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Docosahexaenoic acid production from the acidic hydrolysate of Jerusalem artichoke by an efficient sugar-utilizing *Aurantiochytrium* sp. YLH70

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ABSTRACT

Jerusalem artichoke was first investigated as a non-food and cheap feedback for the production of docosahexaenoic acid (DHA) through *Aurantiochytrium* sp. YLH70. Using the Response surface method, up to 143.8 g/L of reducing sugar was obtained in the acidic hydrolysate of Jerusalem artichoke at 80 °C and 6% (v/v) sulphuric acid after 40 min of hydrolysis. The Plackett-Burman experiment showed that natural components of the hydrolysate of Jerusalem artichoke without the addition of any micronutrient, which is essential in traditional media, were suitable for DHA production by *Aurantiochytrium* sp. YLH70. Moreover, compared to the fructose or glucose medium, the Jerusalem artichoke medium resulted in a higher biomass (32.71 g/L) and DHA content (46.9% of the total fatty acid), which could be associated with the rich nutrition and high osmotic pressure (1306 mOsm/kg H_2O) in the Jerusalem artichoke medium. During fed-batch fermentation under the optimal Jerusalem artichoke medium (695 mL/L hydrolysate of Jerusalem artichoke, 15 g/L yeast extract and 25 g/L sea salt), both a productivity of 3.35 g/L/d and a yield of 0.094 g/g reducing sugar for DHA production were achieved. These results demonstrate that Jerusalem artichoke, when sufficiently utilised by *Aurantiochytrium* sp. YLH70, could indeed be an efficient and cheap option for DHA production.

1. Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) belongs to a tuberous and energy plant that is rich in carbohydrates, of which 70–90% is inulin. As a stress resistant plant, Jerusalem artichoke can grow in poor, desert and frost lands, giving a yield of more than 90 tonnes per hectare by fresh weight. Moreover, as a non-food crop, Jerusalem artichoke does not compete with food crops for arable land and is superior to the other inulin-containing plants in the aspects of biomass, inulin content and stress-resisting properties (Baldini et al., 2004). Inulin is composed of linear fructans, in which fructose units are each linked by β(2,1) bonds, and glucose usually terminates the inulin chain by an α(1,2) bond. This structure is easily hydrolysed by acidic catalysis or microbial inulinases and the resulting fructose monomers are well utilized by many microorganisms (Li et al., 2013). Thus, due to the energy crisis and decreasing cost, Jerusalem artichoke is an excellent cheap and renewable potential raw material for the production of bioethanol, biodiesel, single-cell oil and biochemical products, such as citric acid, L-lactic acid, succinic acid, etc. (Ge et al., 2009; Gunnarsson et al., 2014; Szambelan et al., 2004b; Wang et al., 2013; Zhao et al., 2010). However, the utilization of the Jerusalem artichoke hydrolysate for docosahexaenoic acid (DHA) production has not been investigated. In this study, we investigate the availability of fermenting reducing sugar in the acidic hydrolysate of Jerusalem artichoke to produce DHA by a marine strain in our lab: *Aurantiochytrium* sp. YLH 70.

DHA is a ω-3 polyunsaturated fatty acid (PUFA) and has beneficial effects in preventing cardiovascular diseases, cancer, schizophrenia, and Alzheimer's disease (Riediger et al., 2009; Ruxton et al., 2004). In addition, DHA is an essential component of the cell membrane in human tissues, mainly in the brain and retina (SanGiovanni and Chew, 2005). Moreover, DHA plays a significant role in infant nervous system development and is an essential

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nutrient for cultured animals in the feed industry. Due to its structural and physiological functions, DHA is an important chemical that is widely used in the food, medicine and feed industries (Sijtsma and de Swaaf, 2004).

Aurantiochytrium sp., a heterotrophic thraustochytrid, is ubiquitous and abundant in marine ecology (Raghukumar, 2002). This microorganism can heterotrophically grow on various substrates and shows a remarkable growth rate with a high DHA content in its dry cell weight. Thus, *Aurantiochytrium* sp. have been developed as alternative commercial sources of DHA enriched oils, able to relieve the problems that are caused by traditional marine fish oil products, such as environmental contamination, undesirable taste, diminishing supplies, objections by vegetarians, etc. (Raghukumar, 2008). At present, the traditional carbon source for the fermentative production of DHA is glucose. However, in large-scale fermentation for DHA, it is essential to explore cheap material to lower the cost. Until now, many low-cost materials, such as hydrolyzed potato broth, sweet sorghum stalk juice, shochu distillery wastewater and cane molasses, have been utilized by *Aurantiochytrium* sp. strains for DHA production (Chi et al., 2007; Liang et al., 2010a; Ren et al., 2013; Yamasaki et al., 2006). In addition, many micronutrients, including vitamins and trace elements, are required for the growth of *Aurantiochytrium* sp. and are added to the medium to improve the biomass and DHA yield of *Aurantiochytrium* sp. (Nagano et al., 2013; Song et al., 2015). Therefore, inulin, which is rich in non-food crop plants, such as Jerusalem artichoke, should be considered for the cost-effective production of high-value commodities, such as DHA.

In previous studies, we isolated a marine microorganism strain, named YLH70, which was identified as *Aurantiochytrium* sp. Fructose was the optimal carbon source for the growth and DHA production of *Aurantiochytrium* sp. YLH70. After 5 d of fermentation at the flash level, 15 g/L dry cell weight, 7.77 g/L lipid and 2.5 g/L DHA were achieved using fructose as a carbon source, suggesting that *Aurantiochytrium* sp. YLH70 is a potential DHA producer (Data not published). Thus, Jerusalem artichoke, rich in the inulin, which could be hydrolysed into fructose or oligofructose, is a potential carbon source for *Aurantiochytrium* sp. YLH70 to produce DHA. In this study, we attempted to systematically evaluate whether *Aurantiochytrium* sp. YLH70 can grow and produce DHA using the acidic hydrolysate of Jerusalem artichoke as a carbon source.

2. Materials and methods

2.1. Materials

Jerusalem artichoke was collected from Xuzhou, Jiangsu Province, China at the end of December. The nonadecanoic acid methyl ester and the docosahexaenoic acid methyl ester standards were purchased from Sigma–Aldrich (St. Louis, USA). The BF_3-methanol was purchased from ANPEL Laboratory Technologies Inc. (Shanghai, China). The other chemicals that were used in this study were of analytical grade and purchased from local suppliers.

2.2. Strain, medium and culture conditions

Aurantiochytrium sp. strain YLH70 was isolated from the mangrove ecosystem in Yueqing Bay, Wenzhou, Zhejiang Province, China. This microorganism was collected in the China Centre for Type Culture Collection (CCTCC No.: M2014215) and maintained on GYP medium (20 g/L glucose, 10 g/L yeast extract, 5 g/L peptone, and 20 g/L sea salt).

Jerusalem artichoke medium (JAM), including the hydrolysate of Jerusalem artichoke, yeast extract and sea salt, was used for DHA production by *Aurantiochytrium* sp. YLH70. For comparison, the fructose medium (FM, containing 100 g/L fructose, 15 g/L yeast extract, and 25 g/L sea salt) and glucose medium (GM, containing 100 g/L glucose, 15 g/L yeast extract, and 25 g/L sea salt) were also tested for growth and DHA yield by *Aurantiochytrium* sp. YLH70. The GYP, JAM, FM and GM media were sterilized at 115 °C for 30 min and microorganism cultivation was performed at 200 rpm and 25 °C.

2.3. Pretreatment of Jerusalem artichoke

Approximately 10,000 g of Jerusalem artichoke was washed, peeled, and cut into strips. The cut tubers were dried in an oven at 80 °C for approximately 48 h until the weight was constant. The completely dried Jerusalem artichokes were smashed into fine powder using a crusher at 1000 W and 10,000 rpm for 30 s. For acidic hydrolysis, 500 g of Jerusalem artichoke powder was mixed with 3 L of acidic solutions and incubated at 100 °C for 30 min. Three acids, including sulphuric, nitric and hydrochloric acids, were tested to optimize the concentration of reducing sugar in the hydrolysate. The resulting mixture was centrifuged at 5000 rpm and 4 °C for 10 min, and the pH of the supernatant was adjusted to 6.0 with 1 M NaOH. After centrifugation, the supernatant was diluted with water to a final volume of 1 L and used as the Jerusalem artichoke extract. All of the experiments were performed in triplicate.

2.4. Statistical experimental design

A Box–Benhken design (BBD) for three variables (reaction time, temperature and concentration of sulphuric acid) at three levels (−1, 0, and 1) was applied to optimise the acidic hydrolysis procedure of Jerusalem artichoke. The concentration of reducing sugar was taken as the response. Moreover, a Plackett–Burman design was applied to identify the important factors of the JAM influencing the biomass of *Aurantiochytrium* sp. YLH70. Ten variables, including the concentrations of reducing sugar, yeast extract, $ZnSO_4{\cdot}7H_2O$, $FeCl_3{\cdot}6H_2O$, $CuSO_4{\cdot}5H_2O$, $MnCl_2{\cdot}4H_2O$, thiamine, biotin, sea salt, and cobalamin, at two levels (−1 and +1) were analyzed. Based on the results from the Plackett–Burman experiments, a central composite design (CCD) with three variables (reducing sugar, yeast extract and sea salt) at five levels (−2, −1, 0, 1, and 2) was used to optimise the compositions of the JAM for the biomass of *Aurantiochytrium* sp. YLH70. The Box–Benhken design (BBD), Central Composite Design (CCD) and their analyses were performed via Design Expert (Version 8.0, State-Ease Inc., Minneapolis, MN, USA), and the Plackett–Burman experiments were designed and analysed by the Minitab software (Version 17.10, Minitab. Inc., USA). All of the experiments were conducted in triplicate and the average values were taken as the response.

2.5. Growth with the hydrolysate of Jerusalem artichoke, pure fructose or glucose

Based on the optimized composition of the JAM medium, the *Aurantiochytrium* sp. strain YLH70 that was cultured for 48 h was used as the seed culture to inoculate the JAM medium, containing 695 mL/L of Jerusalem artichoke extract (100 g/L reducing sugar), 15 g/L yeast extract and 25 g/L sea salt. For comparison, the FM and GM media, in which the extract of Jerusalem artichoke in the JAM was replaced by 100 g/L fructose or glucose, respectively, were also tested.

2.6. Fed-batch fermentation using the JAM medium for DHA production

The fed-batch fermentations for biomass and DHA production were carried out in the RALF 5 L Bioreactor (Bioengineering, Swiss).