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OLEAGINOUS YEAST AS POTENTIAL LIPID PRODUCING ORGANISM: A REVIEW

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ABSTRACT

Recently, there has been an increasing demand of oil in fields like food industry, pharmaceutical industries etc. The oil derived from plants and animal requires geographical and climatic conditions, land use, ethical issues, environmental problems and takes longer generation cycle. Hence researchers all over the world have been working on an alternative approach to produce oil, to replace unsustainable fossil fuels with biofuels like biodiesel and alternatives to edible oils and additives. It has been observed that the lipid profile produced by microbes, especially that by oleaginous yeast is similar in type and composition to the oils and fats produced by most plants and animals therefore have many advantages like competitiveness with food, have short growth cycle and their cultivation is independent of climatic factors and can also be genetically modified. Oils extracted from microorganisms is also known as single cell oils (SCO) and yeast, mainly oleaginous yeast have been found with the ability to store lipids over 20% and up to 70% of their cell dry weight and can accumulate lipids up to 40% of their biomass. Hence yeast can be exploited to a great extend using cheap substrates as starting material for the production of oil by fermentation. In this review article, our main focus is on evaluation of oleaginous yeast and understanding their lipid biochemistry, production and also inspecting their potential in industrial applications.
Keywords: Single cell oil, oleaginous yeast, microbial oil, lipid.

Introduction

Yeasts are unicellular, eukaryotic, microscopic organism that has gained significant importance in microbiological world due to their usefulness. In this review article, we will be focusing on a particular type of yeast called oleaginous yeast. More than 600 species of yeast have been identified till date out of which less than 30 are known to be oleaginous yeast (Beopoulos *et al*., 2012). Oleaginous yeast is one of the most desirable micro-organisms due to their ability to accumulate high lipid content i.e. over 20% (Ratledge *et al*., 2002). Most of the lipid accumulated are in the form of triacyl-glycerols (TAGs). Even though the lipid content and profile may differ within species, it was observed that the fatty acid produced by oleaginous yeast are similar to those produced by plants which mainly consisted of: palmitoleic (C16:1), linoleic acid (C18:3) myristic (C14:0), oleic (C18:1), palmitic (C16:0), stearic (C18:0), linoleic (C18:2) (Beopoulos *et al*., 2012). The most thoroughly studied oleaginous yeast is *Yarrowia lipolytica* with promising results as outcome and many other oleaginous yeast have been identified, characterized and studied. The table below presents the lipid content of few oleaginous yeast strains: (Ratlege *et al*., 1990).

SL No.	Species of oleaginous yeast	Lipid content(%)
1	*Rhodotorula glutinis*	72
2	*Trichosporon pullulans*	65
3	*Cryptococcus albidus*	65
4	*Waltomyces lipofer*	64
5	*Lipomyces starkeyi*	63
6	*Cryptococcus curvatus*	58
7	*Rhizopus arrhizus*	57
8	*Candida sp 107*	42
9	*Rhodotorula graminis*	36
10	*Yarrowia lipolytica*	36

The lipid acquired from these yeasts can be used to replace conventional oils like fossil fuels, vegetable oils, animals oil by large scale production, industrially. Also strains can be modified genetically to increase production or to enhance certain characters. For large scale lipid accumulation or oil production, fermentation using cheap substrates is a convenient method. Due to its high productions cost, lipids derived from oleaginous yeast are not much on demand. Hence utilizing cheap substrate can increase its demand commercially by decreasing product cost. Various substrates can be used such as pure sugars, biodiesel derived glycerol, molasses and whey, lignocellulosic materials, starch, lignocellulose hydrolysates. Therefore oleaginous yeast are preferred due high growth rates, can tolerate high sugar concentrations, and use a wide range of substrates as carbon sources and are highly significant in future perspective of industrial products like biodiesel, food industries, cosmetic and chemical industries.

Oleaginous yeasts and biochemistry of lipid accumulation

Yeasts exhibit advantages for lipid production over other oleaginous microorganisms, due to their unicellular form, short duplication times, capability to grow on a large variety of raw materials, and their easy cultivation in large fermenters (Adrio, 2017). The amount of lipid accumulation depends on the individual microorganism, with lipid content ranging from 20% to 70% of the biomass (Ratledge, 2010). For lipid synthesis, precursors (acetyl-CoA, malonyl-CoA, and glycerol) and energy (ATP, NADPH) are required during the lipid accumulation stage (Beopoulos *et al*., 2009).

The oil production in oleaginous yeast are stimulated due to limiting nutrient conditions, usually by the exhaustion of nitrogen in the medium while other nutrients, including carbon remain in excess- leading to accumulation of citric acid in the mitochondria which is then secreted into the cytoplasm of the mitochondria where it is converted to form acetyl-CoA (the precursor for fatty acid synthesis) and oxalo-acetate where it is cleaved by the enzyme ATP-citratelyase(ACL enzyme ATP citrate lyase- Transferases) (Ratledge *et al*., 2008).

Citrate + CoA+ATP = AcetylCoA + oxaloacetate + ADP+Pi

Acetyl CoA + HCO_3 + ATP = malonyl CoA + ADP+ Pi

The natural way of lipid accumulation in yeast mostly occurs through de-novo pathway is induced due to insufficient or a limitation of a particular primary nutrient in the culture medium. Exhaustion of nitrogen reduces the intracellular adenosine monophosphate (AMP) concentration, resulting in isocitrate dehydrogenase (ICDH) inhibition, leading to mitochondrial citrate accumulation (Adrio, 2017). This feature is unique to the oleaginous yeasts. The acetyl-CoA formed is then led to de novo fatty acids synthesis in the fatty acid synthase (FAS) complex (Tehlivets *et al*. 2007). Malonyl-CoA is the elongation unit and acetyl-CoA are the initial biosynthetic unit and are providing two carbons at each step of the growing FA chain to an intermediate level (C:14–C:16), depending on the enzymatic arsenal of each organism. For each step of the carbon chain elongation reaction, FAS requires two molecules of NADPH (Anschau, 2017). FAS complex materials, palmitoyl-CoA and stearoyl-CoA, are shuttled to the endoplasmic reticulum where they are used to generate TGAs or undergo NADPH-dependent desaturation and two carbon elongation prior to synthesis of TAGs (Adrio, 2017). The synthesis of TAGs follows the Kennedy pathway to generate lysophosphatidic acid (LPA), diacylglycerol (DAG), phosphatidic acid (PA), and finally TAGs contained in droplets of lipids. Through the action of intracellular lipases (TGL), free fatty acids (FFA) can be released from TAGs and further activated to yield acyl-CoA acyl-CoA synthetases(ligase) (Beopoulos *et al.*, 2012).

Modes of cultivation for microbial lipid production

Many cultivation methods such as Batch fermentation, fed-batch fermentation and continuous fermentation has been used for microbial lipid production in laboratory scale (Subramaniam *et al*., 2010). Different feeding modes have a major influence on the efficiency of lipids (Zhao *et al*., 2011). The mode of cultivation is an important factor as the efficiency of lipid accumulation differs in different modes, giving different outcomes. Also the importance of using the most effective feedstock as a substrate is immense. Waste products and biomass such as lignocellulosic lysates and other industrial byproducts have been used as substrates in the production of lipid from yeast (Spalvins *et al*., 2019). This significantly effects the future scope of microbial lipid as it decreases product cost. Chemical engineering tools and techniques are very important and the specific details are necessary for Single Cell Oil production on an industrial scale (Christophe *et al*., 2012).

- **Batch Cultivation**

In general, batch fermentation is carried out in a closed system where all the components consisting nutrient, medium, cell, substrate are supplied in the beginning and the end product acquired at the end of fermentation. It is a constant process with no change or variable during the fermentation process. Usually all the parameters such as pH, aeration, temperatures can be controlled. Batch cultivation of oleaginous microorganisms for lipid accumulation is done with a increased amount of C:N ratio to induce the aggregation of extra carbon into lipids at limiting conditions of nitrogen concentration (Anschau, 2017). But it is important to note that, if the concentration is too high, the source of carbon may be inhibitory to cell growth in the culture. Other modes with controlled C/N ratio levels can provide higher yields for better substrate utilization despite the simplicity of batch cultivation. Therefore it indicated that, it was not possible to achieve a high cell density for lipid accumulation in batch culture (Christophe *et al*., 2012).

For instance, *C. curvatus* was grown in batch and fed-batch culture where the amount of iron and nitrogen in the culture was restricted, to produce a high stearic acid lipid content (C18:0) (Hassan *et al*., 1996). The result obtained by that experiment concluded that the highest biomass concentration achieved when grown in fed-batch culture was 70 g/L with 53% (w/w) lipid content, while the highest biomass concentration achieved when grown in batch culture was 17 g/L with 22 % wt lipid content (Hassan *et al*., 1996).

- **Fed-Batch Cultivation :**

In fed-batch fermentation, where one or more nutrient is supplied in the bioreactor during cultivation process, therefore the amount of substrate can be controlled which is more convenient. In increasing both the cell density and lipid content of oleaginous yeasts, Fed-batch culture has proved effective. Additional carbon sources are most frequently added when their levels in the broth are very low. According to Li *et al*. (2007), they researched glucose-based fed-batch cultivation and achieved 106 g / L of Cell Dry Weight and 0.5 g/lipid productivity with *R. Y4 toruloides*. The inhibitory effects of glucose on cell growth were prevented by the combination of low initial glucose concentration (2%) and fed-batch mode during cultivation, and glucose intake increased exponentially. Qu *et al*. (2013) also showed that the fed-batch process was efficient in reducing the inhibition of growth induced by high concentrations of the substrate.

In order to increase the production of lipids by an engineered strain of *Rhodococcus opacus*, Fei *et al.* (2015) carried out a two-stage fed-batch culture. A nitrogen-excess feeding solution was fed in the first stage to raise cell mass with a low accumulation of lipids. The supply of nitrogen was reduced in the second stage of the culture, preventing cell proliferation and triggering excess lipid production. For the subsequent conversion of volatile Fatty Acids to the SCO by *Y. lipolytica*, the two-stage fed-batch method has also been used to achieve high biomass. *Yarrowia lipolytica*, however, is a fairly complex microbe for industrial use (Fontanille *et al*., 2012).

- **Continuous Cultivation**

In continuous fermentation, there is a constant flow of nutrients throughout the reactor. Lipid accumulation can also be done through continuous cultivation under nitrogen-limiting conditions and with stable dilution rates in a continuous culture (Ageitos *et al*., 2011). Fresh nutrients are continuously supplied to a stirred culture in continuous cultivation and the cells and products are continuously removed. Continuous chemostat operations for SCO production has proved to be an efficient fermentation technique. In cultivation under steady-state conditions, high cell yields are obtained using the carbon source with the same efficiency at each residence growth period. Both sources of carbon and nitrogen are given and a steady C/N ratio can be maintained during the steady state. In the fermentation batch of *C. curvature* D for cellular nitrogen accumulation, non-lipid biomass, and lipid were sequential (Brown *et al*., 1989).

It was noted by (Subramaniam *et al*., 2010) that during batch fermentation and with the rate of dilution in continuous fermentation, the rate of lipid production (with respect to the amount of nitrogen present in the cell) increased significantly whereas at the same time as there was a decrease in the rate of accumulation of nonlipid biomass. *Y. lipolytica* can produce a high content of lipids when growing on raw glycerides (Subramaniam *et al*., 2010).

Industrial applications

Oleaginous yeasts have great potential for producing oils that are similar to many organic oil mainly vegetable oil and also different substitutes like cocoa butter etc. They can also be used for biodiesel production. Therefore it has various significances for industrial uses. The high potential is because of their ability to use low cost substrates like waste, rapid growth rate and lipid production, limited requirements for land and water and simple non-climate-affected cultivation methods.

- **Cocoa Butter**

Cocoa butter is widely used in food technology and primarily in the method of making chocolate, though it is also used in various cosmetology applications. Cocoa butter mainly consists of Triacylglycerol (TAG) of POP, POS and SOS (P: Palmitic acid, O: Oleicacid, S: Stearic acid) (Papanikolaou *et al*., 2011). Several researchers have used oleaginous microorganisms, primarily yeasts, which can be regarded as "ideal" candidates for the production of cocoa butter substitutes. These microorganisms store their lipids mainly in the form of Triacylglycerols. The biggest limitation to overcome, however, was how to increase the amount of carbon in the form of total saturated fatty acid C18:0 since yeast cells generally contain unsaturated fatty acids in their total lipids to more than 65 percent w/w (Papanikolaou *et al*., 2011). Cocoa butter contains 55-67% w/w saturated fatty acid. So far, several methods have been introduced to increase the TSFA content in these yeasts (Ykema *et al*., 1989).

- **Biodiesel**

The global need for energy production from various alternative and renewable resources has been raised by the increasing industrialization and reduction of petroleum stocks, and the most promising candidate for replacement is biodiesel, as it can provide an alternate to conventional oils. Also biodiesel is a renewable energy source, hence benefitting the environment by exemption from pollution.

The concept of using SCO for the production of methyl or ethyl ester fatty acids, also known as biodiesel, seems promising and provides many advantages over plant or animal lipid biodiesel. Because of large areas of production land required and the competition for production, fuel demands cannot be met by the use of plant oil or animal lipids.

Because of their high percentage of oleic acid, oleaginous yeasts have a desirable lipid profile for production of biodiesel and are a possible solution to all the sustainability concerns associated with first-generation biodiesel production (Vasconcelos *et al*., 2019). In addition, it has been observed in many experiments conducted on oleaginous yeasts that they can be cultivated in various carbon sources, for e.g. waste materials with rich carbon source like lignocellulosic waste. In the recent years, strains like *Rhodoturula glutinis, Lipomyces starkey* have been used as a potential source for biodiesel production by trans esterification method (Kot *et al*., 2016; Wang *et al*., 2016).

Future scope

Oleaginous yeasts, in most cases are classified as GRAS microorganisms, can be considered as a key and potential source for the production of this 2nd generation biodiesel due to their rapid growth rates and they have show a lot of potential to grow on a low cost substrates such as agricultural and other waste substances rich in carbon source. In addition, there has been increasing demand of cocoa butter in the market but due its high price is a severe drawback. For both of the previously listed yeast lipid applications, the use of low cost materials as substrates to reduce production cost should be contemplated.

We still have long way to go if we consider the current state of yeast oil production as it still needs a lot of research to improve the production cost, considering all the potential they have. Screening or even genetically engineering more effective oleaginous yeasts to produce high lipid content and productivity and also using a wide range of low cost carbon substrates and utilizing effective fermentation methods will help to produce cost effective microbial lipid. Thus it is credible to expect that microbial lipids could still, more than ever, be considered for the growth of a bio-based economy, bypassing the above-mentioned concerns about the conventional production of oil-based production.

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