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Trends in
Biotechnology

Review

What makes *Yarrowia lipolytica* well suited for industry?

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Yarrowia lipolytica **possesses natural and engineered traits that make it a good host for the industrial bioproduction of chemicals, fuels, foods, and pharmaceuticals. In recent years, academic and industrial researchers have assessed its potential, developed synthetic biology techniques, improved its features, scaled its processes, and identified its limitations. Both publications and patents related to *Y. lipolytica* have shown a drastic increase during the past decade. Here, we discuss the characteristics of this yeast that make it suitable for industry and the remaining challenges for its wider use at large scale. We present evidence herein that shows the importance and potential of *Y. lipolytica* in bioproduction such that it may soon be one of the preferred choices of industry.**

Highlights

Selection of the most appropriate microorganism is one of the key aspects for the industrial success of microbial bioprocesses.

Yarrowia lipolytica has gained interest as a chassis strain in academia and industry because of its capacity to make products at high yields, use a broad range of substrates, and be genetically amenable.

Y. lipolytica has many features that are desired at an industrial scale, such as safety, robustness, efficient and stable genetic modifications, capacity to use a variety of substrates, and ability to grow at very high cell density.

To further improve the industrial use of *Y. lipolytica*, some characteristics must be improved through metabolic engineering, such as the high oxygen requirement, byproduct formation, and excessive foam synthesis.

Y. lipolytica in biotechnology

Industrial biotechnology (white biotechnology) includes the application of enzymes, cell extracts, or whole microorganisms in industrial processes that lead to the manufacture of a wide variety of products, including fuels, food ingredients, enzymes, materials, and pharmaceuticals [1,2]. Compared with chemical processes, industrial biotechnology is often more sustainable and environmentally friendly and enables specificity and reactivity that is difficult to achieve otherwise [3]. In the past two decades, along with advances in metabolic engineering and synthetic biology, industrial biotechnology has delivered products and innovations in the chemical, textile, food, packaging, and healthcare sectors [4].

One of the keys to the success of a **bioprocess** (see Glossary) is selection of the microorganism. The selection should consider the natural capacity of the **host** to make the desired products or their intermediates, to consume the preferred substates, and to resist the toxicity of intermediates and/or final products. In addition, it is important to consider the wealth of knowledge on the organism's physiology and metabolism as well as the degree of development of techniques for strain engineering [5]. A given bioprocess can also be improved by the optimization of fermentation conditions and evolutionary adaptation [5–7].

Y. lipolytica has been often considered a nonconventional yeast due to its distinctive genome structure and its relatively large phylogenetic distance to other yeasts while sharing common properties with higher eukaryotes [8]. This yeast was originally isolated from lipid-rich or protein-rich environments such as fermented dairy products (cheese, yogurt), meat, poultry, and also from wastes such as lipid-rich sewage or oil-polluted environments [9]. Since its isolation, *Y. lipolytica* has been used for the production of organic acids and heterologous proteins and for the **bioremediation** of oil-contaminated soil and water [10,11]. Some of the unique characteristics that initially drew the attention of researchers to this yeast are its capacity to accumulate lipids, its dimorphism (with both yeast and pseudo-hyphae forms) [10,12], and its ability to degrade hydrophobic carbon sources (fatty acids, triglycerides, alkanes, alkenes, etc.) [13,14].

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Since the 1990s, publications involving *Y. lipolytica* increased rapidly in parallel with the early development of genetic engineering and the availability of the whole-genome sequence of this yeast that encouraged new research groups to work with this microbe. Together with the development of synthetic biology tools for efficient strain manipulation, the number of published articles has grown exponentially, as shown in Figure 1.

In parallel with academia, industrial research noticed the potential of this yeast, and the number of patents also increased (Figure 2). We have briefly explored the patent landscape of *Y. lipolytica* and found 4536 international patents containing the keywords '*Yarrowia lipolytica*' (Figure 2). As expected, the number of patents has significantly increased since 2001, alongside the development of engineering tools for this yeast, which reflect the increasing interest in this organism as an industrial host. Examples of the products made in *Y. lipolytica* and explored by industry are described in Box 1 (see Box 2 for Survey to industry).

The success and popularity of *Yarrowia* research goes beyond academia, and an increasing number of companies are now choosing this yeast as their preferred workhorse, but what makes *Y. lipolytica* so appealing for bioproduction? Here, we try to answer this question by describing the unique features of *Y. lipolytica* that make it an ideal fit for industrial biomanufacturing, its current use in academic and industrial research, and the challenges yet to be overcome to make it a better host.

Y. lipolytica as a workhorse for bioproduction

Traditionally, wild-type strains of *Y. lipolytica* have been used to produce lipids, organic acids, and polyols. In the past years, the number of applications of this yeast has greatly expanded, as described in Figure 3. The high capacity to divert flux toward acetyl coenzyme A has turned engineered strains into high producers of terpenes and lipid-derived products. In recent years, the high production of metabolites derived from different pathways (e.g., the shikimate pathway and pentose phosphate pathway) has also been demonstrated. The range of compounds made from these pathways have applications in the production of food, additives, cosmetics, chemicals, fuels, pharmaceuticals, and materials (Figure 3).

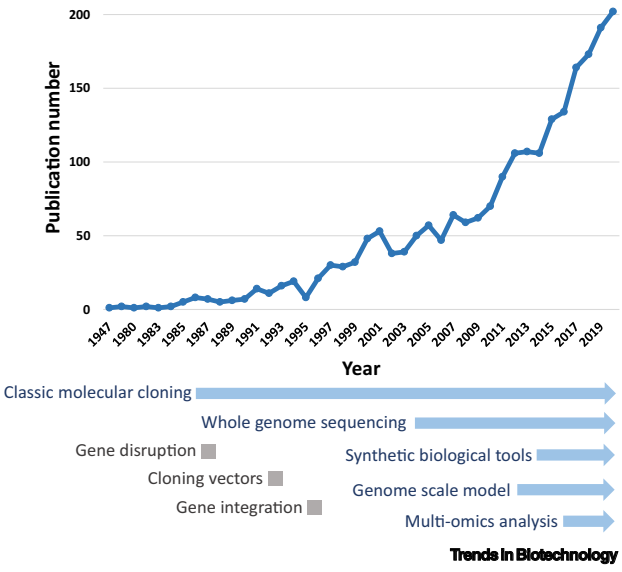

Figure 1. Timeline of *Yarrowia lipolytica* research. The number of research articles published per year. Blue arrows under the graph represent the period of research such as classic molecular cloning since 1987 [including remarkable steps such as gene disruption (1987), cloning vectors (1993), and gene integration (1996)], whole-genome sequencing since 2014, synthetic biological tools since 2014, genome-scale modeling since 2012, and multiomics approach since 2016. The data were obtained using the search term '*Yarrowia lipolytica*' in PubMed.

Glossary

Adaptive laboratory evolution: a process directing microorganisms toward the desired behavior under controlled conditions and selective pressure.
Bioprocess: the process using complete living cells or their components (e.g., enzymes) to obtain desired products.
Bioremediation: the process of absorption or destruction of toxic chemicals and harmful pollutants by using microorganisms or plants.
Biphasic cultivation: a type of cultivation using *in situ* liquid–liquid extraction that separates producing cells and final products (e.g., a liquid organic phase in the aqueous cultivation broth for lipid and terpene production).
Downstream process: recovery of bioproducts from a bioreactor and their purification and modification to a form suitable for final application.
Fed-batch: a type of bioprocess where one or more nutrients are constantly or intermittently supplied during cultivation, which generally results in higher production by achieving high cell growth.
Genome-scale metabolic models: a mathematical model that describes gene–protein–reaction associations for entire metabolic genes and enables the prediction of metabolic fluxes.
Homologous recombination (HR): genetic recombination between two similar or identical sequences that results in targeted gene disruption and integration on the genome – often used in metabolic engineering.
Host: selected microorganism subject to be engineered and cultivated for production in biotechnology.
Nonhomologous end-joining (NHEJ): genetic recombination that does not require a homologous sequence and that results in random integration into the genome.
Pilot scale: precommercial production step for evaluating essential points (feasibility, time, cost, scaling factors, further develop point, etc.) to properly design and implement a full-scale commercial process.
Polyunsaturated fatty acids (PUFAs): fatty acids that contain more than one double bond in their backbone that are beneficial for human nutrition; omega-3 from fish and omega-6 from vegetable oils are well-known examples of PUFAs.

Safety is one of the critical issues for the implementation of industrial applications, especially for products intended for human consumption. *Y. lipolytica* is considered as a 'safe-to-use organism', with a granted GRAS (generally regarded as safe) status for the production of citric acid, erythritol, and eicosapentaenoic acid by the US FDA [15]. In addition, *Y. lipolytica* was attributed the Qualified Presumption of Safety status from European Food Safety Authority and International Dairy Federation, which facilitate its application in food and feed industries [10,11,13,14]. Several studies done on animals have proved the efficacy and safety of both *Yarrowia* biomass and *Yarrowia*-derived products, either purified or contained within the biomass [15–17].

Renewable feedstock: a natural resource derived from living organisms, usually plants, that maintains a neutral carbon supply that is different from depleting fossil fuels.
Transcriptomic, proteomic, and fluxomic: a study of the complete set of RNA transcripts, proteins, and metabolic fluxes under specific circumstances or in a specific strain that will provide thorough understanding of metabolism and potential target genes or reactions for engineering.

Y. lipolytica and its suitability for industry

Desirable industrial traits

The economic feasibility of a microbial bioprocess is often related to three production metrics: titer, rate (also known as productivity), and yield. To achieve greater performance from a microorganism, reduced byproduct formation and high cell density growth (titer), varied and efficient feedstock conversion (yield), and fast growth and production (rate) are necessary [4]. The strain should be safe, genetically stable, and controllable to maintain performance in the production phase and ensure reproducibility. The platform strain needs to tolerate the presence of toxic products, metabolic intermediates, and substrate components [18]. This last point is especially relevant when using **renewable feedstock** as substrates, where the tolerance or capability to eliminate the fermentation inhibitors coming from pretreatment processes is required [4]. More generally, industrial settings are often harsh for cells, and it is important that they show robustness and stability in the fermentation process and in response to environmental fluctuations such as

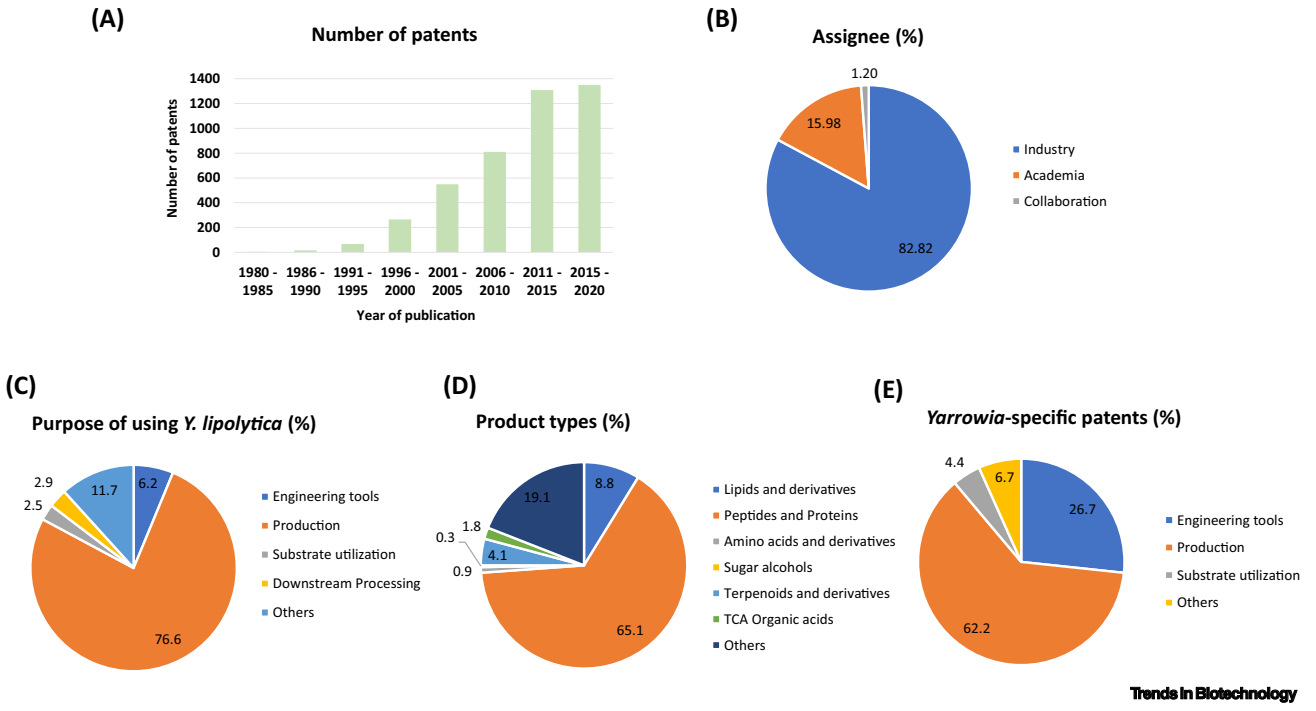

Figure 2. Patent landscape of *Yarrowia lipolytica* research. International patents (4536) have been manually analyzed regarding publication year, assignees, products, and purposes. (A) The number of patents related to *Y. lipolytica* published in 5-year intervals. (B) The types of assignees among academia, industry, or the collaboration of both. (C) The main purpose of patents using *Y. lipolytica* as a host: engineering tools, production, substrate use, downstream processing, and others. (D) The type of products made by *Y. lipolytica* among the production-related patents: lipids, proteins, amino acids, sugar alcohols, terpenoids, organic acids, and others. (E) The main purpose of patent using *Y. lipolytica* in all the patents (44) including '*Yarrowia lipolytica*' in the title: engineering tools, production, substrate use, and others.

Box 1. *Y. lipolytica* commercial products in industry

(i) **Polyunsaturated fatty acids (PUFAs)**, including eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3), also known as long-chain omega-3 fatty acids, have a global market valued at US$2.49 billion in 2019 [70]. E.I. DuPont de Nemours and Company developed a metabolic engineering strategy in *Y. lipolytica*, which expressed heterologous Δ6 desaturase, C18/20 elongase, Δ5 desaturase, and Δ17 desaturase, enabled production of up to 40% EPA [71–73]. Production was further enhanced by eliminating the competitive pathways and introducing several copies of the crucial enzymes, reaching to approximately 25% of dry cell weight and 50% of fatty acid methyl ester [70,74]. This strategy enabled DuPont to commercialize EPA produced from *Y. lipolytica*, New Harvest EPA oil, and Verlasso salmon.

(ii) Skotan SA started the production of single-cell protein (edible protein for human and animals [75]) from waste glycerol and registered the feed product in the EU [15]. Microbial enzymes such as lipases already have their own market valued at US$425 million in 2018 [76]. Commercial recombinant enzymes from *Y. lipolytica* are now available, such as the phospholipase enzyme Lipomod 833L2 by Biocatalysts Ltd., the lipase obtained by *LIP2* gene overexpression by Mayoly, or the human acid α-glucosidase OXY2810 by Oxyrane [44,77].

(iii) Citric acid is widely used by the food industry as additives, preservatives, anticoagulants, antimicrobial agents, fine chemicals, and so forth [78]. The global market volume of citric acid is greater than 2 million tons, and its value is estimated to reach US$6.28 billion by 2030 [79]. Due to easy cultivation, high conversion rate, and tolerance to high product concentrations, *Y. lipolytica* has been proposed as an alternative citric acid producer to *Aspergillus niger* [78,80]. Several companies, such as DSM, Akad Wissenschaften DDR, and OrganoBalance GmbH, own approximately 40 patents on citric acid production by *Y. lipolytica* [78].

(iv) Carotenoids have a number of applications in the food processing, animal feed, pharmaceutical, and cosmetics industries. There is already an enormous global market, which is estimated to be US$1.57 billion in 2022 and reach a valuation of US$2.09 billion by 2027 [81]. The production of carotenoids from *Y. lipolytica* has been described by numerous patents filed by DSM, E.I. DuPont de Nemours and Company, Amyris, and Microbia Inc., among others [82–86]. Microbia (now part of DSM) brought several carotenoid production strains to pilot scales, and a GRAS self-affirmation has been demonstrated for the β-carotene produced with *Y. lipolytica* [15].

changes in the dissolved oxygen concentration, pH, osmolarity, nutrient supply, temperature, etc. [19]. Another desirable feature is tolerance to low pH, which prevents bacterial contamination and reduces the need for neutralizing pH. Many of these properties can be improved through

Box 2. Survey to industry

Although the opinion of academic researchers often can be found in publications, those from industry are often more difficult to obtain. We therefore conducted a survey of companies working with *Y. lipolytica* to find out what they think about this yeast. The survey consisted of several statements or 'ranked questions' and a few 'open questions' where the answer could be typed. The survey was answered by ten companies producing different products at different scales (from lab to pilot and industrial scale).

The first 'ranked question' related to the most suitable target products for *Y. lipolytica* (Figure IA). When considering 'agree' and 'mostly agree' answers, lipids, terpenoids, and proteins were considered the best targets with an agreement of 100%, 80%, and 73%, respectively. Interestingly, the responses to the related 'open question' 'Which products from *Y. lipolytica* are most promising in the near future?' were very diverse, and lipids, terpenoids, amino acids, proteins, and sugar alcohols were mentioned, which shows the high potential of *Y. lipolytica* for a broad range of applications as well as the different interests among companies.

A second 'ranked question' was set to assess industrial features of *Y. lipolytica* (Figure IB). Overall, there is a high agreement ('agree' and 'mostly agree') in the fact that *Y. lipolytica* is good for high cell density cultivation (100%), has a good robustness (100%), engineering tools (70%), and wide substrate utilization (80%), whereas there were less positive answers in regard to downstream processing, byproduct formation, and scaling up, where 30%, 60%, and 40% of the opinions, respectively, were 'neutral' or 'mostly disagree'.

The third 'ranked question' investigated the need for further development of fundamental studies, synthetic biology tools, and fermentation knowledge (Figure IC). Ninety percent of the answers agree with the fact that *Y. lipolytica* still needs more fundamental research and synthetic biology tools. Eighty-two percent of the answers recognized the need of deepening our understanding of *Y. lipolytica* behavior in bioreactors.

These industries use different *Y. lipolytica* strains, being 40% academic strains and 50% wild-type (nonengineered) strains. In addition, an anonymous response stated that '*Y. lipolytica* has a strong body of knowledge, including regulatory approvals for prior products (e.g., is on the short list of microbial chassis that do not need a full safety assessment before manufacture/sale in the EU), which accelerates R & D and manufacturing timelines'.

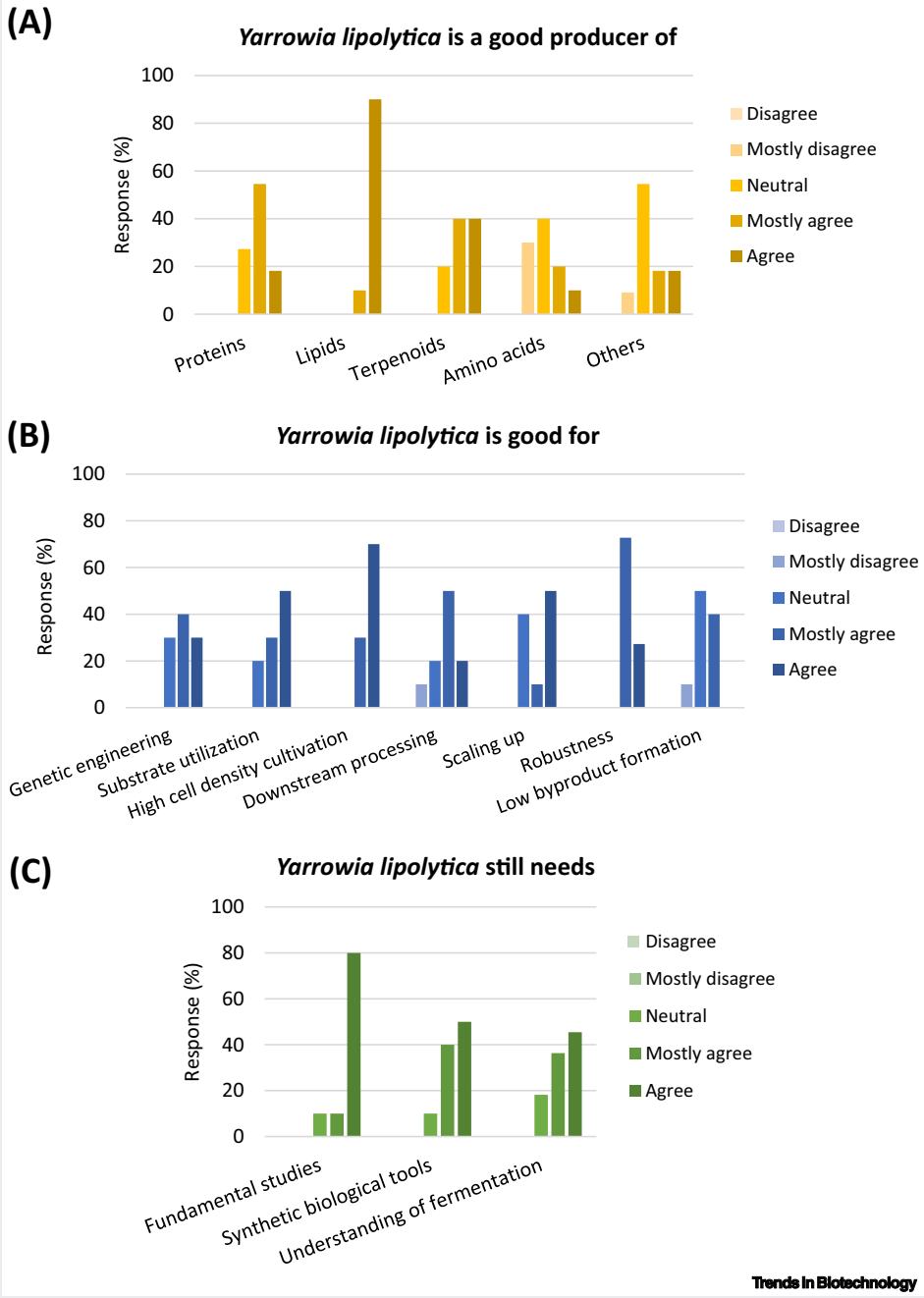

Figure I. Survey of ten companies using *Yarrowia lipolytica*. (A) Agreement or disagreement with the high capacity of *Y. lipolytica* to produce proteins, lipids, terpenoids, amino acids, and other products. (B) Agreement or disagreement with the presence of desired industrial features in *Y. lipolytica*. (C) Agreement or disagreement with the research fields that need further study in *Y. lipolytica*.

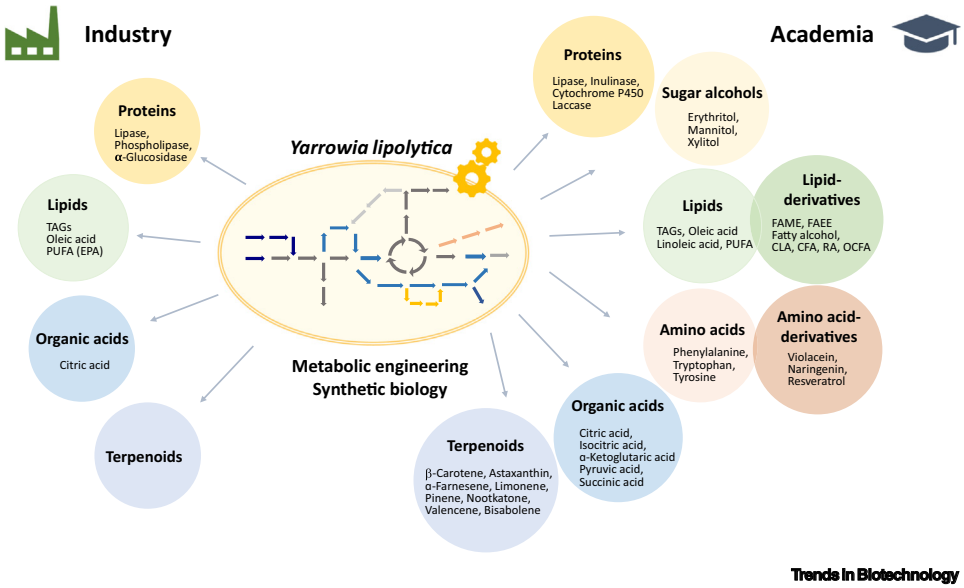

Figure 3. The products from *Yarrowia lipolytica* in academia and industry. Products from *Y. lipolytica* are categorized and illustrated by the type of products such as proteins, sugar alcohols, lipids, amino acids, organic acids, and terpenoids based on research articles and patents. Abbreviations: CFA, cyclopropane fatty acid; CLA, conjugated linoleic acid; EPA, eicosapentaenoic acid; FAEE, fatty acid ethyl ester; FAME, fatty acid methyl ester; OCFA, odd-chain fatty acid; PUFA, polyunsaturated fatty acid; RA, ricinoleic acid; TAGs, triacylglycerols.

genetic and metabolic engineering, and therefore, engineering amenability and the availability of synthetic biology tools are convenient. *Y. lipolytica* meets many of these requirements, which makes it a promising industrial host (Figure 4, Key figure).

Synthetic biological tools

Synthetic biology and metabolic engineering enable the design of microbial strains with desired properties. Therefore, the availability of various tools and protocols can accelerate research and development [20]. In *Y. lipolytica*, there are several homologous and heterologous DNA elements for the construction of gene expression cassettes (promoters, terminators, selective markers, localization tags, etc.), a few synthetic biological techniques that enable multigene assembly and expression (BioBricks, Gibson, Golden Gate, etc.), and genome-editing tools [**homologous recombination (HR)**, TALENs, CRISPR, etc.]. These tools enable rapid and combinatorial genetic engineering and high-throughput screening (for comprehensive reviews, see [20,21]). Several transformation methods of *Y. lipolytica* have been developed, including both the simple chemical (LiAc/PEG) and the efficient electroporation transformation (with high efficiencies of 6×10^5 and 2.8×10^4 transformants per μg DNA, respectively) [22]. Effective transformation methods allow high-throughput engineering approaches, such as library-based screening, to optimize pathway engineering. The level of development of tools and available genetic parts (e.g., selective markers, promoters, terminators, etc.) that can be used for genetic engineering for *Y. lipolytica* is higher than for other nonconventional microorganisms. However, when compared with other highly studied model organisms such as *Saccharomyces cerevisiae*, both the efficiency and tools available for *Y. lipolytica* are far from ideal (further discussed in 'Challenges to overcome' later).

Substrate use

Y. lipolytica can consume a wide range of substrates. Because this yeast has often been isolated from lipid-containing habitats, it has the capability of using hydrophobic substrates such as

Key figure

Advantages and weaknesses of *Yarrowia lipolytica* as an industrial host

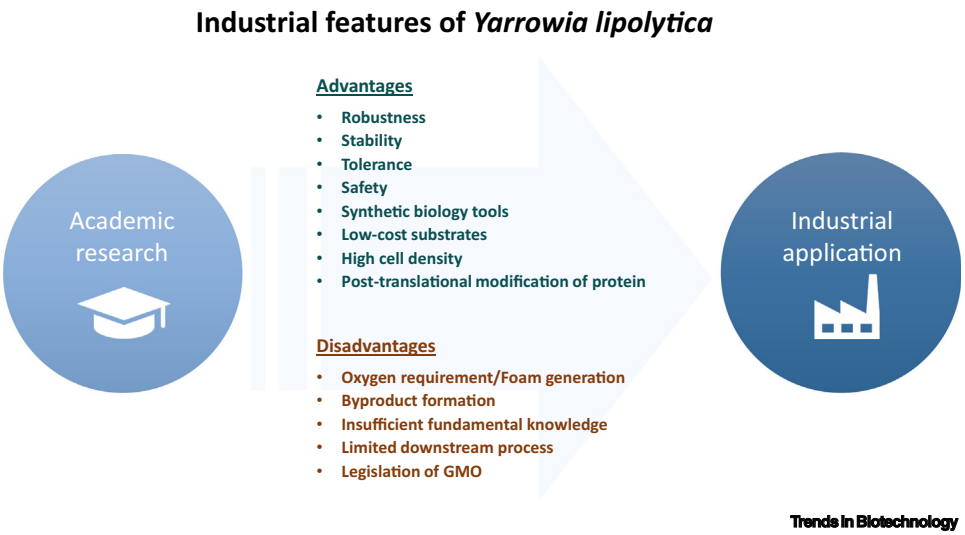

Figure 4. *Y. lipolytica* has many features that are desired at an industrial scale. More research is required to overcome disadvantages and further improve the industrial use of *Y. lipolytica*. Abbreviation: GMO, genetically modified organism.

alkanes, alkenes, fatty acids, triacylglycerides, etc. In addition, it consumes hydrophilic substrates, such as glucose, fructose, and glycerol; and organic acids, including acetate, lactate, citrate, and succinate, among others [23]. The transport and utilization pathways of these hydrophobic substrates have been studied intensively [24]. *Y. lipolytica* can efficiently use some of the preferred low-cost substrates, such as glycerol, oils, or food wastes [12,25]. In recent years, there have been many efforts to expand the substrate range of this yeast through metabolic engineering [23]. Engineering strategies have targeted extracellular degradation, transport, native or heterologous metabolic pathways, and tolerance. As a result, lignocellulose hydrolysates, xylose, cellulose, starch, molasses, and waste oils have now been included in the substrate spectrum of *Y. lipolytica*.

High cell density growth

Y. lipolytica can be cultivated to high cell density, achieving over 100 g/l from glucose (148.0 g/l and 115.8 g/l [26,27]), 120.7 g/l from the mixture of xylose and glycerol [28], 55.4 g/l from inulin [29], and 60 g/l from crude glycerol [30]. This feature is advantageous during scaling because it is often linked to improved titers, reduced culture volume, reduced wastewater, enhanced **downstream processing**, and lower production costs [31].

Tolerance to inhibitors

Raw substrates, including agricultural, food, and chemical wastes, often possess inhibitory compounds such as organic acids, phenolics, and metals that may hamper cellular metabolism [23]. *Y. lipolytica* has good tolerance to most inhibitors. It tolerates high concentrations of salt (25% of salinity, 12% of NaCl [32,33]), organic acids (3% of acetic acid [34]), pH level (2.5–11.5 [35,36]), and methanol (4% [37], a frequent contaminant in raw glycerol). In addition, *Y. lipolytica* has been

used to help detoxify alkanes, aromatics, nitroaromatics, halogenated organophosphate, and metals [38]. Through native extracellular laccases, lignin-derived phenolic compounds can be oxidized, making *Y. lipolytica* more resistant to them [38]. The broad tolerance of *Y. lipolytica* is a great feature for industrial bioprocesses, facilitating the conversion of renewable substrates into value-added compounds. In addition, this feature also makes *Y. lipolytica* a good host for environment-related applications such as bioremediation, and thus, it has been used as a bioabsorbent for treating wastewater and solid wastes [38].

Robustness to fermentation conditions

Y. lipolytica shows robustness to a wide range of fermentation conditions. It has a relatively fast growth (doubling time 1.5 h [39,40]) and tolerance to a variety of aeration rates (0.076–1.0 vvm), pH (pH 2.5–11.5), temperatures (15–38°C [41,42]), and osmotic levels (up to 12% of NaCl) [35,43]. *Y. lipolytica* adapts to a wide range of pH conditions by altering metabolite patterns without impairing growth or the substrate uptake rate [35]. The broad pH range facilitates the production of a variety of compounds, especially organic acids. Depending on the target product, medium composition, pH, temperature, and other operating factors have been optimized to maximize titers, yields, and productivities at bioreactor scale in *Y. lipolytica* [25].

Protein secretion

In protein production, additional features are desired such as specific post-translational modifications (e.g., glycosylation, N-glycan-carrying homogeneous $Man_3GlcNAc_2$ [44]) and high secretion, which facilitates downstream processes. The high secretion efficiency, good product yield (90,500 U/ml lipase secretion [45]), performance reproducibility, and low overglycosylation of *Y. lipolytica* make it an attractive host compared with other species [25].

Challenges to overcome

Optimization of fermentation

The engineered strains in academic laboratories often fail to show similar performance at industrial scale and have encouraged industry to develop their own strains for better performance at large scale. The difference in production performance from laboratory to **pilot** and commercial scale has generally been thought to be one of the main reasons for the so far limited commercialization success of microbial bioprocesses. Thus, process engineering research, including the optimization of fermentation conditions, is required to facilitate scaling up in *Y. lipolytica*. At large-scale fermentation, there are dead zones with poor mixing, heat transfer, and gas–liquid mass transfer, which cause gradients in parameters such as dissolved oxygen, temperature, pH, medium composition, etc. [46]. There are few studies on the optimization of bioprocesses in *Y. lipolytica* at pilot scale. Erythritol production was assessed in a pilot plant (working volume of 0.5 m^3), where the production reached 180.3 kg/l with a productivity of 1.25 kg/m^3·h and a yield of 0.533 kg/kg, which was comparable with that at laboratory scale (working volume of 0.002 m^3) with a production of 165.0 kg/m^3, a productivity of 1.13 kg/m^3·h, and a yield of 0.525 kg/kg [47]. This is the first case study on erythritol production at pilot scale; more development of a production model will accelerate commercialization.

High oxygen requirements

Because *Y. lipolytica* is a strict aerobic microorganism, cultivation needs efficient aeration systems [25]. Finding the right level of oxygen is critical, because large amounts can cause biomass overproduction instead of product formation, and limited amounts of oxygen have shown significant reduction in the target production (e.g., citric acid, γ-decalactone, and lipase) [25,48]. Also, a high oxygen transfer rate is accompanied by excessive formation of foam, which requires

addition of large amounts of antifoam agents, which increase production cost and decrease productivity [48–50]. To maintain the reasonable level of antifoam consumption, several attempts, such as the reduction of oxygen transfer rate or low agitation, were tried. But still they require the optimization between the low foam formation and the high oxygen supply to keep cell viability and good productivity [48,51]. The impact of antifoam itself in production and cell physiology has not been yet characterized, and alternative means to reduce foam formation such as mechanical defoaming need to be studied [48,50].

Limited temperature tolerance

Being able to grow and produce compounds of interest at higher temperatures has the potential to reduce the costs of the process, because it may avoid the use of chilling/cooling systems [52]. Although *Y. lipolytica*, as mentioned earlier, can grow in a relatively wide range of temperatures, it is still far from the capacities of other yeasts such as *Kluyveromyces marxianus*, *Ogataea polymorpha*, and *Blastobotrys adeninivorans*, which can grow at temperatures as high as 45°C [53,54]. Metabolic engineering and **adaptive laboratory evolution** can be used to increase thermotolerance, as it has been done for *S. cerevisiae* [55].

Insufficient fundamental knowledge

Although the genomes of several strains have been published and annotated, functional and biochemical studies are still limited. Out of the 6448 coding genes identified in the sequenced strains, only 44.5% are considered similar or highly similar to known genes and can therefore be confidently annotated. Also, there is much to gain from multiomics approaches (**transcriptomic**, **proteomic**, and **fluxomic**), which are still scarce. It is therefore essential to expand our knowledge about how genetics, metabolism, and cell biology work in this host.

Interestingly, the tolerance of *Y. lipolytica* to several factors, such as pH, temperature, and osmotic pressure, has been, on occasion, linked with improved production in the presence of stress factors, such as organic acids under low oxygenation, enzymes under osmotic stress, or enzymes under temperature stress [56]. However, the mechanisms behind such effects, as well as tolerance and toxicity, are unknown, and further research in this area will help improve production. The combination of systems biology data with **genome-scale metabolic models** may help us deepen our understanding of relevant metabolic control systems. Adaptive evolution and the screening of high-tolerance strains followed by sequencing may also help [46]. This approach has huge potential for the use of complex substrates, which usually have multiple inhibitors such as organic acids and aromatic hydrocarbons originating from pretreatment process. In a recent study, strains tolerant to aromatic aldehyde inhibitors from lignocellulosic hydrolysate were selected by adaptive laboratory evolution [57]. Transcriptome comparisons between the evolved strains and the initial one identified two enzymes (aldehyde ketone reductase and aldehyde dehydrogenase) involved in the enhanced tolerance, which were validated by reverse metabolic engineering.

Byproduct formation

The formation of byproducts is undesired in industrial production because it drains carbon away from the synthesis of the product of interest. *Y. lipolytica* can generate unwanted overflow metabolites under certain conditions, with the most frequent ones being organic acids such as citric acid or polyols such as erythritol and mannitol [58]. Byproducts can be reduced either by metabolic engineering (e.g., removing genes from competitive pathways) or by tuning the culture conditions. For example, nitrogen limitation is a critical condition for both lipid and citric acid production, which means lipids and citric acid can be a byproduct for each other [59]. Thus, strategies to minimize these byproducts are desired [60]. A study screened several *Y. lipolytica* strains

for strains with both high lipid production and minimal citric acid secretion [27]. Using a genome-scale model of *Y. lipolytica*, a **fed-batch** strategy to avoid citrate excretion during the lipid production phase was designed and validated experimentally, resulting in increased lipid yield (from 0.050 g/g glucose to 0.203 g/g glucose) and complete elimination of citrate production [59]. Disrupting the citrate exporter (YALI0D20196g) also resulted in lower citrate production in *Y. lipolytica* [61]. For erythritol production, the gene in anabolic pathways for mannitol and D-arabitol (YALI0D18964g) was disrupted, and the engineered strain showed increased production of erythritol by 8% without byproduct synthesis [62]. Unfortunately, most methods to control undesired byproduct are strain and condition specific and therefore must be developed case by case.

Downstream process optimization

The downstream process is another critical step that significantly contributes to production costs, preventing industrial feasibility. The bioproducts require subsequent processes (isolation, recovery, purification, polishing), which depend on the product itself and the final application [46]. Secreting products to the extracellular media often facilitates their recovery, and therefore, additional research about specific transport mechanisms must be pursued. A metabolic engineering approach that facilitates the secretion and therefore recovery of lipids from *Y. lipolytica* was successfully achieved [63]. The study showed not only the ability to decrease the downstream cost of the process but also the ability to produce a higher amount of lipids than the total cell mass, which would be the equivalent of 120% of the cell dry weight as fatty acids, uncoupling product and biomass formation. **Biphasic cultivation** techniques have been used successfully to recover hydrophobic products, such as lipids and terpenes (limonene, pinene), as well as to overcome toxicity [64,65].

Limited studies on cost-effectiveness

In order to evaluate the cost-competitiveness of production processes, total operating cost must be considered. A model-based analysis of an industrial installation to produce erythritol from *Y. lipolytica* was assessed based on a pilot scale and suggested a production rate of 1075 metric tons/year to achieve economic feasibility [47]. Economic viability must also be considered on a case-by-case basis.

Legislation on genetically modified organisms

More than 100 valuable products have been synthesized so far in *Y. lipolytica* under laboratory conditions. Interestingly, almost all of them are the consequence of genetic and metabolic engineering and are therefore made by genetically modified organisms (GMOs). Legislation around the use of GMOs for various applications will therefore be relevant, and additional studies supporting the long-term safety will be beneficial.

Efficient engineering tools

The metabolic engineering approaches achieved so far have been possible because of the multiple synthetic biology tools and strain engineering protocols described in previous sections. However, in our opinion, the development of molecular and synthetic biology methods is key to optimizing the biotechnological potential of this organism.

Despite the many genetic tools already developed in *Y. lipolytica*, more efficient and diverse techniques are necessary. *Y. lipolytica* prefers **nonhomologous end-joining (NHEJ)** over HR for DNA repair. This makes the overexpression of genes fairly straightforward, which can be randomly integrated in the genome, but targeted deletion and/or integration is highly context dependent and sometimes complicated. The frequency of HR is 0–36% when the target integration site has a size of 0.5–1.0 kb, which is much lower than in *S. cerevisiae* (close to 100%) [66,67]. Thus, efforts for strengthening HR repair by disrupting the enzymes involved in NHEJ [68], introducing a heterologous component of HR machinery [67], or arresting cell cycle in S-phase with high HR

activity [69] have been carried out. However, engineering efficiency still needs to be improved, regardless of wild-type strains and gene lengths. In addition, different research groups use different wild-type strains having different genotypes and phenotypes and their own customized engineering tools, which limits transferability and reproducibility. Therefore, the *Yarrowia* community still needs more consensus and standardized tools.

Concluding remarks

In addition to the use of *Y. lipolytica* in food (e.g., fermented meats, cheeses) and the animal feed industry (e.g., as a single-cell protein source), in the past few years, metabolic engineering has expanded the range of applications of this yeast to the manufacture of more than 100 products. In parallel with the emergence worldwide of new academic labs that work with this organism to create tools that facilitate its manipulation, well-established and new companies have begun to choose *Y. lipolytica* as a production host and unique selling point.

Despite intensive recent research into engineering *Y. lipolytica*, there remains a great need to improve the features of this yeast in order to turn it into an ideal industrial chassis (Figure 4; see Outstanding questions). System-based approaches such as genome-scale modeling and multiomics will allow us to understand its metabolism more completely and apply it to the construction of next-generation strains. The automation of processes such as strain construction, adaptive evolution, screening, and high-throughput analytics will greatly help to accelerate development of engineering approaches, evaluation of strain performance, and optimization of production conditions at the laboratory, pilot, and commercial scales.

In conclusion, *Y. lipolytica* has become an organism of choice for microbial biotechnology research and applications in both academia and industry. This is mostly a consequence of its unique metabolic, genetic, and physiological features. However, there are various characteristics of this yeast that are still far from ideal and require more fundamental research and tool development. We are confident that over the next years, many of the limitations of *Y. lipolytica* will be overcome, and it will play a major role in industrial bioprocesses, contributing to the creation of a more sustainable world.

Outstanding questions

Which strain-related factors contribute the most to the economy of microbial processes?

Can we engineer the perfect chassis strain for any industrial scale process, or is it a product-specific problem?

What is the trade-off between high cell density fermentation and high production?

How many *Yarrowia*-related industrial products and processes are restricted by patents?

How will the quick development of synthetic biology tools in *Yarrowia* impact the creation of novel industrial processes?

How can the scientific community help the creation of new *Yarrowia*-based companies?

Author contributions

R.L-A. conceived the manuscript. Y-K.P. and R.L-A. drafted and edited the manuscript. Y-K.P. prepared figures. All authors read and approved the final manuscript.

Acknowledgments

R.L-A. received funding from BBSRC (BB/R01602X/1, 19-ERACoBioTech-33 SyCoLim BB/T011408/1, and BB/T013176/1), British Council 527429894, Newton Advanced Fellowship (NAF\R1\201187), Yeast4Bio Cost Action 18229, European Research Council (ERC) (DEUSBIO - 949080), and the Bio-based Industries Joint (PERFECOAT-101022370) under the EU's Horizon 2020 research and innovation program. Y-K.P. received funding from the Bio-based Industries Joint (PERFECOAT-101022370) under the EU's Horizon 2020 research and innovation program. We appreciate Biomedican, Acies Bio, Nourish Ingredients, Skotan SA, and the anonymous participants of the survey for their opinions and responses. We appreciate Lucas Coppens for the genome information of *Y. lipolytica*.

Declaration of interests

The authors declare no competing interests.

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