Microbial Bioprocess Method for Sustainable Squalene Production to Replace Shark Liver Oil in Industrial Applications

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Abstract

Squalene is a natural organic compound commonly derived from shark liver oil and widely used in the cosmetic and pharmaceutical industries. As worldwide environmental awareness grows, the usage of shark liver oil is increasingly being criticised since it has the potential to harm marine ecosystems. As a result, new technologies will be demanded in the future to lessen reliance on shark liver squalene manufacturing. Therefore, this paper proposes an alternative process to produce more sustainable squalene to preserve environmental sustainability and reduce the threat of the extinction of rare sharks that are widely hunted for the benefit of the industry. This paper proposes a new method to produce high-quality, non-fish, environmentally friendly, and scalable squalene. The experiment used methods from Lasiana Beach in Kupang, East Nusa Tenggara (NTT) strain. The strain used has the potential to produce squalene from the microalgae Aurantiochytrium. The NTT strain used has the characteristics of round-shaped cells and a yellowish-white cell color. The pure isolate is grown in the nutrient's media, consisting of glucose, yeast extract, reef salt, disodium phosphate, ammonium sulfate, peptone, and a mixture of aquadest. The result of biomass is checked using a microscope, and there are Aurantiochytrium microalgae cells that have the potential to produce squalene. The result cell has a diameter of 17.6 μm on the results of main culture. The total weight of biomass produced was 34.5 grams in 1 liter. The biomass obtained had a fishy smell and a brownish-yellow color. The results of this study show that the biomass organoleptic characteristics and cell micrographs are consistent with the results of previous studies. The resulting product, in the form of squalene, can be further used as a raw material for cosmetics, nutraceuticals, medicines, and vaccines. The results of this research are very interesting for further study as an environmentally friendly alternative raw material. This approach will preserve environmental sustainability and reduce the threat of extinction for the rare shark, which is widely hunted for the benefit of the industry.

Introduction

Squalene is a natural organic substance that is frequently present in both plants and animals [1]. Because of its many positive attributes, it has grown to be very important in the pharmaceutical sector. Squalene has demonstrated several health advantages, such as antioxidant and anti-inflammatory characteristics [2]. It might also aid in the prevention and treatment of some disorders. Squalene is also included in vaccines as an adjuvant to boost the body's immunological response to antigens, which increases their effectiveness [3].

Shark livers, plant oils, and microbes are just a few of the sources from which squalene can be obtained [4]. Compared to shark liver oil, plant-based forms of squalene often contain less of the substance. It may take a lot of agricultural resources, such as land, water, and fertilizers, to extract squalene from plants. If not managed properly, extensive plant cultivation for the sole purpose of producing squalene might potentially compete with food crops or result in deforestation. Due to this, it can become more expensive and more difficult to supply the demand for medicinal applications.

Seasonal fluctuations in the squalene concentration of some plant sources of squalene may have an impact on the supply's stability and dependability. For pharmaceutical companies looking to produce squalene-based products steadily and continuously, this volatility could pose problems. Additionally, squalene generated from plants is sought outside of the pharmaceutical sector. Squalene may also be required by other industries, such as the cosmetics and personal care sectors, for a variety of goods, raising the possibility of competition and supply issues [5].

Since deep-sea sharks have higher squalene concentrations in their livers, the desire for this substance has resulted in extensive shark poaching. Squalene is produced by killing sharks and extracting their liver oil, which has led to questions regarding the viability and conservation of shark populations. Sharks are already susceptible to overfishing because of their modest rates of reproduction and lengthy maturation times [6]. Squalene production's impact on shark populations, along with other issues including habitat degradation and bycatch, has led to decreases in some shark species and altered marine ecosystems.

Conservationists and environmental groups have been pushing for more environmentally friendly substitutes for squalene made from shark liver oil. Squalene, made from plant sources like olive oil, sugarcane, or amaranth seeds, is one of these alternatives. By promoting and utilizing these substitute sources, we can lessen the demand for squalene obtained from sharks and lessen the danger to shark populations.

Environmental worries have been raised by unsustainable shark squalene harvesting, which has been linked to overfishing and a negative impact on marine ecosystems. Animal welfare-related ethical concerns are brought up by the extraction of squalene from sharks and other animals. Squalene is produced sustainably to assure its availability for future generations. The pharmaceutical industry can secure the long-term availability of this priceless substance by using eco-friendly techniques that will lessen its impact on the environment and build a more sustainable supply chain [7].

In conclusion, sustainable squalene production is key to maintaining a steady supply of this vital substance, fostering environmental and moral responsibility, and realizing the compound's potential health advantages. The pharmaceutical sector can promote sustainability and safeguard the planet's biodiversity and ecosystems while promoting a healthier future.

This essay suggests a different method for producing squalene that is high-quality, non-fish, environmentally benign, and scalable. The Aurantiochytrium microalgae isolated from Lasiana Beach in Kupang, East Nusa Tenggara (NTT) strain, is the alternative source suggested in this paper. This paper's conceptual notion is illustrated in Figure 1. Squalene needs in the cosmetics, nutraceuticals, supplements, pharmaceutical, and vaccine industries must be supplied in a more environmentally friendly manner [8]. Around 3000 sharks could be saved if shark liver oil were substituted with a more environmentally friendly substance made from the microalgae Aurantiochytrium [9].

The capacity of Aurantiochytrium microalgae to make squalene has been discussed in several prior articles [10,11]. This research is also anticipated to serve as an inspiration for the advancement of science and technology based on native Aurantiochytrium microalgae from Indonesian mangrove forests since there are currently no international journal publications on their capacity to manufacture squalene.

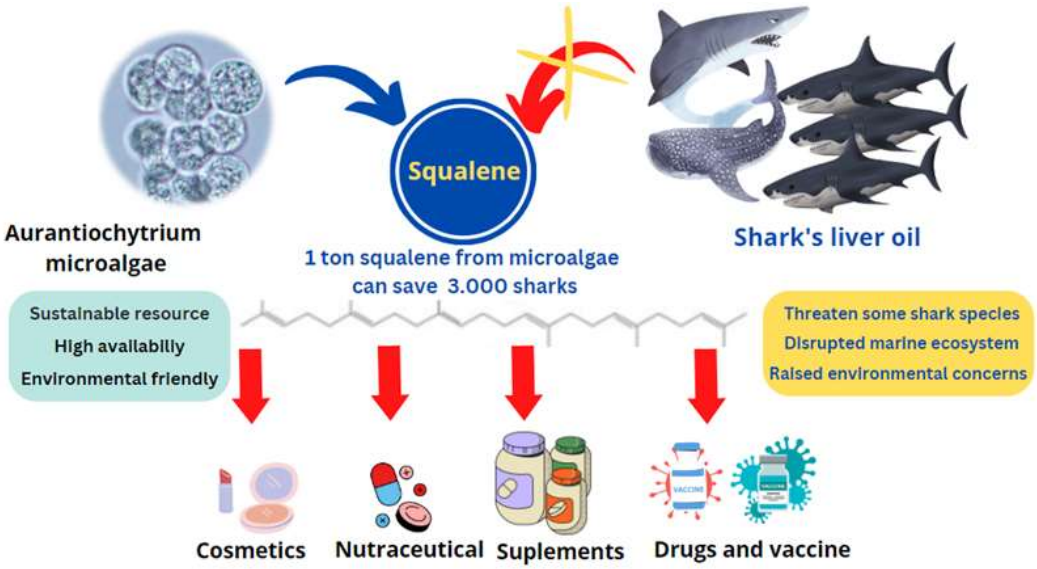

Fig 1. Illustration proposing the development of sustainable squalene production from Aurantiochytrium microalgae.

Experiments

Equipments and Materials

The main instruments used in this research are the Laminar Air Flow (LAF), orbital shaker, autoclave, microscope, Personal Computer, Erlenmeyer flask, centrifuge, tube centrifuge, analytical scale, beaker glass, prepared glass, microliter pipette, volumetric flask, ose disposable inoculation loops, aluminium, funnel glass, shring road, and bottle for saving biomass. The main ingredients used are bacteriological agar, yeast extract, peptone, glucose anhydrous, reef salt, aquades, disodium phosphate, and ammonium sulfate.

Production of Biomass

The study used strains isolated from Lasiana Beach, Kupang, Nusa Tenggara Timur (NTT) Province. The pure strain was isolated using direct plating methode. The batch production of biomass is carried out in a three stage cultivation as illustrate in figure 2. The cultivation stages are namely standing culture (SC), pre-culture (PC), and main culture (MC). The nutrient of culture media consists of glucose, yeast extract, reef salt, disodium phosphate, ammonium sulfate, peptone, and water. The nutritional formulation and operating conditions of biomass production are presented in Table 1. The SC culture is started by adding a snippet of pure isolate into an erlenmeyer flask an orbital shaker of 150 rpm for 48 hours. After 48 hours of cultivation, the culture is observed using a binocular microscope. If the culture is clear without any other microbial contamination, then, the culture is transfered into the PC media. The PC culture is made by pouring 5-10 % of SC culture into the PC media as presente in table 1. The PC culture is then cultivated for 48 hours in an orbital shaker of 200 rpm. Afterwads, the cell growth is observed. If the cell culture seems clearly without any contamination, then the cultivation is continue in MC culture. The MC culture is prepared by pouring 5-10% of the PC culture. The last stage cultivation, the MC culture cultivation, is carried out in an orbital shaker of 200 rpm for 120 hours. The micrograpfh of the cells in each stage of cultivation are captured using a microscope digital camera. The biomass is harveste after completing of MC

cultivation. The wet biomass is separated from ist liquid phase and by a centrifuge at 4000 rpm. The obtainable biomass is dried at a temperature of 40℃ until the weigh is constant.

Table 1. The nutrients formulation in 1 Liter volume and SC, PC, and MC operating conditions

Nutrients	Standing Culture (SC)	Pre Culture (PC)	Main Culture (MC)
Glucose	15 g	30 g	80 g
Yeast Extract	5 g	10 g	18 g
Reef Salt	7,2 g	7,2 g	7,2 g
Disodium Phosphate	-	-	0,75 g
Ammonium Sulfate	-	-	4 g
Peptone			2,5 g
Speed	220 Rpm	220 Rpm	220 Rpm
Temperature	28 ºC	28 ºC	28 ºC
Time	2 Days	2 Days	5 Days

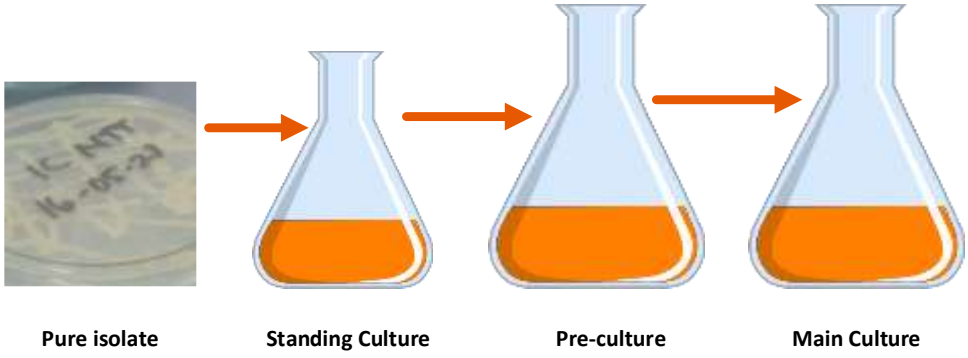

Fig 2. Process Illustration of SC, PC and MC.

Results

Figure 3 shows micrograph of observed cells from each cultivation stages. The micrograph of Auranctiochytrium microalgae cells show that the largest cells diameter of each cultivation stages are 8,62103 µm, 11,9163 µm, 15,9943 and 17,5561 µm for pure isolate, SC, PC an MC respectively. The cells reveal that Aurantiochytrium microalgae can grow and produce certain biomass. The microlagae strain used has the characteristics of a rounded cell, yellowish-white cell colour.

PADA PC SEBESAR 1,2

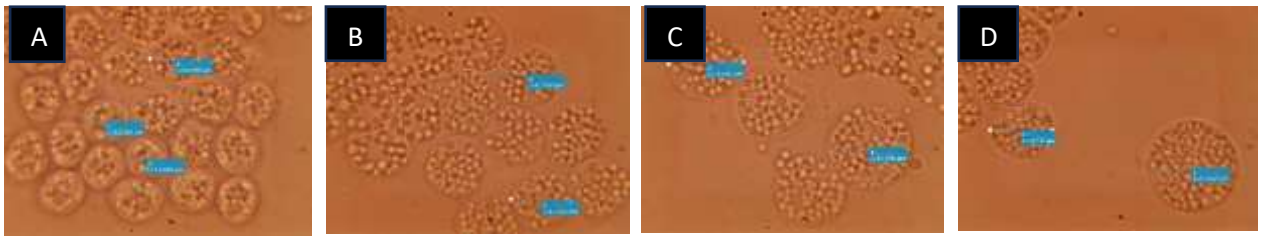

Fig 3. Micrograph of observed cells of each cultivation stages: pure isolate(A), SC(B), PC(C), and MC(D) with 1000x magnification.

Mikrograf sel mikroalga Auranctiochytrium setiap tahapannya menunjukkan bahwa diameter sel dengan perbesaran 1000x yaitu pada isolat murni sebersar 8,62103 µm, SC sebesar 11,9163 µm, PC sebesar 15,9943 µm . dan MC sebesar 17,5561 µm.

Figure 4 shows the products of cultivation process using Aurantiochytrium microalgae. The produced liquid from early stage of cultivation (SC, 4A) has a yellow colour meaning that the inoculation inoculation from pure isolate has succeeded. The liquid product after main culture is shown in figure 4B and it has a fishy smell like fish and is brownish-yellow in colour. The resulting wet biomass (4C) after centrifugation is shown in figure 4B. The total weight of wet biomass was 34,5 gram with a total volume 1 liter. The dried biomass was obtained after the drying process of the wet biomass, as shown in figure 4D.

Fig 4. SC Liquid (A), Biomass liquid (B), Wet biomass (C), Dryed Biomass (D)

Discussion

This paper propsed a microbial approach to produce squalene in order to replace a common process using shark liver oil. The microbe uses native Inonesian Aurantiochytrium microalgae isolated from one of Indonesian mangrove forest. The observed micrograph shows that the strain of Aurantiochytrium microalgae in this experiment can grow in the media presented in this experiment. Previous study observing the potentials of indigenous Indonesian microalgae is rare. One of previous study using Indonesian Aurantiochytrium microalgae has been published before [12] .

Biomass of Aurantiochytrium mcroalgae was successfully produced using the nutritional media proposed in this paper. The results of this study show that the microbial approach succeeded in producing the Aurantiochytrium microalgae biomass based on the results of the micrograph from the isolate used to observe that the cells of Aurantiochytrium microalgae have grown.

The characteristics of biomass and cell micrographs are consistent with the results of previous studies [12,13]. The diameter of the microalgae used was larger than the average in the previous study [14]. So, the strain is potentially used to produce high-value biomass products from Aurantiochytrium microalgae, including squalene [15]. In addition, the sources of carbon and nitrogen used, such as glucose, yeast extract, and reef salt, produce a relatively high level of squalene compared to other sources of coal, nitrogen, or other kinds of microbial substances [16,17].

The resulting squalene potential recent microbial approach can be used further as an alternative raw material for cosmetics, nutraceuticals, pharmaceuticals products.. In these mentioned industries, the demand of squalene is relatively high, therefore the results presente in this recent research are promising for further study. The microbial approach is expected to be considered as a potential environmentally friendly process alternative.

Microalgae are sustainable and renewable sources for squalene production, making it an environmentally friendly alternative to squalene derived from shark liver oil. However, in the future, sources of carbon and nitrogen need to be sought for more economical alternatives, such as cheaper organic materials [18,19]. This microbial approach will be very helpful to fulfill the future demand of squalene while reducing dependence on conventional processes using shark liver. It is hoped that

this approach will preserve environmental sustainability and reduce the threat of extinction for the endangered sharks, which is widely hunted for the benefit of the industry.

Conclusions

Strain from Lasiana beach in Kupang, East Nusa Tenggara, Indonesia, was successfully used in this research to produce the Aurantiochytrium microalgae biomass. This biomass has the potential to produce alternative raw materials to produce squalene. Therefore, further research is needed so that the production of squalene can use Indonesian native microalgae as the raw material the industry needs. Squalene from microalgae is very promising to support marine ecosystems, prevent shark shortages, and serve as a raw material for cosmetics, nutraceuticals, medicines, and vaccines.

Squalene adalah senyawa alami yang sering digunakan sebagai bahan aktif untuk pelembab, anti aging atau suplemen antioksidan

The benefits of squalene can also increase immunity and are used as an adjuvant vaccine

Farmasi dan pangan

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