Journal of Biotechnology 248 (2017) 77–86

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Botryococcus braunii strains compared for biomass productivity, hydrocarbon and carbohydrate content

Joao Diogo Gouveia[a,*], Jesus Ruiz[b], Lambertus A.M. van den Broek[b], Thamara Hesselink[d], Sander Peters[d], Dorinde M.M. Kleinegris[b], Alison G. Smith[e], Douwe van der Veen[a], Maria J. Barbosa[b], Rene H. Wijffels[a,c]

[a] Wageningen University and Research, Bioprocess Engineering Group, AlgaePARC, Droevendaalsesteeg 1, 6708 PB, The Netherlands
[b] Wageningen UR Food & Biobased Research, P.O. Box 17, 6700 AA Wageningen, The Netherlands
[c] University of Nordland, Faculty of Biosciences and Aquaculture, N-8049 Bodø, Norway
[d] Plant Research International, Business Unit of Bioscience, Cluster Applied Bioinformatics, Plant Research International, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
[e] Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK

ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form 1 March 2017
Accepted 11 March 2017
Available online 20 March 2017

Keywords:
Botryococcus braunii
Hydrocarbons
Carbohydrate
Galactose
Fucose
Microalgae

ABSTRACT

Botryococcus braunii can produce both long-chain hydrocarbons as well as carbohydrates in large quantities, and is therefore a promising industrial organism for the production of biopolymer building blocks. Many studies describe the use of different strains of *Botryococcus braunii* but differences in handling and cultivation conditions make the comparison between strains difficult. In this study, 16 *B. braunii* strains obtained from six culture collections were compared for their biomass productivity and hydrocarbon and carbohydrate content. Biomass productivity was highest for AC768 strain with 1.8 g L^{-1} day^{-1}, while hydrocarbon production ranged from none to up to 42% per gram biomass dry weight, with Showa showing the highest hydrocarbon content followed by AC761. The total carbohydrate content varied from 20% to 76% per gram of the biomass dry weight, with CCALA777 as the highest producer. Glucose and galactose are the main monosaccharides in most strains and fucose content reached 463 mg L^{-1} in CCALA778.

1. Introduction

Human activities greatly depend on petroleum as both an energy source and industrial raw material (Dale, 2007). Petroleum usage in the long term is both unsustainable due to depleting economically relevant sources and by rapid release of carbon dioxide in the environment. One potential source of biofuels and other biobased raw material compounds are microalgae. These photoautotrophic organisms are able to transform inorganic carbon into lipids such as triacylglycerols (TAGs) at a faster rate than agricultural oleaginous crops, and do not compete for arable land (Wijffels and Barbosa, 2010). Besides lipids, other products of interest that microalgae may produce in large quantities are hydrocarbons and carbohydrate. Hydrocarbons are natural occurring compounds consisting entirely of hydrogen and carbon, and are one of the most important energy resources (Timmis and Qin, 2010). Hydrocarbons derived from microalgae can be hydrocracked and transformed into aviation turbine fuel (Hillen et al., 1982). Carbohydrate have a range of industrial uses, including as thickeners, stabilisers and gelling agents in food products (Donot et al., 2012), as well as in the pharmaceutical and cosmeceutical industries (Borowitzka, 2013; Buono et al., 2012).

One promising production host of biofuels and biobased materials is *Botryococcus braunii*. This eukaryotic microalga specie can be found across the world as a variety of strains with different physiological characteristics. Some strains of *B. braunii* can produce to up to 86% hydrocarbons on cell dry weight basis (Brown et al., 1969), whereas other strains can produce to up to 4.5 g L^{-1} carbohydrates into the medium (Fernandes et al., 1989). One advantage of *B. braunii* is that it secrets extracellular hydrocarbons and carbohydrates (Kalacheva et al., 2002; Lupi et al., 1994; Volova et al., 1998; Weiss et al., 2012; Wolf, 1983) which allows the development of strategies for *in situ* extraction such as “milking” (Moheimani et al., 2013).

B. braunii can produce hydrocarbons with different chemical structures. These hydrocarbons play a role in the natural growth cycle of *B. braunii* (Khatri et al., 2014). Depending on what type

* Corresponding author.
E-mail address: joao.gouveia@wur.nl (J.D. Gouveia).

http://dx.doi.org/10.1016/j.jbiotec.2017.03.008

Table 1
Origen of *Botryococcus braunii* strains. Information regarding the individual strains and the culture collections from where they were purchased. * strains used for growth in bubble columns photobioreactors.

Culture collection	*Botryococcus braunii* Strain		Race	Location	Isolation, date of isolation	Reference
Berkeley	Showa	*	Race B	culturing tanks, Berkley	By unknown, 1980	Wolf et al. (1985)
Scandinavian Culture Collection of Algae and Protozoa (SCCAP)	SCCAP K-1489		Not know	Belgium, Nieuwoort	By G. Hansen, 2008	No reference
Culture Collection of Algae at Goettingen University (SAG)	SAG 30.81		Race A	Peru, Dpto. Cuzco, Laguna Huaypo	By E. Hegewald, 1977	Dayananda et al. (2007)
Culture Collection of Autotrophic Organisms (CCALA)	CCALA-777	*	Not know	Porto da Castanheira (Poço dos Basilios) Portugal	By Santos, 1975	Fernandes et al. (1989)
	CCALA-778	*	Not know	Serra da Estrela (Barragem da Erva da Fome) Portugal	By Santos, 1997	No reference
	CCALA-835		Race A	Peru, Dpto. Cuzco, Laguna Huaypo	By E. Hegewald, 1977	Dayananda et al. (2007)
Culture Collection of Algae and Protozoa (CCAP)	CCAP-807-2	*	Race A	Grasmere, Cumbria, England	By Jaworski, 1984	Hilton et al. (1989)
UTEX Culture Collection of Algae	UTEX Bb 572		Race A	Madingley, Cambridge, England	By M.R. Droop, 1950	Eroglu and Melis (2010)
	UTEX Bb LB572		Race A	Cambridge, England	By M.R. Droop, 1950	Eroglu et al. (2011)
ALGOBANK-CANE	AC755	*	Race A	Lingoult-Morvan, France	By Pierre Metzger, 1981	Metzger et al. (1985a,b)
	AC759	*	Race B	Ayame, Ivory Coast	By Pierre Metzger, 1984	Metzger et al. (1988)
	AC760		Race B	Kossou, Ivory Coast	By Pierre Metzger, 1984	Metzger et al. (1988)
	AC761	*	Race B	Paquemar, Martinique, France	By Pierre Metzger, 1983	Metzger et al. (1985a,b)
	AC765		Race L	Kossou, Ivory Coast	By Pierre Metzger, 1984	Metzger et al. (1988)
	AC767		Race L	Songkla Nakarin, Thailand	By Pierre Metzger, 1985	Metzger et al. (1987)
	AC768		Race L	Yamoussoukro, Ivory Coast	By Pierre Metzger, 1984	Metzger et al. (1987)

of hydrocarbons are produced, *B. braunii* is subclassified into four chemical races, designated A, B and L (Metzger and Largeau, 2005), and S, a recent assignment (Kawachi et al., 2012). Race A strains synthesize odd-numbered alkadienes and trienes (C_{25} to C_{31}) (Dayananda et al., 2007; Eroglu and Melis, 2010; Hilton et al., 1988; Metzger et al., 1986; Metzger et al., 1989), race B strains synthesize a class of isoprenoid derived compounds termed botryococcenes (C_{30} to C_{37}) and methylated squalenes (C_{31} to C_{34}) (Metzger and Casadevall, 1983; Metzger et al., 1985b; Metzger et al., 1987; Nanamura, 1988), race L strains synthesize lycopadiene (C_{40}) and race S strains synthesize C_{18} epoxy-*n*-alkanes and C_{20} saturated *n*-alkanes (Metzger and Casadevall, 1987).

In addition to hydrocarbons, *B. braunii* strains can produce large amounts of carbohydrates with the highest amounts so far reported as 4.0–4.5 g L^{-1} (Fernandes et al., 1989). Extensive carbohydrate production was first observed by an increase of broth viscosity during growth (Casadevall et al., 1985). Since this initial report, other strains were found to produce carbohydrates with yields of 250 mg L^{-1} for race A and B strains, and 1 g L^{-1} for a race L strain (Allard and Casadevall, 1990). Later, 1.6 g L^{-1} for *B. braunii* LB572 and 0.7 g L^{-1} for SAG30.81 (Dayananda et al., 2007) were reported. Galactose was identified as the main monomeric sugar constituent of all carbohydrates examined, with fucose and rhamnose as accompanying monomers. Glucose was detected in the L strain only (Allard and Casadevall, 1990).

One drawback of using *B. braunii* as an industrial host is its slow growth compared to other photoautotrophic microorganisms. *B. braunii* biomass productivities range between 0.1 and 0.2 g L^{-1} d^{-1} (Cabanelas et al., 2015; Eroglu et al., 2011) where other green microalgae such as *Chlorella* sp. can achieve 0.5 g L^{-1} d^{-1} (Hempel et al., 2012). One commonly reported hypothesis for the slow growth is due to the synthesis of energetically expensive hydrocarbons (Banerjee et al., 2002).

There is an extensive body of work in last few decades describing different strains of *B. braunii* and it is clear that there is a high degree of morphological plasticity and physiological diversity amount the genus. It is probably due to this high diversity in the genus that *B. braunii* is not an easy organism to maintain and grow under laboratory conditions. Many methods of cultivation, different types of growth medium or culture conditions have been reported in the literature to study more in depth *B. braunii* individual strains as well as for comparing strains diversity (Allard and Casadevall, 1990;