Chapter

The Oleaginous Red Yeast *Rhodotorula/Rhodosporidium*: A Factory for Industrial Bioproducts

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Abstract

Rhodotorula genus, amended in 2015, is polyphyletic and contains *Rhodotorula* species that grow as single-cell yeast (monomorphic) and reproduce asexually via budding/fission (anamorphic); it also contains *Rhodosporidium* species that reproduce sexually (teleomorphic) and alternate between a yeast phase and dikaryotic filamentous phase (dimorphic). Several species of these "red yeast" produce industrial bioproducts, namely biofuel feedstocks, carotenoids, enzymes, and biosurfactants. This chapter highlights the biotechnology areas that *Rhodotorula/Rhodosporidium* contributes to and the future market value of those industries. The primary yeast species to be discussed include *Rhodosporidium toruloides, Rhodotorula glutinis, Rhodosporidium diobovatum, Rhodosporidium kratochvilovae, Rhodotorula graminis, Rhodotorula babjevae, and Rhodotorula taiwanensis.*

Keywords: *Rhodotorula*, *Rhodosporidium*, red yeast, oleaginous, carotenoids, biofuels, biodiesel, antioxidants, DAAO enzyme, PAL enzyme, PEFA, surfactants, antagonistic yeast

1. Introduction

The aim of this chapter is to discuss the *Rhodotorula* genus in the context of biotechnology; it is not meant to be an academic "deep-dive" into all things known about the topic (their discovery and classification in the early 1900s could be a separate chapter unto itself [1]). The word "oleaginous" used in the chapter title is important, and it means "oil producing" or "rich in oils." Much of the interest in these yeast stems from the fact that they often store excess carbon as triacylglycerols (TAG), not as polysaccharides [2]. These lipids can account for up to 70% of the cells dry mass depending on growth conditions, and these oils can be harvested and used as raw material in second-generation biodiesel production. Some *Rhodotorula* species also produce glycolipids, known as polyol esters of fatty acids (PEFA), which have broad interest in the biosurfactant industry. Several emerging publications and patents have been reported in this area. In addition, these yeasts are indeed "pinkish red." These hues are carotenoid compounds produced by the yeast; these natural dyes can be extracted from *Rhodotorula/Rhodosporidium* and used in the food and vitamin industries. This chapter will further expand upon these intriguing facts and illustrate other examples that are in the scientific and patent literature, all while highlighting the future market potential of these "industrious" yeast.

1.1 A brief introduction to the *Rhodotorula* genus

There was a major revision of the subphylum *Pucciniomycotina* (Phylum *Basidiomycota*, Kingdom *Fungi*) published by Wang et al. in 2015; therefore, this chapter will focus solely on species that are categorized under the revised *Rhodotorula* genus in the Sporidiobolaceae family. Species to be discussed include *Rhodosporidium toruloides, Rhodotorula glutinis, Rhodosporidium diobovatum, Rhodosporidium kratochvilovae, Rhodotorula graminis, Rhodotorula babjevae, and Rhodotorula taiwanensis*. Species that were removed from the *Rhodotorula* genus in 2015, for example, *Rhodotorula bogoriensis* (reclassified as *Pseudohyphozyma bogoriensis*), will not be discussed in detail, even though it produces sophorolipid biosurfactants and is of industrial interest [3–5].

It is noteworthy that the *Rhodotorula* genus is polyphyletic. It contains *Rhodotorula* species that grow as single-cell yeast (monomorphic) and reproduce asexually via budding/fission (anamorphic) [6]. It also contains *Rhodosporidium* species that reproduce sexually (teleomorphic) and alternate between a yeast phase and dikaryotic filamentous phase (dimorphic). For *Rhodosporidium*, sexual reproduction begins with the fusing of compatible haploid yeast cells. They then grow as dikaryotic hyphae/mycelium, during which diploid teliospores are produced. Teliospores germinate forming basidium (where karyogamy and meiosis occur) and then extrude haploid basidiospores. Germination of basidiospores then restores the yeast phase of growth [7, 8]. Thus, the yeast phases of *Rhodotorula* and *Rhodosporidium* species are virtually indistinguishable. It is notable that "all known *Rhodosporidium* species [have been] isolated as haploid yeasts and have a bipolar mating behavior, i.e., their strains belong to either one of two complementary mating types, designated *A1* and *A2* or *A* and *a*" [9]. Due to this mating behavior, *Rhodosporidium toruloides* is being developed as an alternative biotechnology platform to *Saccharomyces cerevisiae* [10] with unique biochemical pathways for the production of biofuels, carotenoids, and industrial enzymes.

1.2 The industrial markets of the genus *Rhodotorula*

The most relevant question for the purposes of this chapter, regardless of the details of any given species, is "what biotechnology markets are impacted by the *Rhodotorula* genus?" **Figure 1** summarizes the five major industrial markets where *Rhodotorula* yeast is utilized (or will likely be utilized in the future). These include biofuels, carotenoids, enzyme production (e.g. D-Amino acid oxidase (DAAO) and L-Phenylalanine ammonia lyase (PAL)), biosurfactants, and antagonistic yeast.

1.2.1 Biofuels

Biofuels continue to grow as a global industry, despite the challenges in production capacity and automotive engine compatibility [11]. The biofuels market size is expected to reach USD ~218 billion by 2022 [12]. For example, United Airlines announced in 2018 that it would begin blending more biofuel with conventional fuel, with a goal to reduce greenhouse emissions by 50% on all flights by 2050. It is expected that other airlines will follow suit, further increasing biofuel demand. Thus, there continues to be a need for microbial factories to produce biofuels [13, 14], especially given the market competition of using vegetable oils in both the food and biodiesel sectors [15]. Microorganisms may contribute to biofuel production in several ways; bisabolene (a diesel alternative) can be produced through bioengineering the isoprenoid pathway [16], and alkanes and alkenes can be produced through fatty acid biosynthesis inside the cell [15, 17]. Furthermore, microbial triglycerides,

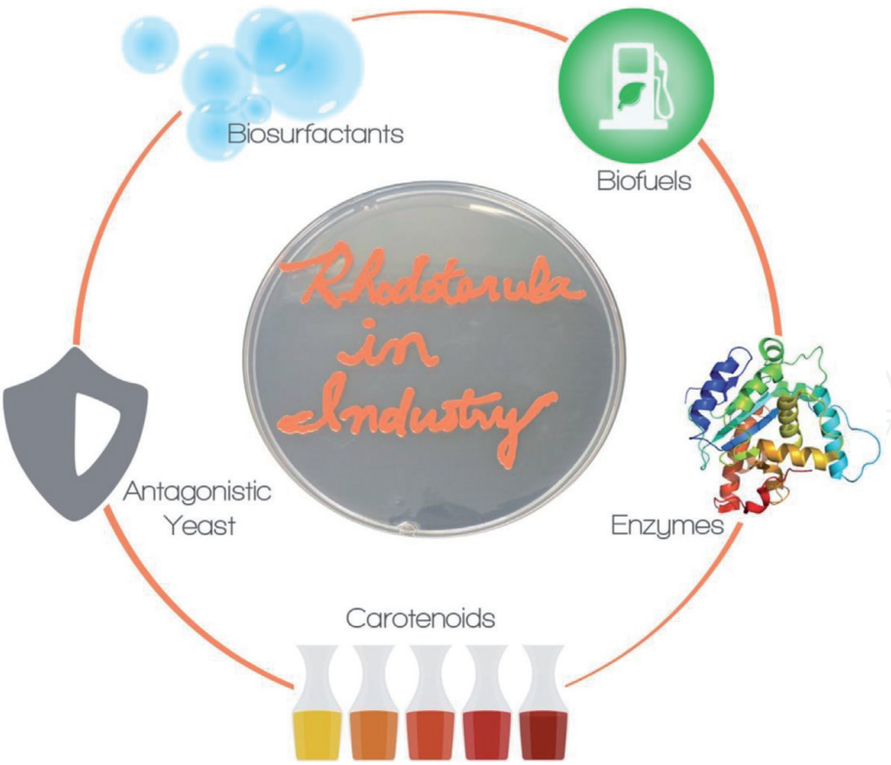

Figure 1.
The primary biotechnology industries impacted by the Rhodotorula genus. "Rhodotorula in Industry" was written in Rhodotorula taiwanensis MD1149 on a Hommel minimal salt (HMS) agar plate supplemented with glucose.

fatty acids, and other lipids can be converted to fatty acid methyl esters (FAME) by transesterification with methanol; FAME molecules are the primary component in biodiesel fuel.

The biofuels industry is largely divided between two types of fuel: biodiesel and bioethanol. These biofuels are further separated as "first generation, second generation, third generation, or fourth generation." First-generation biofuels are produced using feedstocks that can also be used for food (e.g. soy, canola, or sunflower oils). These oils are transesterified into FAME by reacting a triacylglyceride with a short alcohol—usually methanol—in the presence of a catalyst (strong base) and heat. By contrast, second-generation biofuels are produced using *non-food/non-edible* sources such as wood, organic waste, and food crop waste. Second-generation biofuels are preferred as they do not utilize food crops for fuel. Third-generation biofuels are produced by engineered algae; fourth-generation biofuels are aimed at using biomass that captures CO_2 while producing lipids that can be converted into biofuel. In this context, *Rhodotorula/Rhodosporidium* species that are used in biodiesel production are classified as "second generation" biodiesel producers that accumulate single-cell oils (SCOs); they will be discussed later in this chapter.

1.2.2 Carotenoids

In addition to lipids, these yeasts also produce carotenoids, which are of industrial interest. A recent report entitled "Global Carotenoid Market – Growth, Trends, and Forecast (2018–2023)" predicted a global carotenoid market of USD 2 billion by 2023 [18]. Carotenoids are yellow, orange, and red pigments produced by microorganisms, algae, higher plants, and some animals; these compounds are responsible for giving *Rhodotorula* species their "Red Yeast" moniker. Common examples of carotenoids include alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein,

lycopene, torulene, and torularhodin. Carotenoids are effective "quenchers" of reactive oxygen species (ROS), and the protective health effects of dietary carotenoids (as antioxidants) are of intense interest to the industrial research community [19]. Some carotenoids found in fruits and vegetables can be converted into vitamin A, namely beta-carotene, and to a lesser extent alpha-carotene and beta-cryptoxanthin (note: these compounds are often referred to as "provitamin A"). The impact of Vitamin A on human health is nicely summarized by Ulbricht et al. [20]. Carotenoids may also be used as natural coloring agents in the food, cosmetic, and pharmaceutical industries [21].

1.2.3 Enzymes

The *Rhodotorula/Rhodosporidium* enzymes, D-Amino acid oxidase (DAAO) and L-Phenylalanine ammonia lyase (PAL), have notable utility within industry. DAAO is a "FAD-dependent oxidoreductase that catalyzes stereospecifically the oxidative deamination of d-amino acids to α-keto acids, ammonia, and hydrogen peroxide" [22]. Even though DAAOs have been identified in a wide range of living organisms (e.g. bacteria, fungi, humans) [23], DAAO from certain *Rhodotorula* strains—discussed later in this chapter—has advantages over others, mainly due to a higher turnover rate and their increased stability of FAD binding [24]. This makes them ideal for the enzymatic deamination of cephalosporin C to 7-(5-oxoadipoamido)-cephalosporanic acid, an important intermediate for the production of cephalosporin [25]. Cephalosporin antibiotics are the largest selling class of antibiotics in a market, which is expected to reach USD 57 billion by 2024 [26, 27]. The PAL enzyme is also a major contributor to the enzymatic synthesis of "industrially relevant biomolecules," namely pure L-phenylalanine (L-Phe), L-phenylalanine methyl ester (L-PM), and para-hydroxycinnamic acid (p-HCA) [28]. L-Phe, being an essential amino acid, is used in food formulations, feed for livestock, dietary supplements, and nutraceuticals [29]. L-PM is a precursor in the production of the sweetener aspartame [28], and p-HCA has utility in the cosmetic, health, and flavoring industries [30].

1.2.4 Biosurfactants

The biosurfactant market is estimated to be worth over USD 2.7 billion by 2024, primarily driven by their usage in the personal care and cosmetic industries [31]. Biosurfactants are small molecules that contain both hydrophilic and hydrophobic moieties and reduce the surface tension between oil and water mixtures (colloquially they are known as "green detergents" or "bio-soaps"); they are produced by several microbial species, viz., bacteria, yeast, and fungi [32, 33], and they are utilized extensively in multiple industries: cosmetics, food, explosive, pharmaceutical, detergents, and paints [34, 35]. The primary market barrier for biosurfactants is the vast library of existing chemical surfactants produced from petroleum feedstocks; end users can choose from hundreds of synthetic surfactants to fulfill their industry need. A major drawback is that chemical/synthetic surfactants can accumulate in the environment and are toxic to microbes, plants, aquatic life, and higher vertebrates including humans [36]. Therefore, because biosurfactants are biodegradable and petroleum-independent, their value will likely increase as pollution increases.

1.2.5 Antagonistic yeast

Rhodotorula species also play a role in the market of biocontrol agents, also known as antagonistic yeast. Significant losses in harvested fruit occur from decay by filamentous fungi such as *Botrytis cinerea* and *Penicillium expansum* [37]. In order to

replace or augment chemical fungicides, biological control agents have been studied heavily the past two decades, with some yeast-based biocontrol products commercially available for specific commodities [38]. The ability to control different rots, on different fruits/vegetables, is still an important goal when developing postharvest biocontrol products. Therefore, antagonistic yeasts continue to be an active area of research, and several *Rhodotorula* species have shown promise in this field.

2. Species utilized in biotechnology

2.1 *Rhodosporidium toruloides*

Rhodosporidium toruloides has tremendous potential as a workhorse for multiple industrial applications [39]. It can grow on a variety of carbon sources associated with modern waste streams, and its bioproducts can be used in antibiotic manufacturing, biofuel synthesis, and the food industry.

R. toruloides is a nonpathogenic, aerobic, oleaginous red yeast that has been isolated from a variety of sources, e.g. conifers, soil, wood pulp, dry leaves, and a salt farm [2]. It is able to accumulate lipids to more than 70% of its dry cell weight [40–43]; this occurs when carbon is in excess during growth, and other key nutrients such as nitrogen are sparse [44]. This lipid production can occur on a variety of different carbon sources, e.g. sugarcane juice, crude glycerol, lignocellulosic hydrolysates, vegetable market waste, and Jerusalem artichoke plants [45–49].

Due to the ability of *R. toruloides* to use different types of carbon sources for growth and lipid production, several studies have been carried out on growing this organism using "cheap" carbon sources that are the waste streams from other large industries, namely food waste and crude glycerol waste. Food wastes are an appealing nutrient source, because they are cheap, abundant, and decrease the environmental impact of disposing of these materials using traditional consumer and agricultural waste infrastructure [50, 51]. Crude glycerol is of interest as a "waste" source as the biofuel industry generates ~10% w/w of glycerol for every batch of biodiesel produced; *R. toruloides* has been shown to use crude glycerol as a carbon source for the production of microbial lipids of interest, even in the presence of impurities [52, 53]. In addition, it has been reported that *R. toruloides* has the ability to degrade/utilize hydrocarbon fuels [54]. This opens the possibility of using this yeast as a bioremediation tool for oil contaminated soil.

The bioproducts produced by *R. toruloides* are also of intense interest, namely lipids, enzymes, and carotenoids. Wild-type and engineered strains of *R. toruloides* are truly oleaginous. They produce lipids at high titer making them promising organisms for the production of lipid-based chemicals such as biofuels, lubricants, surfactants, solvents, waxes, creams, and adhesives [2, 55, 56]. It has been shown that under low nitrogen conditions, growth on simple sugars, like glucose, fructose, xylose, or the carbohydrate glycerol, can increase TAG production [55]. To this end, several groups are working to further increase lipid production using engineered strains of *R. toruloides,* primarily for biofuels [45, 56, 57]. As a practical example, scientists in Brazil have performed a successful diesel engine test using biodiesel manufactured from lipids produced by *R. toruloides* when the organism was grown in sugarcane juice (carbon source) and urea (nitrogen source) [48].

R. toruloides is also a "gold mine" for industrial enzymes. It produces a high titer of esterase enzymes, which makes this organism of immense interest to the drug industry. For example, the process of making antibiotics in the cephalosporin class of compounds requires 3-acyloxymethyl cephalosporins to be enzymatically deacylated to the more stable intermediary 3-acyloxymethyl cephalosporins. This can be achieved

by the esterase enzymes produced by *R. toruloides*. Another enzyme produced by *R. toruloides* is phenylalanine ammonia-lyase (PAL), which is a major contributor to the enzymatic synthesis of pure L-phenylalanine (L-Phe), L-phenylalanine methyl ester (L-PM), and para-hydroxycinnamic acid (p-HCA) [28].

R. toruloides has also played an important role in the development of an enzyme substitution treatment of Phenylketonuria (PKU) [58, 59], a genetic mutation manifesting in the inability to metabolize L-Phe. In the human diet, L-phenylalanine is found naturally in protein foods, such as eggs, meats, fish, cheese, and soybeans, and is also produced as a product that can be added to foods, in the case of aspartame. The buildup of L-Phe in the blood is extremely toxic and impacts neurological function in the form of seizures, tremors, and loss of muscle coordination, especially in the extremities [58]. Newborns are screened for PKU at birth to increase the chances of early discovery and attempt to limit the effects of PKU. A new drug, Palynziq™, was developed based on some of the research that involved *R. toruloides* along with other PAL producing species; it was approved by the FDA in May 2018 as an enzyme substitution therapy for PKU [59].

2.2 *Rhodotorula glutinis*

Rhodotorula glutinis, like *R. toruloides*, is of high industrial importance as it also synthesizes numerous valuable compounds: lipids (SCO, single-cell oils), enzymes (in particular, PAL), and carotenoids (lycopene, β-carotene, torulene, and torularhodin). An example workflow of harvesting these bioproducts from these species is shown in **Figure 2**.

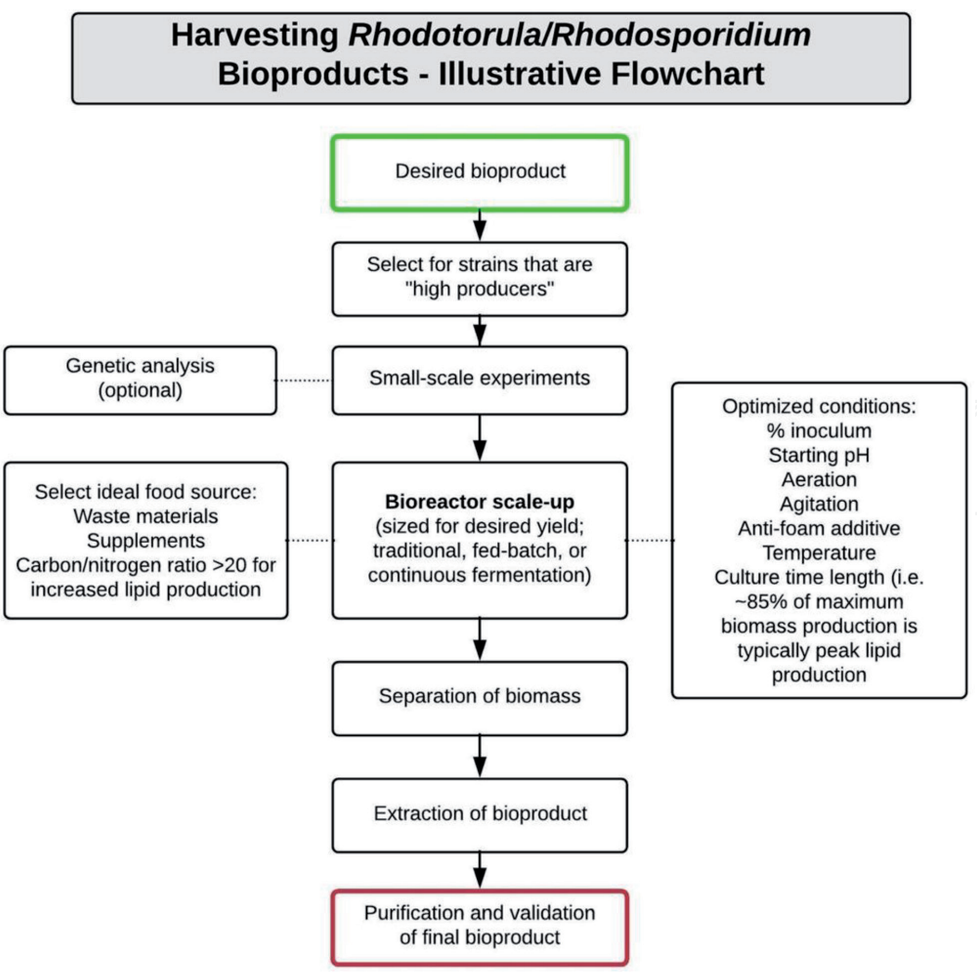

Figure 2.
An example workflow of harvesting bioproducts from members of the Rhodotorula genus.

R. glutinis isolates have been isolated from air, fruit, seawater, soil, grass, milk, and cheese products [60, 61]. Previously, 370 collection strains were assigned to this species based upon color and growth properties; however, additional methods—namely genetic sequencing—have pared this list to a few dozen strains [61]. The majority of these strains are spherical, ellipsoidal, or elongated in shape; aerobic; and mesophilic, although some can thrive under lower temperatures. They use different sources of carbon for growth: glucose, galactose, sucrose, maltose, trehalose, ethanol, glycerol, and hexadecane. *R. glutinis* lack the capacity to perform sugar fermentation and can grow in salt (10% NaCl) but cannot tolerate high sugar (>50% glucose). Depending on strain and growth conditions, their colonies are smooth, moist, and mucoid. As with most species in the *Rhodotorula* genus, color can be creamy, yellow, salmon, pink, orange coral, and blood red due to the production of carotenoids [60].

R. glutinis strains also produce several microbial oils—dominated by oleic, linoleic, palmitic, and stearic acid [62, 63], and the lipid content in their biomass can reach up to 72% [64]. Numerous factors can affect the lipid content and distribution including choice of strain, carbon sources (molasses, glucose, sucrose, glycerol, or waste materials), nitrogen sources (ammonium sulfate or chloride, yeast extract, or monosodium glutamate wastewater), C/N ratio, and cultivation times. These factors result in a wide lipid content distribution between 18 and 66% [60]. Additionally, investigators have worked to increase microbial oil production by either improving culture medium (Chinese patent CN102559788A) or by genetically engineering *R. glutinis* (Chinese patent CN102796675B).

It is noteworthy that several conditions were evaluated for the co-production of both lipid and carotenoids; variables tested included irradiation (as a stressor), temperatures, and C/N ratios. This allowed for the development of a two-stage cultivation strategy where the first stage maximized biomass and carotenoid production under irradiation/high temperature and then maximized lipid content when switched to dark/low temperature [63]. Additional growth studies have evaluated the effect of pH with potato wastewater and glycerol [65], modulating air flow rates using glycerol with yeast extract as the nutrient supply without pH control [66] and using lignocellulosic biomass [67]. Other parameters for scale-up were explored using an airlift bioreactor with mixed carbon sources [68]. *R. glutinis* has also been genetically engineered by introducing both the beta-carotene biosynthesis genes and cellulase genes to increase yields and co-production [69].

Per other industrial applications, *R. glutinis* has been evaluated as a biocontrol agent for post-harvest microbial diseases of fruit (US patent US5525132A). To this end, it has been shown to significantly reduce the incidence of the gray mold, *Botrytis cinerea* on strawberries and apples [70, 71] possibly due to the attachment capability of the antagonistic *R. glutinis* to *B. cinerea* [72]. *R. glutinis* was also used in combination with rhamnolipids to be more efficacious against *Alternaria alternata* infection in cherry tomato fruit than either agent alone [73].

It is also interesting that *R. glutinis* has been isolated during the production of olive oil and during the fermentation and storage of green and black olives in the USA [74, 75]. Olive processing results in the generation of large quantities of olive mill wastewater (OMW) high in phenol. Advantageously, *R. glutinis* has been utilized to treat OMW by dephenolization [76].

2.3 *Rhodosporidium diobovatum*

Rhodosporidium diobovatum has been identified as being a top-tier lipid producer among a group of 69 varied oleaginous yeast strains when glucose is used as the sole carbon source; it is also amenable to scalability [77, 78]. However, biofuel research has also focused on *R. diobovatum* as a second-generation biodiesel producer due

to its ability to consume the impure glycerol waste that is created as a byproduct of first-generation biodiesel production [77]. For example, it was recently reported that *R. diobovatum* could be an "effective strain for production of neutral lipids" given its high yields of oleic, palmitic, and linoleic acid [79], although growth on glucose produced more TAGs than glycerol. Importantly, a glycerol consumption strategy would allow for the continued use and optimization of the first-generation biodiesel production pipelines, while simultaneously allowing investment in the development of second-generation approaches.

Interestingly, it has been reported that up to 70% of the cost to make biodiesel could be accrued *after* the production of the cell biomass, when chemical methods of extracting TAGs are used [80]. This is a costly process that requires the complete drying of biomass, full chemical diffusion of solvent into the cells [81], yeast cell wall disruption, and lipid extraction [2]. The *direct* conversion of wet biomass to biodiesel is the ideal solution, but current methods for that are not scalable, require high temperatures, have lengthy reaction periods, or utilize expensive catalysts [82]. Recent advances with microalgae utilized an ionic liquid for wet cell disruption and lipid extraction in less than 1.5 hours [83]. This same method was effective for *R. diobovatum* and was optimized to produce 97.1% conversion of maximum FAME yields in 2.5 hours at 65°C. They did note a loss of ~40% of their KOH catalyst but conclude that switching from a homogenous to a heterogeneous catalyst could mitigate this [80]. Therefore, research is ongoing to develop *R. diobovatum* as top biofuel production species.

R. diobovatum is also being examined in several novel applications. These include as a way to produce the vitamin supplement glutathione (GSH) [84], which has reported antioxidant properties, and also can be utilized as an anti-toxicant, as a cell metabolism modulator, and potentially as a neuromediator [85]. GSH is synthesized by canonical yeasts but was found in *R. diobovatum* using high performance liquid chromatography (HPLC). Consequently, the *R. diobovatum* synthase genes have been identified and characterized, with the expectation that it could be a valuable industrial producer of GSH in future [84]. This species also has potential as a bioremediation agent for fertilizer pollution. It efficiently assimilates nitrogen at higher rates than other yeasts and may become a useful tool for treating agriculture wastewater [86].

2.4 *Rhodosporidium kratochvilovae* and *Rhodotorula graminis*

Rhodosporidium kratochvilovae cultures have been used to create single-cell oil biodiesel when cultured on cane molasses, a sugar refinery waste product [87]. This biodiesel meets standard specifications in Europe and the USA for quality and purity (ASTM D6751, EN14214). The strain was originally discovered in a screen for oleaginous yeasts in Ethiopia. Of the 340 yeast isolates screened, 18 tested positive for oil production, and a *R. kratochvilovae* strain was one of three chosen as best candidates for further optimization based on productivity [88]. Multiple parameters of its cultivation were optimized for lipid production [89] prior to being used to create SCO.

Rhodotorula graminis has also been developed for biodiesel using crude glycerol and undetoxified lignocellulosic hydrolysate (hydrolyzed corn stover; aka, the stalks, leaves, and cobs that remain in fields after harvest) [90]. This strain produced a high lipid content (34% w/w) on hydrolysate and increased this production on waste crude glycerol (54% w/w) [90]. Further optimization of this production pipeline was discussed in patent US9322038B2 granted in 2016 to Washington State University for "Simultaneous saccharification and fermentation (SSF) of lignocellulosic biomass for single-cell oil production by oleaginous microorganisms," with details of strains and culture conditions therein.

R. kratochvilovae and *R. graminis* also produce carotenoids (specifically beta-carotene, torularhodin, and torulene) in order to mitigate cellular damage done by ROS [91]. The benefits of beta-carotene have been understood for some time (antioxidant), but those of torularhodin and torulene only been elucidated recently, namely their potent anti-cancer [92] and anti-microbial activity [93]. Importantly, *R. graminis* and other *Rhodotorula* species have the ability to produce these rare carotenoids at high levels [94] and represent viable options for industrial production of these compounds.

R. kratochvilovae and *R. graminis* are also involved in the biological control of plant pathogens [95]. In a screen for yeasts that control pathogenic fungal strains, *R. kratochvilovae* LS11 was found to have high antagonistic activity [96]. Further development of this strain is hoped to be achieved, and its detoxification pathways are currently being examined in detail [97]. *R. graminis* was also been examined for this application, alone and in combination with fungicide [98].

The PAL enzyme of *R. graminis* (RgrPAL) has industrial interest as it is highly stable in comparison to that of other species, and the organism has flexible culturing requirements. Mutagenesis was employed to create mutants that expressed higher levels of PAL, including an isolate that showed a fourfold increase in production [99]. RgrPAL is also useful to pharmaceutical production, as it accepts analogues of its substrate L-Phe, which can be included in peptidomimetic drugs. The enzymatic activity of RgrPAL was recently increased 28-fold using directed evolution methodology in a recent study [100].

2.5 *Rhodotorula babjevae* and *Rhodotorula taiwanensis*

It was recently published that strains of *Rhodotorula babjevae*, and other *Rhodotorula* species, produce polyol esters of fatty acids (PEFA) [101, 102], similar in composition to extracellular glycolipids reported in the 1960s [103]. The authors stated that "discovery of these PEFA-secreting yeasts may aid in improving production of renewable, sustainable, environmentally friendly surfactants for use in household and industrial cleaning products, as well as many other applications" [102]. To this end, a full patent application was submitted in 2017 by the University of California for "Methods of producing polyol lipids," with details of *Rhodotorula* strains and culture conditions that optimize PEFA production (WO2017184884A1).

It is important to note that the general utility of surfactants in industry—whether they be chemical surfactants or biosurfactants—is determined by their hydrophilic-lipophilic balance (HLB). The HLB concept was first published in 1948 brochure by Atlas Powder Company, along with a follow-up journal paper by William Griffin (an Atlas chemist) [104]. Griffin described HLB as follows: "emulsifiers consist of a molecule that combines both hydrophilic and lipophilic groups and the balance of the size and strength of these two opposite groups is called HLB. For the purpose of convenience, the effective balance of these groups is assigned a numeric value [105]." Griffin then went on to develop the HLB number system, on a scale 0–20, based on polyoxyethylene (POE)-type surfactants. Although the correct classification of surfactants in the HLB system is still being refined and debated [104, 106, 107], the concept of different surfactants having different HLB values remains the benchmark for surfactant science. An example of different surfactant HLB values, their solubility, applications, and use in industry is provided in **Table 1**.

Perhaps the greatest market barrier that biosurfactants face is their limited coverage of the HLB scale. Therefore, they can only be used in very targeted commercial applications. By contrast, hundreds of petroleum-derived chemical surfactants

Surfactant HLB	Solubility	Applications	Industry examples
1.5–3	Oil soluble	Antifoaming agents (defoamers)	Hydraulics, paper, oil drilling, machine tools
3–6	Oil soluble	Water-in-oil emulsions	Cosmetics, sunscreen, margarine
7–9	Oil soluble	Wetting and spreading agents	Herbicides, fertilizers
12–16	Water soluble	Oil-in-water-emulsions	Mayonnaise, cosmetics, dispersants
13–15	Water soluble	Detergents	Laundry and dishwashing detergents
15–18	Water soluble	Solubilizing agents	Pharmaceuticals

Table 1.
The hydrophilic-lipophilic balance (HLB) scale covers 0–20, with different industrial applications depending on the HLB value.

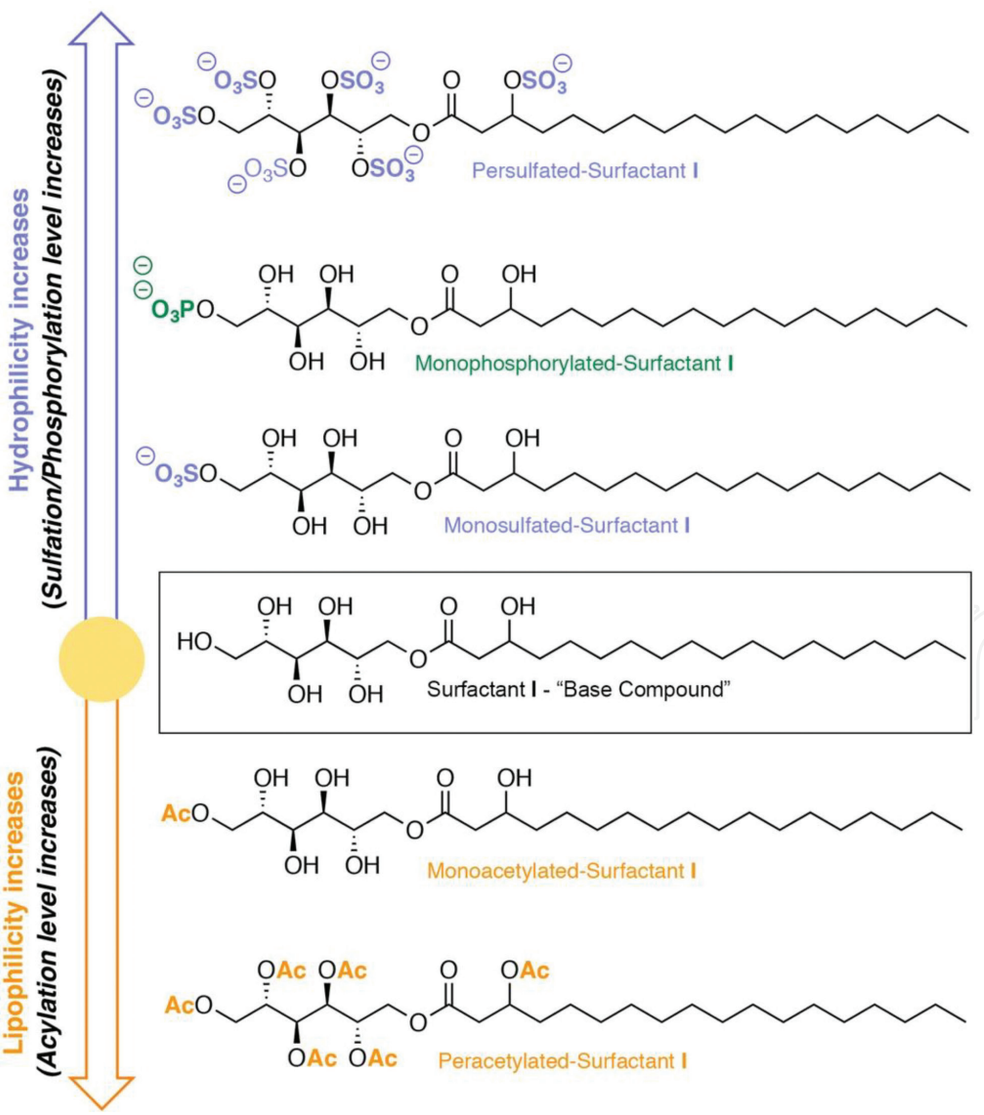

Figure 3.
A "tunable" PEFA biosurfactant. Rhodotorula taiwanensis would be genetically engineered to produce a single PEFA "base compound" and then be systematically modified to move up and down the HLB scale.

conveniently cover the entire HLB scale, to such an extent that their massive usage is now poisoning the environment [36]. Biosurfactants offer a more environmentally friendly option (biodegradable and petroleum-independent); however, there are a limited number of commercially available biosurfactants, and each one covers only a small portion of the HLB scale.

Therefore, in order to expand PEFA coverage on the HLB scale, it was recently published that a new strain of *Rhodotorula taiwanensis* produced hypoacetylated PEFA compounds compared to those produced by *Rhodotorula babjevae* [108, 109]; the difference in their acetylation profiles resulted in different surface tension of the growth medium, i.e. a different hydrophilic-lipophilic balance. It was noted in a subsequent patent filing that although the current *Rhodotorula taiwanensis* strain produced a complex mixture of acetylated and non-acetylated biosurfactants, it could be genetically engineered to produce an unmodified (non-acetylated) "base compound." This base compound could then be "tuned" to produce the full range of biosurfactants that could more extensively cover the HLB scale (**Figure 3**).

This "tunable" biosurfactant approach, using a single species of PEFA compound that can be systematically modified, provides a viable option for competing directly with all types of chemical surfactants, across all market sectors. The biosurfactant's chemical topology, highlighted by the carbohydrate unit's hydroxyl groups, offers further opportunity for chemical modifications that have a direct impact on the physical properties of the surfactant (e.g. antifoaming, emulsifying, wetting, detergent, and solubilizing). Each hydroxyl (OH) group on the molecule has the ability to be acetylated, sulfated, or phosphorylated, leading to modified versions of the parent molecule with different physicochemical properties. Therefore, modification of the hydroxyl groups by chemical and/or biochemical means will enable scientists to produce various biosurfactant products best suited for their unique industrial applications—serving markets not currently served by existing biosurfactants.

3. Conclusions

The *Rhodotorula* genus currently plays a significant role in yeast biotechnology and is poised to expand into various industrial markets: biofuels, carotenoids, biocontrol agents, enzymes, bioremediation, cosmetics, and others. Several advanced genetic systems are currently being developed for these red yeast, and they will likely become an alternative biotechnology platform to *Saccharomyces cerevisiae* [10]. The future looks bright (red) for these fascinating, diverse, and versatile oleaginous microbes.

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Conflict of interest

The authors declare no conflict of interest.

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