice bran (5.5 g), sucrose (18.6 g L^{-1}), T 30 °C and pH = 5.4, in the dark for 12 days.	320 μg g^{-1}	Husseiny et al. (2018)
	R. glutinis CTT 2182	Cassava wastewater diluted to have a reduces sugar content of 10 g L^{-1}, $(NH_4)_2SO_4$ (5 g L^{-1}),	200 rpm	Batch culture in shake flasks Cassava wastewater diluted to have a reduces sugar content of 10 g L^{-1}, $(NH_4)_2SO_4$ (5 g L^{-1}), 30 °C and pH = 5.98 ± 0.3 for 120 h in the dark.	167.23 μg g^{-1}	Ribeiro et al. (2019)
	R. mucilaginosa SML	Food industry effluent	50–250 rpm Optimum 200 rpm	Batch culture, food industry effluent, DBO: 620 mg L^{-1}, DCO: 2704 mg L^{-1}, Glucose 70 g L^{-1}, 2 % inoculum size and incubated at 27 °C at 200 rpm	645 μg g^{-1}	Sundaramahalingam and Sivashanmugam (2023)
Light/ irradiation	*R. mucilaginosa* K-1	Glucose, yeast extract	Different intensities of white bio-lamp: dark, 1700 lx and 3500 lx Higher concentration in 3500 lx	Batch fermentation in shake flasks glucose 40 g L^{-1}, yeast extract 1.54 g L^{-1}, $(NH_4)_2SO_4$ 2 g L^{-1}, pH = 5.5, 150 rpm and 30 ± 1 °C for 168 h	120 μg g^{-1}	Kong et al. (2019)
	R. mucilaginosa	/YPG (20 g L^{-1} peptone, 10 g L^{-1} yeast extract, 20 mL/L glycerol),	White light, Free light Higher concentration with white light.	Batch fermentation in shake flasks Medium: YPG (20 g L^{-1} peptone, 10 g L^{-1} yeast extract, 20 mL/L glycerol), pH 6.0, 150 rpm for 28 °C, 120 h.	228 μg g^{-1}	Allahkarami et al. (2021)
	R. glutinis CGMCC No. 2258	glucose, 4 %; yeast extract, 0.15 %; $(NH_4)_2SO_4$, 0.2 %;	Three different irradiation conditions: without light, two LEDs, three LEDs Maximum	Batch fermentation in 5 L fermentor the medium contained (w/v): glucose, 4 %; yeast extract, 0.15 %; $(NH_4)_2SO_4$, 0.2 %; For 24 h at 30 °C and 180 rpm.	2.6 μg g^{-1}	Zhang et al. (2014)

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Table 4 (*continued*)

Factors	Yeast	Substrates	Concentration/ Value/ intensity	Conditions	Carotenoids	References
			carotenoids production with three LEDs			
	R. glutinis CGMCC No. 2258	Sodium acetate (20 g L^{-1}), yeast extract (1.5 g L^{-1})	Three different irradiation conditions: Without light With 4000 lx With 8000 lx Maximum production in 8000 lx	Batch culture in Erlenmeyer Flask Sodium acetate (20 g L^{-1}), yeast extract (1.5 g L^{-1}) pH 6.0, at 200 rpm, T = 30 °C.	200.53 μg g^{-1} of β -Carotene	Gong et al. (2020)
	R. glutinis CGMCC No. 2258	Glucose	Without and with continuous irradiation (3400 lx) Maximum carotenoids production with continuous irradiation	Batch culture in shake flasks, medium composition: Glucose 40 g L^{-1}, yeast extract 1.5 g L^{-1}, $(NH_4)_2SO_4$ 2 g L^{-1}, pH 6.0, at 180 rpm for 168h.	≈240 μg g^{-1} of β -Carotene	(Gong et al., 2019a, 2019b)

(NTG), have also been explored. Zhang et al. (2021) observed no significant fatty acid changes without stress selection, however, Qi et al. (2020) reported that combining ARTP with stress selection led to a strain with increased α-linolenic acid content. These findings underscore the importance of pairing mutagenesis with stress selection to enhance lipid productivity in *R. toruloides*.

More recently, genetic engineering has opens new opportunities for optimizing lipids biosynthesis. Schultz et al. (2022) applied a CRISPR/Cas9 toolkit in *R. toruloides* along with fatty acyl-CoA reductase expression, to target key metabolic nodes. Overexpression of ACL1 (ATP-citrate lyase) and ACC1 (acetyl-CoA carboxylase) increased precursor supply, while deletion of DGA1 and LRO1 (diacylglycerol acyltransferases) reduced the TAG accumulation due to the carbon flux redirection. These modifications enabled the yeast *R. toruloides* to reach a fatty alcohol titer of 3.7 g L^{-1} under bioreactor conditions. Brink et al. (2023) further identified and characterized six native promoters in *R. toruloides* that activated during nitrogen starvation, including genes linked to lipid metabolism and stress response (example those linked to acetyl-CoA metabolism and storage lipid formation). Since nitrogen limitation is the major driver of lipid accumulation in oleaginous yeasts, these promoters represent valuable tools for precisely regulating lipids biosynthesis under conditions that promote lipid accumulation.

8. Challenges and knowledge gaps in *Rhodotorula* industrialization

Yeasts of the genus *Rhodotorula* have exhibited substantial biotechnological potential primarily owing to their capacity to synthesize high-value metabolites, particularly carotenoids. These compounds hold a significant share of the global market, which was valued at 1.44 billion USD in 2019 and is projected to reach 2.3 billion USD by 2032, with a compound annual growth rate of 3.76 % during the forecast period. Astaxanthin, β-carotene, and capsanthin collectively account for approximately 90 % of the market's total value, with animal feed being the dominant sector, contributing around 41 % of total revenue. The food and dietary supplement industries follow as key application areas (Mussagy et al., 2022). Moreover, the capacity of *Rhodotorula* species to synthesize oils with distinct lipid offers a promising platform for further biotechnological applications. Optimizing lipid production could enable their application across various industries, including biodiesel synthesis, vegetable oil alternatives, food additives, biopolymer development, as well as pharmaceutical and cosmetic formulations (Lee et al., 2017; Almuhayawi et al., 2023).

Despite the significant market potential of yeast biomass and yeast-derived biomolecules, the industrial-scale implementation of *Rhodotorula* upstream and downstream bioprocesses remains constrained by several critical challenges (Lopes da Silva et al., 2023). Nevertheless, growing demand in this sector continues to drive efforts toward improving economic viability to achieve targeted outcomes.

A key challenge in the upstream and downstream bioprocesses of *Rhodotorula* is ensuring their feasibility, sustainability and profitability at large scale. Although this yeast holds great promise for sustainable applications, upstream and downstream bioprocesses often face cost and environmental constraints. To improve the overall viability of *Rhodotorula*-based bioprocesses, it is essential to optimize biomass generation and metabolite biosynthesis (Karamerou et al., 2016; Shanmugam et al., 2020; Mussagy et al., 2021).

Recent advancements in synthetic biology and omics technologies have significantly contributed to optimizing yeast-based bioproduction of high-value biomolecules (Wan et al., 2023). The development of genome-editing tools, particularly the CRISPR-Cas system, has revolutionized strain engineering by enabling accurate and efficient genetic modifications. Unlike conventional genetic engineering approaches that rely on the cellular DNA repair machinery, CRISPR-Cas allows for targeted genetic alterations in non-conventional yeast species, thereby accelerating strain improvement and metabolic pathway optimization (Xu et al., 2023). Significant progress has been made in *Rhodosporidium toruloides* (synonym of *Rhodotorula toruloides*) where Otoupal et al. (2019) successfully implemented a CRISPR-Cas9 system to disrupt the *URA3* gene in *R. toruloides*, achieving a 364-fold increase in editing efficiency following optimization of the transformation process. Additionally, the study demonstrated the feasibility of multiplexed genome editing by simultaneously targeting *CAR2* and *URA3* genes. Jiao et al. (2019) further advanced CRISPR applications in *R. toruloides* by integrating a codon-optimized Cas9 gene into its genome, identifying functional U6 promoters for sgRNA transcription, and achieving over 60 % success in targeted gene modifications, including disruptions of *CRTI*, *CAR2*, and *CLYBL*. Furthermore, the study by Pi et al. (2018) represents the only reported work utilizing CRISPR for *R. glutinis* engineering. This study successfully integrated β-carotene biosynthetic genes (*crtI, crtE, crtYB,* and *tHMG1*) and cellulase genes (*CBHI, CBHII, EgI, EgIII, EglA,* and *BGS*), leading to enhanced β-carotene production (27.13 ± 0.66 mg g^{-1}) compared to the wild-type strain, alongside increased cellulase activity. However, further research is required to optimize CRISPR-based genetic modifications in *Rhodotorula* with a focus on understanding of its genetic background remains, editing efficiency, simplifying plasmid construction, and reducing associated costs, thus facilitating the large-scale deployment of *Rhodotorula* as a microbial cell factory for biotechnological applications (Zhang et al., 2020).

Given the inherent challenges associated with metabolic

Table 5
Key cultivation parameters affecting lipids accumulation in *Rhodotorula* biomass.

Factor	Strain	Carbon and/or nitrogen Source	Concentration/ Value	Cultivation medium and conditions	Lipidproduction	References
Carbon source Carbon source	*R. glutinis* R 4	Glucose	40 g L^{-1}	**Batch culture.** GMY medium: glycerol (40 g L^{-1}, 81.6 %), KH_2PO_4 (8 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.5 g L^{-1}), yeast extract (3 g L^{-1}), C/N: 40, pH 5.5, T° 25 °C, 250 rpm, 120 h.	6.80 g L^{-1}	Maza et al. (2020)
	R. glutinis (CGMCC No. 2258)	Glucose + xylose	40 g L^{-1} (Glucose) 20 g L^{-1}(Xylose)	**Batch culture in shake flasks.** Medium: $(NH_4)_2SO_4$ (2 g L^{-1}), yeast extract (1.5 g L^{-1}), pH 6.0, T° 30 °C, 264 h. Glucose and sucrose: 216 h; xylose and arabinose: 240 h; fructose: 168 h; glycerol: 268 h.	2.68 g L^{-1}	(Gong et al., 2019a, 2019b)
		Glucose Fructose Sucrose Xylose Arabinose Glycerol	40 g L^{-1}		1.75 g L^{-1} 2.1 g L^{-1} 2.5 g L^{-1} 1.25 g L^{-1} 0.75 g L^{-1} 0.75 g L^{-1}	
	R. glutinis TISTR 5159	Crude glycerol (Purity 50 %)	Crude glycerol diluted to obtain 10 % of the glycerol concentration	Batch culture in shake flasks. Medium: $(NH_4)_2SO_4$, C/N 60, pH 6.0, inoculum size 10 % (v/v), T° 30 °C, 200 rpm, 72 h.	35.22 %	(Saenge et al., 2011a)
	R. glutinis BCRC22360	Crude glycerol + fresh palm oil	Glycerol : 60 g L^{-1} Fresh palm oil: 30 g L^{-1}	Batch culture. Medium: $(NH_4)_2SO_4$ (2 g L^{-1}), pH 5.5, T° 24 °C, agitation 200–500 rpm, aeration rate- 1 vvm.	≈35 %	Yen et al. (2019)
	R. glutinis BCRC 22360	Glycerol Crude + used cooking oil	Glycerol : 60 g L^{-1} Used cooking oil: 50 g L^{-1}	Batch culture. Medium: $(NH_4)_2SO_4$ (2 g L^{-1}), pH 5.5, T° 24 °C, agitation 200–500 rpm, aeration rate- 1 vvm.	30 ± 1.2 %	(Yen et al., 2019) (Yen et al., 2019)
	R. glutinis BCRC 22360	Glycérol Brute + used cooking oil.	Glycerol: 60 g L^{-1} Used cooking oil: 30 g L^{-1}	Fermentation in 5 L air agitator. Medium: yeast extract (20 g L^{-1}), $(NH_4)_2SO_4$ (2 g L^{-1}), pH 5.5, T° 24 °C before 200 h, aeration 1.5 vvm.	46 ± 5 %	
	R. glutinis R4	Sugar cane molasses	40 g L^{-1} (60 %reducing sugars)	Batch fermentation in shake flasks. MediumGMY: yeast extract 3g L^{-1}; f pH 5.5,	40.66 % 8.68 g L^{-1}	Sineli et al. (2022)
	R.glutinis R4	Residual glycerol from the biodiesel industry	40 g L^{-1}	Agitation : 250 rpmT°C : 25 °Cfor 120h, inoculum size 10 % v/v.	46.86 % 5.72 g L^{-1}	
	R. glutinis	Sugar cane molasses	80 g L^{-1}	Batch fermentation in shake flasks. Medium: yeast extract (0.2 g L^{-1}), $(NH_4)_2SO_4$ (12 g L^{-1}), pH 6.0, T° 30 °C, agitation 120 rpm for 96 h.	45.0 %	Lakshmidevi et al. (2021)
Nitrogen source	*R. glutinis* CCT 2186	Nitrate d'ammonium (NH_4NO_3) (Inorganic source)	6g L^{-1}	Batch fermentation in shake flasks. Medium: glucose (20 g L^{-1}), pH 4.0, T° 30 °C, agitation 170 rpm for 72 h.	48.99 %	(Mussagy al. 2021)
	R. glutinis R4	Yeast extract	3g L^{-1}	Batch mode aerobic,GMY medium: glucose (40 g L^{-1}), C/N: 40, pH 5.5, T° 25 °C, agitation 250 rpm for 120 h.	6.80 ± 1.84 g L^{-1}	Maza et al. (2020)
	R. glutinis CGMCC 2.703	Ammonium sulphate $(NH_4)_2SO_4$	4g L^{-} + Continuous power supply with 22 g L^{-1} $(NH_4)_2SO_4$	Fed-batch culture, corn cob hydrolysate with 2.31 g L^{-1} glucose and 39.0 g L^{-1} xylose, fed with corn cob hydrolysate concentrate containing 40.5 g L^{-1} glucose and 790.2 g L^{-1} xylose, pH 6, T 30 °C for 201 h.	14.6 %	Liu et al. (2015)
	R. glutinis BCRC 22360	fine vinasse (residual waste from the rice wine-making process)	Total nitrogen: 1.97 g L^{-1}	Batch culture in a medium consisting of a mixture of fine vinasse (reducing sugars 0.112 %) and crude glycerol 30g L-1, inoculum size 10 %, incubation temperature 24 °C, shaking at 200–400 rpm, pH 5.1 after 80h.	36.5 %	Yen et al. (2019)
C:N ratio	*R. glutinis* JMT21978	Glucose/ yeast extract	70:1	Batch culture, 0.5 % yeast extract, 7 % glucose, 28 °C for 192 h, shaking at 180 rpm.	4.6 g L^{-1}	Tkáčová et al. (2017)
	R. glutinis R4	Glucose/ yeast extract	40	Culture under aerobic conditions in GMY medium (yeast extract 3 g $L^{-1,}$ glucose 40 g L^{-1}), pH 5.5, temperature 25 °C for 120h	6.80 ± 1.84 g L^{-1} 47.24 %	(Maza et al., 2020)
	R. glutinis (CGMCC No. 2258)	Glucose/ yeast extract	80- 100 (90)	Batch culture medium components (w/v): glucose 4 %; yeast extract 0.15 %; $(NH_4)_2SO_4$ 0.2 %; pH 5.5, shaking: 180 rpm, inoculum size 10 % (v/v), time: 45 h.	29.7 %	Zhang et al. (2014)
	R. glutinis TR29	Molasses/$(NH_4)_2SO_4$	14:94	Molasses concentration 20 % (94.4 g L^{-1} total sugar, $(NH_4)_2SO_4$: 4g L^{-1}), pH 5.0, T 25 °C, stirring speed: 200 rpm and incubation time: 96 h.	8.8 g L^{-1} 55.3 %	Taskin et al. (2016)
	R. glutinis CICC 31596	Glycerol/Yeast extract	137	Batch culture in shaken flasks, glycerol: 60 g L^{-1}, yeast extract 2g L^{-1}, shaking 3.3 Hz, pH 5.5, temperature 30 °C for 72 h.	1.21 g L^{-1} 29.8 %	Karamerou et al. (2016)
	R. glutinis TISTR 5159	Glycerol/$(NH_4)_2SO_4$	85	Batch culure in shake flasks, crude glycerol diluted with a concentration of 9.5 % glycerol, $(NH_4)_2SO_4$, C/N: 60, inoculum size: 10 % (v/v), T°:30 °C, 200 rpm, pH 6, for 72 h.	41.78 %	Saenge et al. (2011)
	R.glutinis	Sugar cane molasses/ Yeast extract	11	Batch fermentation in shake flasks, 80g L^{-1}sugar cane molasses, 0,2 g L^{-1} of yeast	45.0 %	Lakshmidevi et al. (2021)

(*continued on next page*)

Table 5 (*continued*)

Factor	Strain	Carbon and/or nitrogen Source	Concentration/ Value	Cultivation medium and conditions	Lipidproduction	References
				extract, 12 g L^{-1} of $(NH_4)_2SO_4$, pH 6, T: 30 °C, agitation 120 rpm, for 96 h.		
Agitation	*R. glutinis* R 4	Glucose/ yeast extract	250 rpm	Batch mode, aerobic culture in GMY medium (3g L^{-1} yeast extract, 40 g L^{-1} glucose), C/N: 40, pH 5.5.	6.80 ± 1.84 g L^{-1} 47.24 %	Maza et al. (2020)
	R. glutinis CCT 2186	Glucose/Asparagine	170 rpm	Batch culture, Glucose: 20 g L^{-1}, Asparagine 2g L^{-1}, pH 4, T: 30 °C, for 72h.	61.60 %	(Mussagy, al. 2021a)
	R. glutinis BCRC 22360	Glycerol/ Yeast extract	200–500 rpm	Batch culture (shaker trials), Glycerol: 60 g L^{-1} + Used cooking oil: 30 g L^{-1}, Yeast extract: 20 g L^{-1}, $(NH_4)_2SO_4$- 2g L^{-1}, pH 5.5, T: 24 °C, Stirring speed 200–500 rpm, Aeration rate: 1 vvm.	46 ± 5 %	(Yen et al., 2019)
	R. glutinis CICC 31596	Yest extract/ Ammonium sulphate	3.3 Hz	Batch culture in shaken flasks, 1.5 g L^{-1} yeast extract, ammonium sulphate (purity 98.5 %), glycerol: 30 g L^{-1}, pH 5.5, temperature 30 °C for 72 h.	28.7 % 2.08 g L^{-1}	(Karamerou et al., 2016)
pH	*R. glutinis* KCTC 7989	Glucose/Asparagine	3	Batch fermentation in shake flasks, Glucose: 10 g L^{-1}, Asparagine: 20.6 g L^{-1}, NH_4NO_3: 4 g L^{-1}, T: 30 °C, 170 rpm for 72h.	44.08 %	(Mussagyal. 2021a)
	R. glutinis TR29	Sugar beet molasses/ ammonium sulphate /	4	Batch culture in medium containing 16 % sugar beet molasses, ammonium sulphate: 3 g L^{-1}, temperature: 30 °C, agitation speed: 200 rpm and incubation time: 96 h.	5.9 g L^{-1} 41.2 %	Taskin et al. (2016)
			5.0	Batch culture in medium containing 20 % sugar beet molasses, ammonium sulphate: 3 g L^{-1}, temperature: 30 °C, stirring speed: 200 rpm and incubation time: 96 h	7.6 g L^{-1} 52.6 %	
	R. glutinis (CGMCC No. 2258)	Glucose/Yeast extract	6	Batch culture in medium containing 40 g L^{-1} Glucose, 20 g L^{-1} Xylose, 2g L^{-1} $(NH_4)_2SO_4$, 1.5 g L^{-1} yeast extract, T:30 °C, pH 6, after 264h	2.68 g L^{-1}	(Gong et al., 2019a, 2019b)
Temperature	*R. glutinis* (CGMCC No. 2258)	Glucose/Yeast extract	24 °C	Batch culture medium constituents (w/v): glucose 4 %; yeast extract 0.15 %; $(NH_4)_2SO_4$- 0.2 %; pH 5.5, shaking: 180 rpm, inoculum size 10 % (v/v), time: 45 h	6.2 g L^{-1} 38.8 %	Zhang et al. (2014)
			30 °C		5.1 g L^{-1} 32.5 %	
	R. glutinis TR29	Molasses/Ammonium sulphate	10 °C	Batch culture, molasses concentration 20 %, ammonium sulphate: 3 g L^{-1}, pH 5.0, shaking speed: 200 rpm and incubation time: 96 h	4.5 g L^{-1} 55.7 %	Taskin et al. (2016)
	R. glutinis JMT 21978	Glucose/Yeast extract	28 °C	Batch culture, 0.5 % yeast extract, glucose, for 192 days, shaking at 180 rpm	4.6 g L^{-1}	Tkáčová et al. (2017)
Inoculum size	*R. glutinis* (CGMCC No. 2258)	Glucose/Yeast extract	10 % (v/v)	Batch culture medium constituents (w/v): glucose 4 %; yeast extract 0.15 %; $(NH_4)_2SO_4$ 0.2 %; pH 5.5, shaking: 180 rpm, inoculum size 10 % (v/v), time: 45 h	29.7 %	Zhang et al. (2014)
	R.glutinis	Glucose/Yeast extract	5 %(v/v)	Batch culture, GYM medium : 60 g L^{-1} Glucose, 0.2 g L^{-1} yeast extract, 12 g L^{-1} de $(NH_4)_2SO_4$, pH 6, T : 30 °C, agitation 120 rpm for 96 h	42.80 %	Lakshmidevi et al. (2021)
	R. glutinis CGMCC 2.703	/	10 % (v/v)	Culture in shaken flasks in a culture medium based on non-detoxified maize cob hydrolysate (2g L^{-1}: xylose, 40 g L^{-1} Glucose),4 g L^{-1} $(NH_4)_2SO_4$, pH 6.0, T°: 30 °C for 79 h and a shaking speed of 180 rpm, C/N: 75	32.6 %.	Liu et al. (2015)

engineering, researchers have turned to alternative approaches such as adaptive laboratory evolution (ALE) and mutagenesis using physical or chemical methods. These techniques have proven effective in generating mutant strains with enhanced lipid accumulation, offering a complementary strategy to genetic engineering for improving metabolites yield and industrial applicability (Shi et al., 2017; Wen et al., 2020; Koppram and Olsson, 2020).

Furthermore, one major barrier is the high cost of fermentation processes, including expenses related to large bioreactors, aeration, pH control, and strain preservation (Mussagy et al., 2022). Additionally, while *Rhodotorula* sp. demonstrates very high substrate versatility, industrial production still relies on costly culture media, making large scale upstream and downstream bioprocesses economically unfeasible (Bautista et al., 2012; Mussagy et al., 2019). Although various studies have explored the use of low-cost substrates such as lignocellulosic biomass and agro-industrial residues (Kot et al., 2016; Kot et al., 2017; Ghilardi et al., 2022; Li et al., 2022; Keskin et al., 2023).

It is also important to note that several challenges remain before full scale industrial integration of agro-industrial wastes-based cultivation media tht often show wide variations in chemical composition and can contain inhibitory compounds which some of them may require detoxification prior to fermentation (Papanikolaou and Aggelis, 2011). Additionally, seasonal fluctuations and logistics challenges of waste collection, transportation and storage can affect process reproducibility and scalability (Halim et al., 2025). On the downstream perspective, impurities present in crude substrate may negatively affect the product quality and increase purification costs.

Further research is required to optimize these alternative feedstocks for consistent and efficient production by focusing on composition analysis, advanced pretreatment methods (chemical, physical, and biological approaches), standardization techniques (Halim et al., 2025) as well as on integrating *Rhodotorula* fermentations with existing

agro-industrial waste management systems (Abeln and Chuck, 2021; Priyadarshini and Kataria, 2025). Additionally, conducting a life cycle assessment (LCA) is crucial to evaluate the environmental and economic impact of utilizing these alternative feedstocks sustainably. Moreover, an integrated strategy for the concurrent production of carotenoids, lipids, and enzymes remains unavailable in a cost-effective manner (Kot et al., 2019). Overcoming these limitations requires a shift towards sustainable and affordable cultivation strategies, with a focus on scaling up processes that utilize low-cost substrates while maintaining high productivity.

The optimization of cultivation conditions for *Rhodotorula* strains presents a significant challenge, as conventional optimization techniques are frequently labor-intensive and constrained in their ability to assess multivariable interactions. These limitations make it difficult to systematically evaluate the complex interactions between various factors affecting growth and product yield. High-throughput screening methods offer a promising solution by enabling rapid testing of numerous conditions to identify the most favorable environments for different strains and their specific products (Saenge et al., 2011; Zhao et al., 2023)

Regulatory and safety standards also play a crucial role in the widespread industrial use of *Rhodotorula*. Ensuring that both native and genetically modified strains meet regulatory requirements and adhere to stringent biosafety protocols is necessary for gaining approval and ensuring safe practices. This includes addressing potential biosafety concerns and developing comprehensive safety measures to facilitate the regulatory approval process (Jansson and Hofmockel, 2018; EFSA, 2020). Finally, consumer acceptance is a vital factor in the commercialization of *Rhodotorula*-derived products. Understanding and addressing consumer perceptions is essential for market adoption. Conducting consumer acceptance studies can provide valuable insights into public attitudes and concerns, thereby supporting the alignment of product development with consumer expectations and regulatory frameworks (Aschemann-Witzel and Peschel, 2019; Siegrist, 2021).

9. Conclusion and future research perspectives

Rhodotorula strains are an adaptable and flexible microbial platform due to their ability to produce valuable products such as carotenoids and lipids. Because of their ability to use a wide range of substrates and tolerate different and sustained conditions, they are strong candidates for next-generation applications in biotechnology. However, to realize their potential with large-scale and cost-effective applications will require advancements in many areas. On the genetic side, there is a need for better and more affordable tools that will enable precise and efficient modifications of the target organism. Better and advanced CRISPR systems and synthetic biology techniques could facilitate pathways to modify multiple pathways simultaneously. Complementary to these are strategies such as adaptive laboratory evolution and site-directed mutagenesis to select for improved tolerance, resilience and performance under industrial conditions. Cultivation approaches and operational strategies are also important facets of improvement. Using more renewable and affordable feedstocks while maintaining and improving productivity and sustainability will be a priority. Advanced bioprocessing such as continuous fermentation systems, data driven modelling and high-throughput screening to stabilize yields and, decreased overall costs will improve their feasibility. Another exciting area of development is the creation of integrated biorefineries which produce multiple valuable metabolites in one continuous spin, increasing efficiency and reuse and reduction. When considering the creation and development of *Rhodotorula* products, there are social and regulatory considerations. Ultimately, biosafety tests, clarity on the legal guidelines, and consumer acceptance studies will be necessary to enable wider acceptance and commercialization of the products. If we can combine genetic based advances, sustainable cultivation strategies and proactive regulation, future endeavours could establish *Rhodotorula* activity as fundamental to Wales biotechnology industry delivering products in food, feed, pharmaceuticals and renewable energy.

Author's contribution

IC: Funding acquisition, conceptualization, literature review, writing - original draft preparation, writing-review and editing, visualization, supervision. **YBM, AMK, PN, CUM, FA:** Writing-review and editing. **GM:** Review and editing, visualization.

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Declaration of competing interest

None.

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