Algal Research 30 (2018) 113–120

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Review article

Fluorescence activated cell-sorting principles and applications in microalgal biotechnology

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ARTICLE INFO

Keywords:
FACS
Flow cytometry
Microalgae
Bioprospection
Strain improvement
High-throughput

ABSTRACT

Microalgal biotechnology has gained increasing attention over the last few decades as a next-generation driver for obtaining food, feed and biofuels and to carry out bioremediation of effluents and CO_2 mitigation. Flow cytometry (FC) and fluorescence-activated cell sorting (FACS) have recently acquired outstanding importance in the development of high-throughput methodologies. For example, bioprospecting novel species using FACS widened the current portfolio of available strains for drug discovery and biomass production in large-scale production systems. Moreover, FACS has recently prompted several approaches for the effective improvement of microalgal strains by means of genetic engineering, serial selection and random mutagenesis. In the upcoming years, routine implementation of FC and FACS is expected to further bring forward the field of microalgal biotechnological research as occurred with mammalian cells in biomedical sciences. This review highlights the recent developments of FACS applications to different biotechnological goals, as well as the principles and details of FACS-based microalgal analysis. In addition, the future perspectives of novel and innovative approaches of FACS applications in microalgal biotechnology are suggested and discussed.

1. Introduction

Microalgal biotechnology is a green alternative to produce foods, feeds and fuels with high potential to close the loop of CO_2 or phosphorus when flue gases or wastewaters are used [1]. Nevertheless, the production costs of the biomass still restrict the commercialization of microalgal products to the manufacture of pigments, fatty acids or whole microalgal cells for niche markets application in the food and feed sectors. For companies to enter larger markets, high-throughput methods to screen the high diversity of microalgae found in the aquatic environment are required [2,3,4]. In particular, these methods are needed to select improved algal strains for producing one or more biotechnologically relevant biomolecules [4,5,6] with enhanced volumetric and areal biomass productivities [7].

Classic methodologies for the isolation of microalgal strains and selection of genetically engineered cells commonly employed are time-consuming techniques, such as agar streaking [8]. Alternatively, flow cytometry (FC) coupled with fluorescence-activated cell sorting (FACS) methodologies were developed by researchers and the microalgal industry [2,9,10]. Previously, FC and FACS gained increasing popularity in biomedical sciences, leading to expedite methodologies that revolutionized the analysis of mammalian cells (e.g., efficacy of vaccination) [11].

FC coupled to FACS can process and measure up to 20 different light scatter and fluorescence properties of thousand cells per second, enabling screening procedures at high speed (thousands of cells per second), with cell recovery and axenicity higher than 99% [12]. Therefore, FACS-based approaches are key to expediting the bioprospection of novel strains and selection of strains with improved traits. The need for novel strains may be long overdue, considering that in 30 years of microalgal biotechnology only ~20 species are commercially exploited, but the globally available pool of microalgae is no smaller than 72,500 species [13,14]. Among these species, microalgal strains have evolved and adapted to specific environments that led to unique metabolic pathways, which could be used for biotechnological purposes [15]. In fact, these numbers emphasize the biodiversity of microalgae and the need to develop FACS-based approaches, as mining of this bioresource requires the isolation and scale-up of strains that produce the desired biocomponents in large amounts within a short period of time.

Recently, Hyka et al. [10] reviewed FC methods and probes applied to microalgal biotechnology, while Hildebrand et al. [16] and Dashkova

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https://doi.org/10.1016/j.algal.2017.12.013
Received 8 August 2017; Received in revised form 22 December 2017; Accepted 30 December 2017

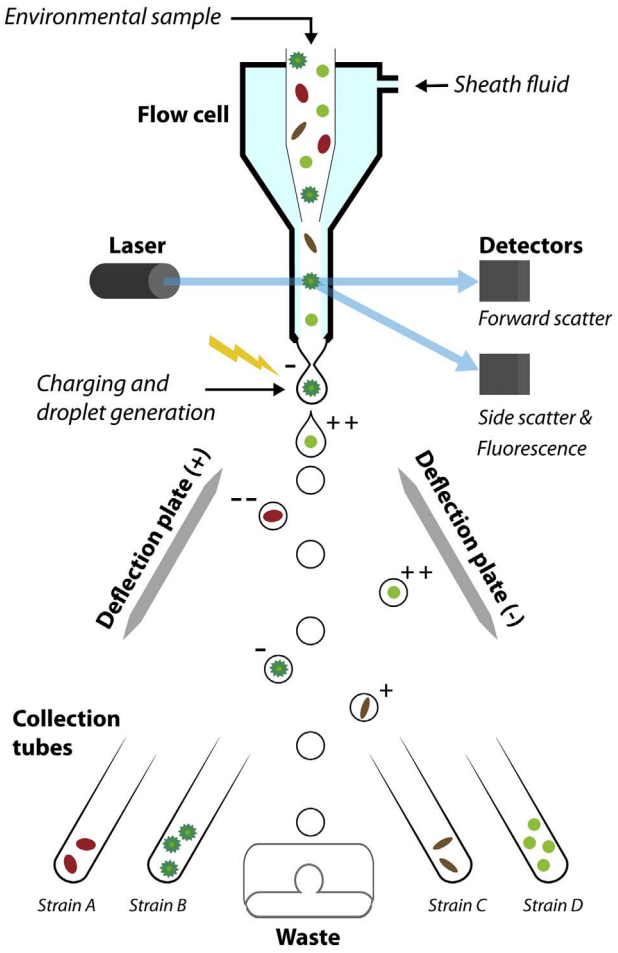

Fig. 1. Fluorescent activated cell sorting of environmental samples containing microalgae. Cells are restricted to a narrow band by a liquid stream (sheath liquid) in the flow cell. Through high-speed vibration of the nozzle of the flow cell, the liquid is divided into droplets usually containing no more than one cell, which is either positively or negatively charged. The cells are diverted into specific collection tubes by deflection plates according to the type and intensity of the electrical charge.

et al. [17] discussed the applications of imaging FC. The present review summarizes the principles of FACS and its application for microalgal bioprospection and strain improvement. In addition, we discuss and point out future directions and possibilities of FACS in microalgal biotechnology.

2. Principles of FC and FACS

FC enables the acquirement of cellular suspensions for detailed analysis of mono- or plurialgal populations as well as complex environmental samples. Samples are acquired through a draining system into the flow cell (Fig.1). Inside the flow cell, a narrow stream guides the sample into the sheath fluid (e.g., PBS or culture medium), where cells are coordinated to pass one at a time through a process called hydrodynamic focusing. At the interrogation point, each cell intercepts the lasers and the optics collect cell specific data. In microalgal research, the blue (488 nm) and red (633 nm) lasers are usually selected to emit light, which is then measured by a series of detectors (see below) that can assess parameters such as the scattering of light due to the interaction of photons with the sample. Subsequently, detected light is converted to an electrical signal with a specific voltage. The generated data can be used to perform multiparametric analysis of all cells within the sample. At a later stage, signals representing events (e.g,. a cell, salt grain or debris particle) detected by the apparatus can be processed via gating on multiple two-dimensional dot plots [18], which allow the selection of subpopulations with shared properties in a multidimensional parameter space.

Coupling FC with a cell sorter enables FACS (Fig. 1) by which separation of cells or populations is achieved in an expedited fashion. Through high-speed vibration of the nozzle of the flow cell, the liquid stream is split into droplets containing individual cells that have been selected by the gating procedure. As the apparatus recognizes a cell defined in the gate(s) at the interrogation point, an electrical charge is later applied to that specific event when the droplet is generated in the nozzle. Afterwards, the stream of droplets passes through the deflection plates, and the droplets containing the cells are deviated to the desired collecting system based on the charge previously applied (Fig. 1).

Cell sorting can be performed onto a wide array of collecting systems (e.g., 96-well plates, different tubes, microscopic slides) containing the desired (solid or liquid) growth medium.

3. Light scattering and fluorescence detectors

When the light emitted by the laser hits a cell, the photons are scattered into multiple directions. Different detectors are used to measure the light scattered by the cell, such as the forward scatter (FSC) and side scatter (SSC) sensors (Fig. 2). It is often stated that FSC and SSC can be used to measure the relative cell size and inner cell complexity, respectively (Fig. 2A). However, this is an oversimplification of how

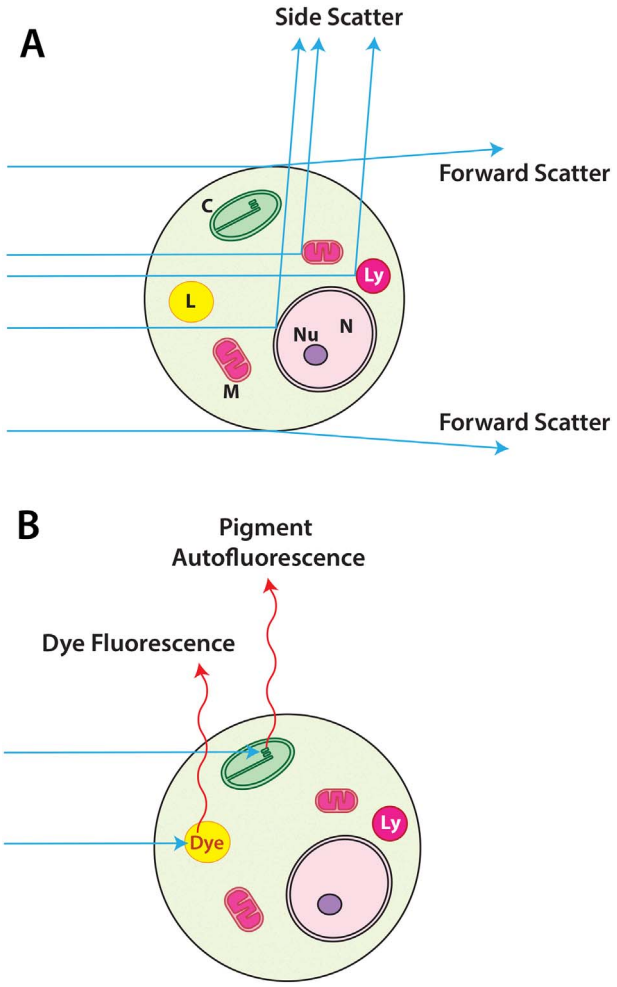

Fig. 2. Light scattering (A) and fluorescence (B) detected by flow cytometry. The cell wall was omitted for simplicity's sake and because there are microalgae that do not possess this cell covering. Pigment autofluorescence is usually detected by photomultipliers at high angles. C, chloroplast; L, lipid body; Ly, lysosome; M, mitochondrion; N, nucleus; Nu, nucleolus.