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Review

Progress toward isolation of strains and genetically engineered strains of microalgae for production of biofuel and other value added chemicals: A review

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ABSTRACT

Microalgae and cyanobacteria are promising sources of biodiesel because of their high oil content (~10 fold higher) and shorter cultivation time (~4 fold lesser) than conventional oil producing territorial plants (e.g., soybean, corn and jatropha). These organisms also provide source of several valuable natural chemicals including pigments, food supplements like eicosapentanoic acid [EPA], decosahexaenoic acid [DHA] and vitamins. In addition, many cellular components of these organisms are associated with therapeutic properties like antioxidant, anti-inflammatory, immunostimulating, and antiviral. Isolation and identification of high-yielding strains with the faster growth rate is the key for successful implementation of algal biodiesel (or other products) at a commercial level. A number of research groups in Europe, America, and Australia are thus extensively involved in exploration of novel microalgal strain. Further, genetic engineering provides a tool to engineer the native strain resulting in transgenic strain with higher yields. Despite these efforts, no consensus has yet been reached so far in zeroing on the best microalgal strain for sustainable production of biofuel at reasonable cost. The search for novel microalgal strain and transgenesis of microalgae, are continuing side by side with the hope of commercial scale production of microalgae biofuel in near future. However, no consolidated review report exists which guides to isolate and identify a uncontaminated microalgal strain along with their transgenesis. The present review is focused on: (i) key factors for sample collection, isolation, and identification to obtain a pure microalgal species, (ii) present status for isolation of microalgal strains worldwide based on geographical location and habitat, (iii) the current research for application of genetic engineering tools for enhanced production of biodiesel and value added chemicals, and (iv) the comparison of different cultivation systems for genetically modified strain.

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1. Introduction

With improvement of living standards, energy consumption across the world is expected to rise sharply in the near future [1–3]. On the other hand, accessibility of crude oil from reserves will be difficult in the coming decades [2,4]. Further, conventional fossil fuel based energy resources are being questioned as they are directly linked with environmental pollution and global warming [5–7]. In this scenario, environment friendly renewable energy sources (e.g. solar, hydro, wind, and biofuels) are destined to be dominant in the immediate future. Therefore, efforts toward search of renewable and sustainable fuels are being promoted by government, industrial, and energy sector [8,9].

Biofuels synthesized from biomass are regarded as potential fuel and currently contribute around 10% of the world's energy demand [10]. Biofuels are categorized into first generation (derived edible biomass), second generation (derived from inedible biomass) and third generation (through sequestration of carbon dioxide; e.g., biodiesel from microalgae) [7,11–13]. Production of bioethanol and biobutanol from non-food crops (i.e. second generation biofuels) has the ability to replace gasoline and other gaseous fuels (e.g. liquefied petroleum gas). Third-generation biofuels offer a strategic alternative for high energy density biofuels (e.g., biodiesel) [14–18]. Further, biodiesel is considered as better alternative for environmental sustainability than petroleum based fuels because of its nontoxic and biodegradable nature [19].

Biodiesel is produced through trans-esterification of oils or fats, which are extracted from animal fats, vegetable oils, soya, rapeseed, jatropha, mahua, mustard, flax, sunflower, palm oil, *Pongamia*, etc. [20–26]. However, microalgae (here the term 'microalgae' represents both microalgae and cyanobacteria) are considered as the third generation biofuel feedstock where in the average yield of biodiesel will be 10–20 times higher than the yield obtained from oleaginous seeds or vegetable oils [27–30]. Beside this, microalgae have a shorter harvesting cycle (10–30 days) compared with conventional crop plants, which are usually harvested once or twice a year [31]. These microalgae utilize about ten times lesser land compared to land plants. For example, low lipid containing microalgae land requirement is 0.2 m^2 year/kg biodiesel whereas palm oil land requirement is estimated about 2 m^2 year/kg biodiesel [32]. Biodiesel derived from microalgal biomass can reduce 78% CO_2 emission on a life-cycle basis as compared to traditional diesel fuels [33,34]. Unfortunately, despite of increased production in biodiesel from microalgae, it is still not a feasible substitute for fossil fuels due to high production costs [35]. However, a number of research institutes and industries are in the process to overcome these limitations. For example, Sapphire Energy and Institute for Systems Biology, Seattle, USA, is aiming to increase oil yield and improving resistance to crop predators and environmental factors in order to commercialized algae biofuel production [36]. One multinational industry, Reliance Industry, invested 2.4 million US dollar in the year 2014 toward commercialization of algal based biodiesel [37].

It has been reported that only limited species are well studied and characterized for biofuel production while large number of algal species are identified from different geographical locations throughout the world [27,38,39]. It may be noted that microalgae are also the source of value-added chemicals such as chlorophylls, carotenoids, antioxidants, enzymes, polymers, toxins, sterols and fatty acids. Genetic engineering of the promising isolated strain would likely lead to higher production of biofuel and other value added chemicals. Research efforts (e.g. National Renewable Energy Laboratory and The National Alliance for Advanced Biofuels and Bioproducts from United States are working on isolation and identification of microalgal species) have been initiated to isolate and identify new microalgal species from diverse natural resources and further efforts toward search for novel microalgae species are to be explored [40,41]. In addition, limited scientific review reports have been found, which would guide the use of genetic engineering tools to modify the native microalgal strain for enhanced yields. Therefore, in this review, we focus on techniques associated with the collection of sample specimen, isolation and identification methods to obtain a pure microalgal strain. Further, the present status of isolated microalgae strains from across the world is discussed. Finally, latest achievements in genetic engineering strategies for the manipulation of desired strains with potential precursors of biofuels and value-added chemicals are been reviewed.

2. Natural habitat, collection, isolation and identification of microalgal species

2.1. Natural habitat

Native microalgal strains are most preferred for production purpose as they have the superior adaptability to the environmental conditions prevailing in a particular geographical location [42]. Microalgae are typically found in aquatic bodies, which include freshwater, marine and brackish water systems. While most microalgae are photoautotrophic with a great diversity in the natural habitat and can be found and collected from lakes, ocean, river, pond, and other water bodies [43]. In addition, they may also be found in environmental conditions such as hot spring lakes, acid mine drainage and ice-lakes [44–46]. Some microalgal strains can