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Microbial lipid production by oleaginous yeast *Cryptococcus* sp. in the batch cultures using corncob hydrolysate as carbon source

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ABSTRACT

To realize the feasibility of biodiesel production from high-lipid cell culture, microbial lipid production by the oleaginous yeasts was studied using glucose and sucrose as carbon source. Among the tested strains, *Cryptococcus* sp. SM5S05 accumulated the highest levels of intracellular lipids. The crude lipid contents of *Cryptococcus* sp. cultured in yeast malt agar reached 30% on a dry weight basis. The accumulation of lipids strongly depended on carbon/nitrogen ratio and nitrogen concentration. The highest content of lipids, measured at a carbon/nitrogen ratio of 60–90 and at a nitrogen concentration of 0.2%, was 60–57% lipids in the dry biomass. Batch cultures using corncob hydrolysate demonstrated that there was minimal inhibitory effect with a reducing sugar concentration of 60 g l^{-1} or higher. Batch cultures of *Cryptococcus* sp. SM5S05 in the corncob hydrolysate medium with 60 g l^{-1} glucose resulted in a dry biomass, lipid yields, and content of 12.6 g l^{-1}, 7.6 g l^{-1}, and 60.2%, respectively. The lipids contained mainly long-chain saturated and unsaturated fatty acids with 16 and 18 carbon atoms. The fatty acid profile of *Cryptococcus* oils was quite similar to that of conventional vegetable oil. The cost of lipid production could be further reduced with corncob hydrolysate being utilized as the raw material for the oleaginous yeast. The results showed that the microbial lipid from *Cryptococcus* sp. was a potential alternative resource for biodiesel production.

1. Introduction

Biodiesel derived from oil crops is a potential renewable and carbon-neutral alternative to petroleum fuels. However, biodiesel from oil crops, animal fat, and waste cooking oils cannot realistically satisfy even a small fraction of the existing demand for transport fuels. As a consequence, recent demand for biodiesel worldwide has turned triacylglycerol (TAG) into an ever-growing and substantial consumption resource [1].

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The quest for non-traditional TAG production processes, especially those that can be operated continuously and with no extensive arable land requirement, is crucial for a sustainable biodiesel industry. In this context, microbial lipids appear to be an ideal source of renewable biodiesel to meet the global demand for transport fuels. Recent advances have shown that some microbial species such as yeast, fungi and microalgae can be used as potential sources for biodiesel as they can synthesize and store large amounts of fatty acids in their biomass [2–4]. Like plants, microorganisms utilize organic carbon or sunlight to produce oils. Even the oil productivity of many microorganisms greatly exceeds that of the most productive crops. Therefore, microbial TAGs may be a prospective alternative feedstock for energy sources.

Microorganisms that accumulate lipids at more than 20% of their biomass are defined as oleaginous species [5]. Among these oleaginous microorganisms, some yeasts, such as *Candida curvata*, *Trichosporon cutaneum*, *Rhodosporidium toruloides*, and *Lipomyces starkeyi* seem to store the largest quantities of lipids and have the potential for use in biodiesel production [6–9]. Lipids serve as storage materials in some lipid-accumulating yeasts, which can accumulate intracellular lipids up to 70% of their biomass dry weight [10]. The majority of those lipids are TAG-contained, long-chain fatty acids that are comparable to conventional vegetable oils [11]. Further, some of those oleaginous species show the ability to metabolize pentoses, demonstrating the potential to produce TAGs from lignocellulosic biomass and other cheap materials [12,13]. Though the costs of microbial oil production are currently higher than those of vegetable oil, several options have been proposed to drastically improve the techno-economics of microbial oil production processes. In particular, the development of lignocellulose-based carbohydrates as feedstock may greatly lower the costs of biofuel production. Further optimization of the operating processes also contributes to a higher lipid production rate and cellular lipid content.

Organic carbon of different feedstocks can significantly increase the cell growth rate and microbial biomass and dramatically enhance the lipid content of the biomass. It has been studied that lipid accumulation in many oleaginous yeasts, using different organic substrates, such as industrial glycerol [12], whey permeate [14], rice straw hydrolysate [4], and sewage sludge [6]. During the past years, agricultural feedstocks contribute a large part of renewable resource for biodiesel production. Organic material, such as lignocellulosic biomass from agriculture, is an ideal source of energy because it is both renewable and available in large quantities around the world. Lignocellulosic materials are mainly composed of cellulose, hemicellulose, and lignin, which make up approximately 90% of the dry weight of most plant materials [15]. Cellulose and hemicellulose can be converted to fermentable sugars for microbial lipid production. Recent studies detailed conversion of hemicellulose hydrolysate into lipids by oleaginous yeast strains and their tolerance degrees to lignocellulose degradation compounds [16,17]. According to previous reports, the raw materials for biodiesel production account for almost 75% of the total biodiesel cost [18,19]. The scale-up of biodiesel production is also limited by insufficient and expensive raw materials. The advantage of yeast lipid production from cellulosic agriculture wastes is that it helps for cost reduction. In addition, yeast oil used as the source for biodiesel is that the feedstock is comparatively cheap and easy to obtain for further industrial- scale production. Further, because the fatty acid profile of microbial oils is similar to that of conventional vegetable oils, oleaginous microorganisms are a favorable feedstock for the biodiesel industry [20].

Certain microorganisms are capable of accumulating huge quantities of lipid when they are cultured on nitrogen-limited, sugar-based media. However, effects of carbon sources and carbon to nitrogen (C/N) ratios on the crude lipid content need to be studied. It has been reported that under nitrogen-limiting conditions and in the presence of an excess carbon source, microorganisms started to store lipids [21,22]. It is known that lipid production requires media with an excess of sugars or similar components (e.g. polysaccharides, glycerol, etc.) and other limited nutrients, usually nitrogen. Thus, the microbial oleaginous potential is majorly affected by the C/N ratio of the culture and by other factors such as aeration, inorganic salt presence, etc. [23]. Various microorganisms are capable of accumulating huge quantities of lipid when cultured on hydrophobic materials, but lipid production is restricted when cultivation is carried out in nitrogen-limited, sugar-based media [24,25].

In this study, the biomass with high crude lipid contents from the yeast cultures was obtained through the selection of potential producing strains that were isolated from soil and leaves in Taiwan. The aim of this study was to obtain a biomass with high crude lipid contents from the yeast cultures by applying various concentrations of the organic carbon source to the cultural medium. A cheaper raw material, corncob hydrolysate, was used in our study as a carbon and energy source, to reduce the cost of lipid production. We have developed an effective, yet simple batch fermentation system to obtain optimum operating conditions for lipid production, with glucose or corncob hydrolysate as the sole carbon source. These improvements would make the process of microbial lipid production more competitive. They also provide an economical opportunity to produce microbial lipid using agricultural waste materials as carbon sources.

2. Materials and methods

2.1. Microorganisms and cultivation

Five candidate yeast strains, including *Cryptococcus* sp. SM5S05, *Moesziomyces eriocauli* SJ3L01 and SJ4L09, and *Pseudozyma* spp. FN20L03 and SJ4L03, were collected from peka, leaves, and forest soil samples in Taiwan, and then isolated. Isolation of these strains was performed by the method of Liu et al. [21]. One-tenth of a milliliter of successive decimal dilutions was spread on acidified yeast malt agar (YMA) (1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 1.5% agar, pH 5.5). The plates were then incubated at 25 °C for 3 d. Representative colonies were picked and preserved on YMA slant at 4 °C or −70 °C.