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Disruption of *Botryococcus braunii* colonies by glycoside hydrolases

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Abstract

Microalgae are a promising alternative resource to fossil-based products. *Botryoccocus braunii* is a colonial green microalga having the ability to convert CO_2

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by photosynthesis into long chain hydrocarbons. These are excreted and trapped in an extracellular matrix (ECM). A panel of glycosidases ranging from arabinanase, galactananase to endoglucanase was tested for their ability to lyse the polysaccharides maintaining the *B. braunii* colony integrity in order to release the hydrocarbons present in the extracellular matrix without harming the cells. The *Bp*GH9 endoglucanase from *Bacillus pumilus* was fused with *Ct*CBM3a from *Clostridium thermocellum* and yellow fluorescent protein to probe the presence of microcrystalline cellulose in the cell wall of *B. braunii* and to increase the efficacy of the endoglucanase. All the tested enzymes were able to some extent to dissociate the cells from the extracellular matrix while keeping them alive, suggesting the feasibility of a semi-continuous *in situ* recovery of hydrocarbons.

Keywords

Botryococcus braunii, microalga, glycosidase, flow cytometry, extracellular matrix, hydrocarbons.

1. Introduction

Microalgae and cyanobacteria were originally produced for food and animal feed, and have been cultivated in large scale for some decades, notably in Asia. They contain valuable compounds such as proteins, sugar, vitamins, pigments and fatty acids among other metabolites (Spolaore et al., 2006; Borowitzka, 2013; Pulz and Gross, 2004). The interest in green microalgae comes from their ability to convert carbon dioxide and sunlight to biomass and valuable products. If many microalgae produce useful proteins, carbohydrates and lipids, a few produce specific long-chain hydrocarbons such as botryococcens (Kumar et al., 2020). Some of these excreted molecules appear to have promising biological activities.(Oliva et al., 2022; Sasaki et al., 2017)

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Botryococcus braunii is a fresh water colonial oleaginous green microalga and is classified in four different races A, B, L and S, according to the chemical structure of the hydrocarbons it produces (Metzger and Largeau, 2005; Jin et al., 2016b). Although this microalga is known to deliver large amount of exopolysaccharides and carotenoids (Cheng et al., 2019), the interest in *B. braunii* rests mainly on hydrocarbons. The production of such energetically demanding hydrocarbons is supposed to be responsible to the low growth of *B. braunii* in comparison to other microalgae (Banerjee et al., 2002). Nevertheless, this microalga is considered as a great candidate for the production of liquid hydrocarbons that can represent up to 61% of dry biomass *e.g*. in race A (Jin et al., 2016b). In contrast to other oleaginous microalgae, hydrocarbons synthesized in *B. braunii* are mostly secreted in the extracellular space and not accumulated as lipid bodies in the cytoplasm (Hirose et al., 2013). However, these hydrocarbons of interest are retained in an extracellular matrix (ECM) consisting in three different components: the liquid hydrocarbon containing extracellular space, a colony retaining wall and a polysaccharide colony sheath (Weiss et al., 2012).

Because of this structural specificity and its slow growth, the exploitation of *B. braunii* is therefore not very profitable by conventional methods of hydrocarbon extraction (Banerjee et al., 2002). The colony sheath and retaining wall have to be deconstructed in order to release the liquid hydrocarbons from the extracellular matrix of *B. braunii*. These components are mainly composed of carbohydrates (97%) where arabinose (42%) and galactose (39%) are predominant. The polysaccharide consists in a backbone made of galactose with mainly 1,2 linked side chains and ends composed of arabinofuranose. However the galactose in the backbone shows an unusual substitution pattern where linkages in position 2 and 3

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are the most abundant, in comparison to the more common 1,4 or 1,6 linkages (Weiss et al., 2012). One can also assume the *B. braunii* cells interact with the ECM through their cell wall, which prevent their efficient release from the ECM. Thus targeting the cell wall polysaccharide consisting in β-1,4 and/or β-1,3 linked glucans in addition to the polysaccharide sheath is an appealing strategy to favor hydrocarbon release (Weiss et al., 2012).

The aim of this work was therefore to use glycosidases to hydrolyze the polysaccharides involved in the different components of a colony in a targeted and gentle manner in order to make accessible the hydrocarbons from the extracellular space. We demonstrate that specific endoglycanases (cellulases, arabinanases and galactanases) promote a significant disruption of the size of *B. Braunii* colonies. The most efficient disruption was observed with endocellulases that have been fused with carbohydrate binding module (CBM3a), which allowed the cellulases to target the crystalline cellulose of the cell walls.

2. Material and methods

2.1 Enzymes

Cellulase (C-0901) EC 232.734.4 from *Penicillium funicolosum*, (5 units/mg solid) and Hemicellulase (H7649) EC 232.799.9 from *Aspergillus niger,* (1.5 units/mg solid) were purchased from Sigma-Aldrich® (France). Endo-α1,5-arabinanase *(Bt*ABN43A) , endo-β1,4-galactanase *(Bs*Gan53A) and exo-β1,3-galactanase (*Ct*Gan43A) were acquired from Nzytech (Portugal).

2.2 Botryococcus braunii suspension cell culture

B. braunii Kützing SAG 30.81 race A was provided by the Culture Collection of Algae at Gottingen University (Sammlung von Algenkulturen, SAG). This race A strain was previously purified and studied (Jin et al., 2016a). The colonies were grown in the

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buffered AF6 culture medium (Provasoli and Pintner 1959) within Erlenmeyer flasks on a rotating shaker (100 rpm). Some assays were conducted with colonies from a continuous culture within a labscale photobioreactor (0.35 L) at 20°C and under continuous illumination corresponding to a Photon Flux Density of 150 µmol m^{-2} s^{-1}. To prevent the interaction of possible exopolysaccharides with enzymatic activities, cell-colonies were previously rinsed and transferred to new buffered culture medium.

2.3 Gene cloning and YFP fusion

The *Bp*GH9 and CBM3a-*Bp*GH9 constructs containing an in frame C-terminal poly-histidine tag for purification by immobilized ion metal affinity chromatography (IMAC) were obtained as described previously (Ogonda et al., 2021). For *in vitro* studies and imaging, the YFP gene was inserted at the 5' extremity of the constructs in the original pECa plasmid used in the previous study. These constructs were assembled using Gibson Assembly® protocol (New England BioLabs®Inc) with recombinant primers to amplify by PCR (Polymerase Chain Reaction) the YFP gene and to insert it in the pECa already containing the *Bp*GH9 and CBM3a-*Bp*GH9 genes. The successful constructs were then confirmed by colony PCR and sequencing.

2.4 Production and purification of YFP-BpGH9 and YFP-CBM3a-BpGH9 recombinant enzymes

Recombinant strains of *E. coli* BL21(DE3) expressing the *Bp*GH9 variants (YFP-*Bp*GH9 or YFP-CBM3a-*Bp*GH9) were grown in 1 L of LB medium supplemented with ampicillin (resistance antibiotic) at 37°C to 0.6 of OD_{600nm} (Optical Density measured at λ=600nm) and IPTG at 0.5 mM was added to induce the expression at 30°C overnight under stirring (200 rpm). Then the culture was centrifuged and the pellet was frozen and resuspended in 20 ml of lysis buffer 20mM Tris-HCl pH 8.0 containing 10mM Imidazole, 5mM DNaseI, 1mg/ml lysozyme and protease inhibitors

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cocktail (Sigma-Aldrich®). After sonication and centrifugation, the recombinant proteins with His-tag contained in supernatant (soluble fraction) were purified by IMAC: 300 µl of Ni-NTA Superflow (Qiagen), preliminary equilibrated into 20mM Tris-HCl pH 8.0 containing 10 mM imidazole, were added to the supernatant and stirred for 1 h at 4°C, then loaded onto a manual column. The column was washed using 25 mL of washing buffer (20mM Tris-HCl pH 8.0, 25 mM imidazole), then elution buffer (5-fold 500 µl = 2,5 ml) was added (20mM Tris-HCl pH 8.0, 250 mM imidazole). The purity of the final product was checked by sodium dodecyl sulfate-polyacrylamide 12% gel electrophoresis (SDS-PAGE). Enzyme concentration was determined by UV absorbance at 280 nm using a NanoDrop 1000 (ThermoScientific) with the protein extinction coefficient (ε) calculated using http://atchimiebiologie.free.fr/proteinecalc/proteinecalc.html.

2.5 Enzymatic activity characterization

To assess the enzymatic activities of *Bp*GH9 constructs (YFP-*Bp*GH9 and YFP-CBM3a-*Bp*GH9), enzymatic assays were conducted using the insoluble microcrystalline cellulosic substrate Avicel PH-101 (Sigma-Aldrich®). The enzymatic activities were tested at an enzyme concentration of 3µM in 10mM Tris/HCl Buffer pH 8.0 and with 5% (w/v) of Avicel at 37°C during 24h. For the assays, 70µl of the reaction medium were withdrawn at regular time intervals and added to 70µl of DNS (Dinitro salicylic acid solution). The mixture was heated at 95°C for 10 minutes (Miller 1959). The OD was then read at 540nm by spectrophotometry (Magellan, Tecan®), reflecting the reducing end of released sugars.

2.6 Affinity pull down assay

The functionality of the CBM3a module following fusion to the bi-modular wild type endoglucanase was ascertained by binding of the CBM to the microcrystalline

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cellulosic substrate Avicel PH-101 according to the method by Stern with modifications (Stern et al. 2015). The enzymes (YFP-*Bp*GH9 and YFP-CBM3a-*Bp*GH9) at 5µM were placed under orbital stirring (200 rpm /4°C) for two hours in presence of 5% (w/v) insoluble cellulose (Avicel). The mixture was then centrifuged for two minutes on a bench-top mini-centrifuge (Extragen). The supernatant was then recovered and the pellet was washed with 10 mM Tris/HCl buffer, pH8.0 containing 0.05% (v/v) Tween®20 (Sigma) in order to remove the non-specifically bound proteins. The mixture was centrifuged again for two minutes on a bench-top mini-centrifuge. The supernatant was eliminated and the pellet was taken up with 10 mM Tris/HCl buffer, pH8.0. The fractions containing the pellets (P) and the supernatants (S) were denatured for 3 minutes at 95°C in loading buffer (SDS 2% (w/v); 0.08 M Tris-HCl; pH 6.8; 10% glycerol (v/v), ß-mercaptoethanol 0.06% (v/v)) then loaded onto an SDS-PAGE gel containing 12% acrylamide under denaturing conditions.

2.7 Fluorescence microscopy

B. braunii cell suspension was incubated with a 10µM enzyme solution of YFP-*Bp*GH9 or YFP-CBM3a-*Bp*GH9 with a 2:1 ratio (v/v). The mix loaded between blade and coverslip was observed at ×40 lens with optical microscope at optical fiber Nikon Ni-E, in transmission light to observe the colonies. A TRITC filter (excitation: 543/22 nm; emission: 593/40 nm) was used to observe chlorophyll auto-fluorescence and a FITC filter (excitation: 482/35 nm; emission: 536/40 nm) was used to observe YFP fluorescence. The pictures of chlorophyll fluorescence and Bright Field Dual Imaging were analyzed to estimate the cell viability with Nis-Elements Viewer software (Wang et al., 2021).

(https://www.nikoninstruments.com/fr_FR/Produits/Logiciels/NIS-Elements-

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Advanced-Research/NIS-Elements-Viewer).

2.8 Confocal imaging

Multicolour images of colonies were acquired with a Leica SP8 inverted confocal microscope (Leica) and a 63× oil-immersion objective (NA 1.4) or a 20x oil immersion (NA 0.75) driven by the Leica Acquisition Suite software. The excitation and emission wavelengths for Nile Red were 488 and 565/25, respectively; for autofluorescence from chlorophyll, they were 561 and 700/50 nm.

2.9 Cytometry

Cytometry analyses were carried out on a Beckman Coulter CytoFLEX flow cytometer on the same cell suspension treated with *Bp*GH9 constucts and observed in fluorescent microscopy (see 2.7). For other enzymes 150µL of microalgal cell suspension were treated with 75µl of hemicellulase (20mg/ml, Merck-Sigma®), cellulase (20mg/ml, Merck-Sigma®), *Ct*Gan43A (2mg/ml, Nzytech®), *Bs*Gan53A (1mg/ml, Nzytech®) and *Bt*Abn43A (1mg/ml, Nzytech®). The measurements of frontal diffusion ("Forward SCatter" or FSC) and lateral diffusion ("Side SCatter" or SSC) were taken with a blue laser at 488nm. A 660nm filter was used to detect the fluorescence emission of chlorophyll in the microalgae. The flow rate was fixed at 10µL/min and the recording was done on 1000 events.

3. Results and discussion

B. braunii is a microalga which forms colonies in which cells are embedded in an ECM mainly composed of polymerized hydrocarbons in which are trapped the liquid hydrocarbons excreted by the cells (Hirose et al., 2013). By separating the cells from the ECM one can assume that extraction of the hydrocarbons should be feasible while keeping the cells alive for continuing the microalgae culture, making possible to envisage the development of a semi-continuous process. Such semi-continuous

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process cannot be considered with most of microalgae that must be killed prior to recover their hydrocarbon production as lipids usually accumulate in lipid bodies inside the cell.

The cell wall, the retaining wall and the colony sheath are all composed of polysaccharides whose partial hydrolysis is a potential strategy for releasing the hydrocarbons from the colonies. To this aim, different glycosidases were chosen according to the carbohydrate composition of these colony components. On the one hand, arabinanases and galactanases are obvious candidates to be active on the retaining wall and colony sheath which consists of arabinose and galactose residues. Therefore the endo-α1,5-arabinanase from *Bacteroides thetaiotaomicron* (*Bt*Abn43A) (Cartmell et al. 2011), the endo-β1,4-galactanase from *Bacillus subtilis* (*Bs*GAn53A) (Nakano et al., 1990) and the exo-β1,3-galactanase from *Clostridium thermocellum* (*Ct*Gan43A) (Ichinose et al., 2006; Jiang et al., 2012) were selected and tested. On the another hand cellulases and hemicellulases are expected to be active on the cell wall made of β1,3 and β1,4-glucans, so commercial cellulases and hemicellulases and the cloned cellulase from *Bacillus pumilus* (*Bp*GH9) (Ogonda *et al.*, 2021) were included in the assay.

3.1 Binding of BpGH9 cellulase on B.braunii

The endo-β-1,4-D-glucanase *Bp*GH9 from *B. pumilus* (NCBI Gene databank AVG70969) was chosen in order to genetically fuse this cellulase with various functional modules for detection (YFP) or binding (CBM3a). This enzyme was previously isolated and identified in our laboratory from a new strain of *B. pumilus* collected from the hot springs of Lake Bogoria in Kenya. It consists of a catalytic domain flanked by a CBM3c at its C-terminal end. It was previously shown that this enzyme was very tolerant to fusion at either the N-terminus or the C-terminus of the

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catalytic domain (Ogonda et al., 2021).

3.1.1 Fusion of BpGH9 with YFP and CBM3a

The *Bp*GH9 was shown to be active on cellulose substrates (carboxymethylcellulose, Na-CMC and phosphoric acid swollen cellulose, PASC) but very weakly on crystalline cellulose (Avicel) despite the presence of the CBM3c module in its structure (Ogonda et al., 2021). To our knowledge the presence of crystalline cellulose in the cell wall of *B. braunii* has not been checked but has been identified in different green microalgae. If such crystalline cellulose exists in the cell wall of *B. braunii*, the fusion of *Bp*GH9 with the CBM3a from *Clostridium thermocellum* (*Ct*CBM3a), known to have high affinity for such cellulose, should improve the *Bp*GH9 activity by fixing the enzyme to its polymeric substrate.

Moreover, since the discovery of the green fluorescent protein (GFP) from the jellysfish *Aequorea victoria*, fluorescent proteins (FP) have been largely used for biotechnological applications such as cell imaging and protein interaction studies. A set of GFP variants that are fluorescing along the visible spectrum is now available like BFP (blue), CFP (cyan) and YFP (yellow). In the carbohydrate field fluorescent proteins have been fused with CBM for localizing exopolysaccharides, controlling the accessibility of cellulose to cellulases and hemicelullases, or imaging complex carbohydrates (Kawakubo et al., 2010).

To test the presence of microcrystalline cellulose and the effect of *Ct*CBM3a on the *Bp*GH9 activity, two constructs comprising fused YFP and *Bp*GH9 with (YFP-CBM3a-*Bp*GH9) or without (YFP-*Bp*GH9) *Ct*CBM3a were expressed in *E. coli*.

3.1.2 Enzymatic and affinity pull down assays on Avicel

Before testing the *Bp*GH9 constructs on *B. braunii* cells, it was necessary to verify that adding YFP to CBM3a-*Bp*GH9 and *Bp*GH9 did not impair the fixation to

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crystalline cellulose nor the endocellulase activity.

A pull-down assay was used to show the affinity of different constructs of *Bp*GH9 for insoluble crystalline microcellulose (Avicel PH-101). When YFP-*Bp*GH9 was mixed with Avicel (Figure 1), most of the protein was found in the supernatant, showing that YFP-*Bp*GH9 possesses low affinity for Avicel. In contrast the YFP-CBM3a-*Bp*GH9 construct was located mainly in the pellet fraction, demonstrating good affinity for insoluble microcrystalline cellulose. Thus adding YFP to the *Bp*GH9 constructs did not modify the affinity of the original constructs (Ogonda et al., 2021).

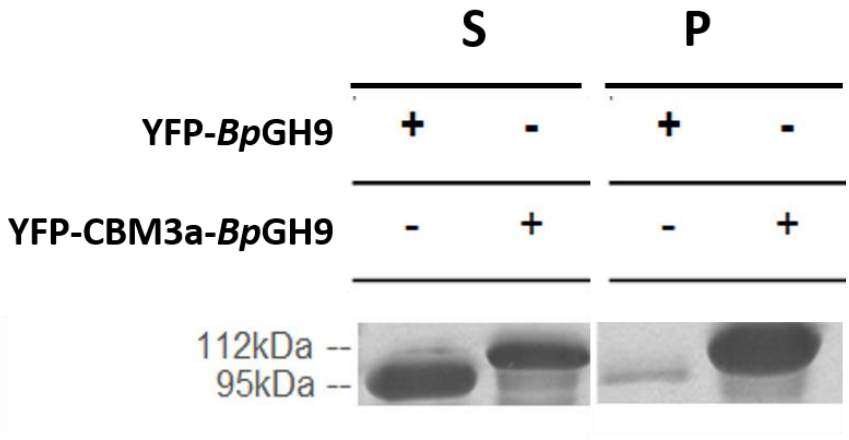

Fig. 1. Cellulose based affinity pull down assay for YFP-*Bp*GH9 (95 kDa) and YFP-CBM3a-*Bp*GH9 (112 kDa) over microcrystalline cellulose (Avicel), observed by SDS-PAGE with Coomassie Blue staining (**P**: pelleted fractions with Avicel, **S**: supernatant soluble fractions).

The activity of the constructs was determined on Avicel for checking that addition of YFP does not impair with the enzymatic activity and the effect of CBM3a. As shown on figure 2, the YFP-*Bp*GH9 construct retains activity and the activity is increased when fused with *Ct*CBM3a (YFP-CBM3a-*Bp*GH9).

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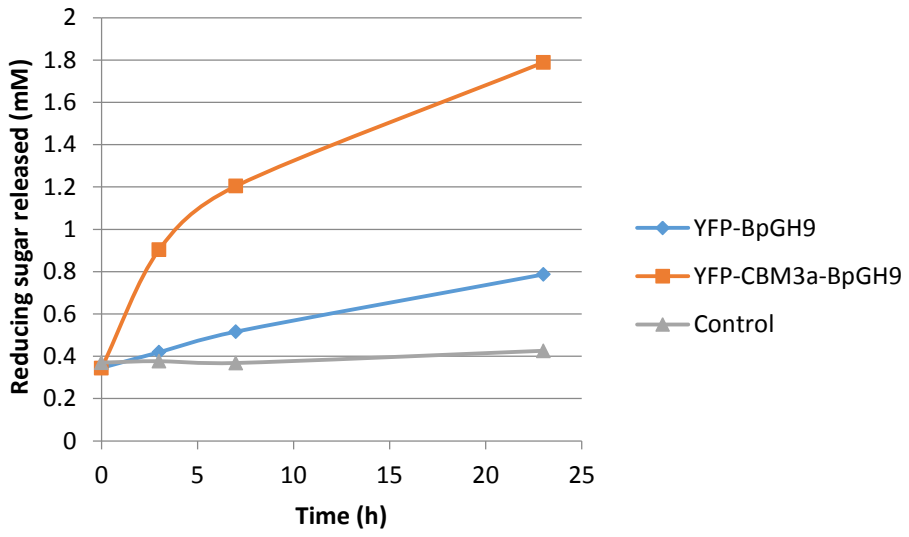

Fig. 2. Comparison of the enzymatic activities of YFP-*Bp*GH9 and YFP-CBM3a-*Bp*GH9 on Avicel at 37°C by measuring the released reduced sugar (OD, optical density at λ=540nm) in function of time (hours), compared with a negative control without enzyme (control).

3.1.3 Fluorescence microscopy

The YFP-CBM3a-*Bp*GH9 and YFP-*Bp*GH9 constructs were incubated with *B. braunii* cell suspension and analyzed by fluorescence microscopy to verify the effect of *Ct*CBM3a on the fixation of the construct to *B. braunii* cells and thus highlight the presence of microcrystalline cellulose. By comparing pictures 3c and 3d corresponding to the YFP signal of the *Bp*GH9 constructs with and without fused *Ct*CBM3a respectively, it appears that YFP-CBM3a-*Bp*GH9 is localized at the cell surface (see arrow and square box in figure 3c), suggesting binding to crystalline cellulose present in the cell wall. In absence of *Ct*CBM3a the localization seems much more diffuse and may be due to weaker unspecific binding or binding of the native CBM3c present in *Bp*GH9 to amorphous or soluble cellulose (Figure 3d).

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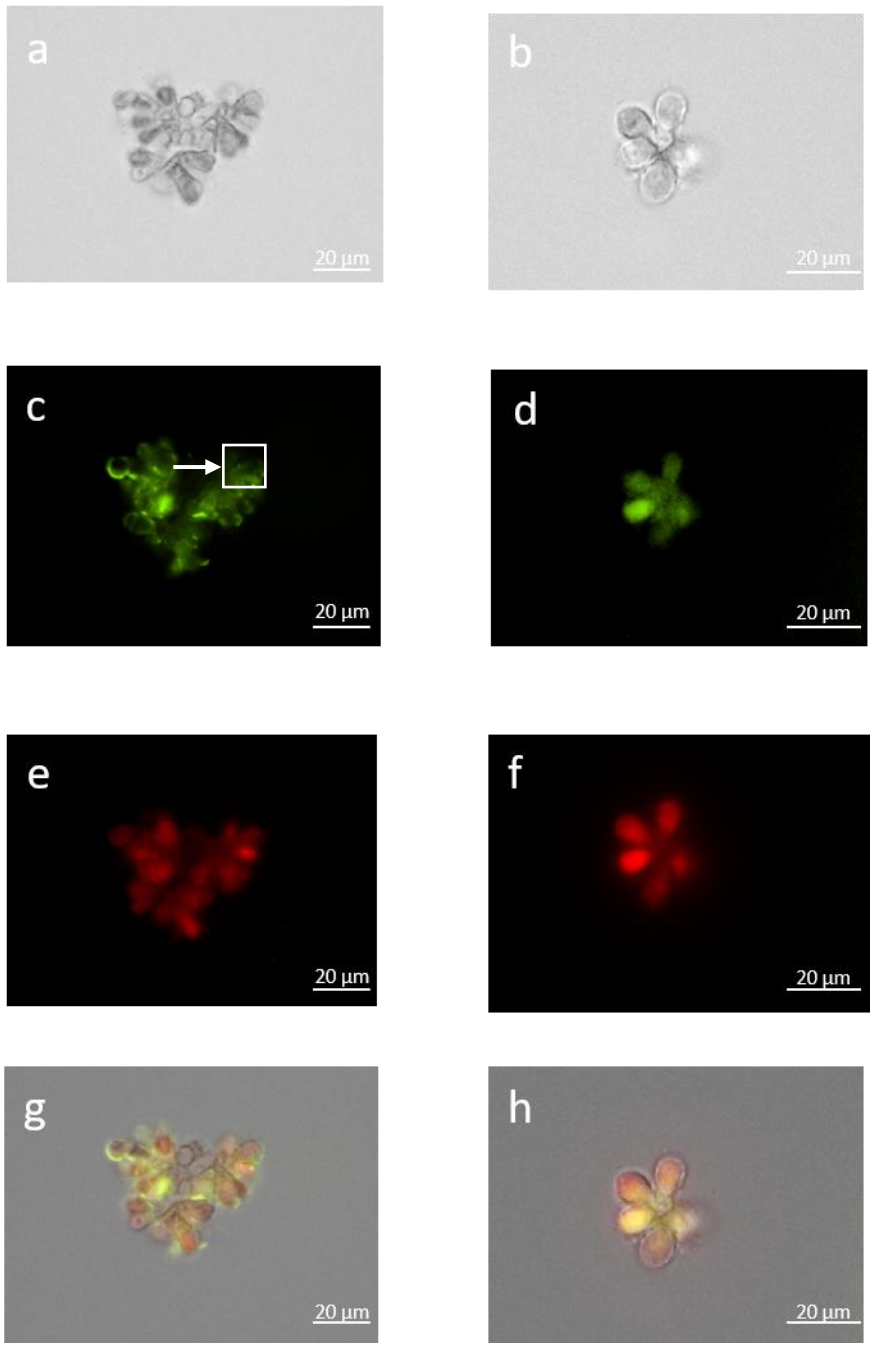

Fig. 3. Fluorescence microscopy observations of cellular localization of YFP-CBM3a-*Bp*GH9 (a, c, e, g pictures) and YFP-*Bp*GH9 (b, d, f, h pictures) in *B. braunii* cell suspension. a and b pictures correspond to the observation in transmission light, c and d correspond to the observation with FITC filter adapted to the YFP signal, e and f correspond to the observation with TRITC filter for the chlorophyll autofluorescence. g and h pictures are artificial merge of light transmission, FITC and TRITC signals. In absence of YFP the cells showed no

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fluorescence with FITC filter (not shown) (scale bars 20 µm).

3.2 Evaluation of enzymatic colony disruption by flow Cytometry

Cytometry is the method of choice for determining size of cells in suspension as the quantity of scattered light (Forward Scatter or FSC) increases with cell size (Peniuk et al. 2016). We therefore used this technique to evaluate the impact of glycosidases on the organization of *B. braunii* colonies. Indeed if enzymes are able to disaggregate the *B. braunii* colonies, the cell suspension should evolve from large colonies to smaller ones and eventually to single cell. By injecting a control cell suspension of *B. braunii* in the cytometer three populations P1 (Large, 18.8%), P2 (Medium, 29.7%) and P3 (small, 42.0%) were discriminated according to the scattered light (Figure 4).

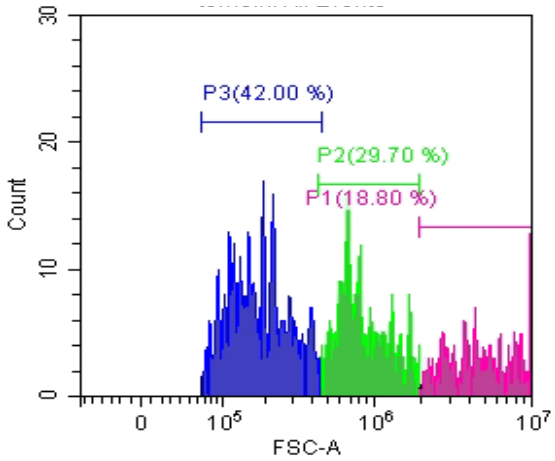

Fig. 4. Distribution of colonies size in *B. braunii* cell culture before enzyme treatment.

When enzymes were applied on *B. braunii* cell suspension the P3 population increased indicating that enzymes effectively reduced the size of colonies. Doubling the enzyme activity did not change the percentage of P3 population significantly, which suggests that the colony disruption reached a plateau. All enzymes targeting

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the cell wall were active by increasing the P3 population from around 40% for the untreated cells to 60-75% (Figure 5). The three enzymes (cellulase, hemicellulase and YFP-*Bp*GH9) were almost as active but fusion of *Ct*CBM3a to *Bp*GH9 (YFP-CBM3a-*Bp*GH9) even further increased the enzyme efficiency by around 15%. Concerning the enzymes targeting the colony retaining wall and sheath, the endo-α1,5-arabinanase *(Bt*Abn43A) was almost as efficient as the cellulases. The endo-β1,4-galactanase *(Bs*Gan53A) and the exo-β1,3-galactanase *(Ct*Gan43A) were also effective but in a less extent, with an increase in P3 population from 40% (control) to 45-50%.

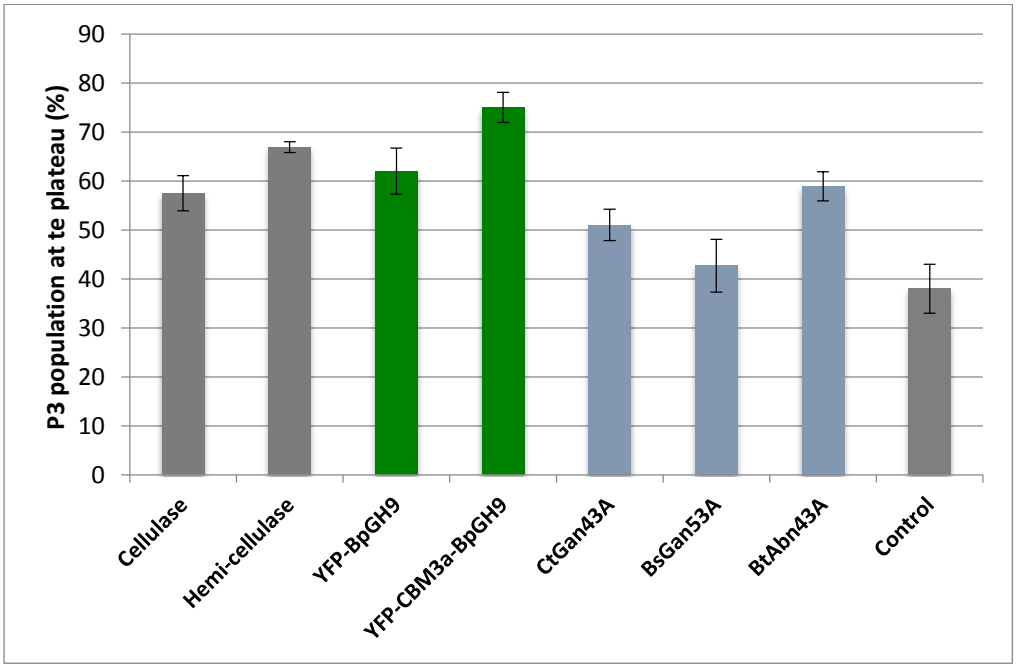

Fig. 5. Quantification of the *B. braunii* colonies disorganization by flow cytometry.

Surprinsingly the enzymes supposed to act on the cell wall (cellulase, hemicellulase and *Bp*GH9) were as much or even more effective than those supposed to act on the retaining wall and colony sheath (*Bs*Gan53A, *Bt*Abn43A, *Ct*Gan43A). This demonstrates the importance of targeting the cell wall for releasing the cells from the

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colony.

To confirm the effect of enzymes, the cell suspension treated with cellulase and hemicellulase were inspected by fluorescence microscopy. The presence of colonies was revealed by staining the lipids and hydrocarbons present in the ECM with Nile Red and the cell healthiness was controlled by verifying the autofluorescence from chlorophyll. The untreated cells consisted in colonies (Figure 6b) with cells surrounded by hydrocarbons as demonstrated by the presence of a green color due to Nile red (false color) in comparison to cell suspensions treated with cellulase (Figure 6d) and hemicellulase (Figure 6f) where cells appeared relatively more isolated. The autofluorescence from chlorophyll (red color) indicated that the cells were still alive after 1 h enzymatic treatment. (Wang et al., 2021)

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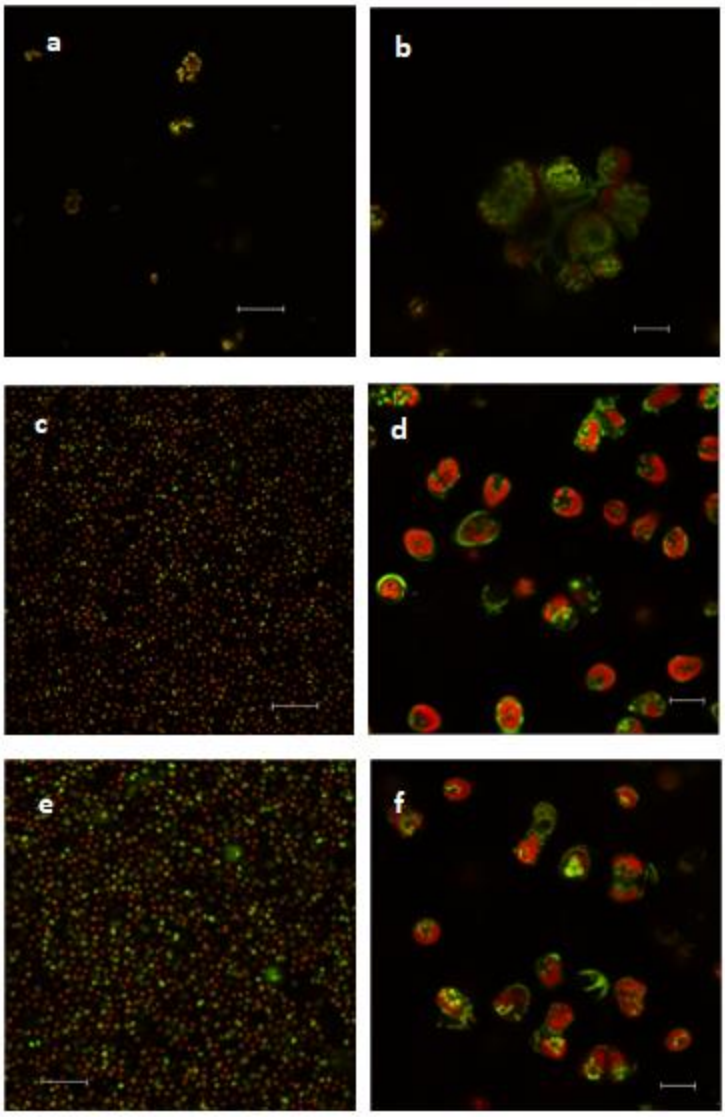

Fig. 6. *Botryococcus braunii* colonies and cell suspensions with enzymatic treatments. Merge of red autofluorescence from chlorophyll, green fluorescence from intracellular lipids and extracellular hydrocarbons with Nile Red staining. Images **a-b** control without enzyme, enzymatic treatment **c-d** with cellulase, **e-f** with hemicellulase. (objective lens x20 (a, c, e), x63 (b, d, f)); scale bars 100 µm (a, c, e), 10 µm (b, d, f), observations done after 1h incubation.

When the cells were left growing some days after the enzymatic treatment the distribution of the different populations tended to come back to the initial proportion before the treatment (see supplementary material).

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4. Conclusions

A set of glycosidases, *i.e*. arabinanase, galactanases, cellulase, hemicellulase and endoglucanase (*Bp*GH9) were shown to be active on the disruption of *B. braunii* colonies, separating the cells from the ECM containing valuable high energy hydrocarbons that can be used as biofuels. The fusion of *Bp*GHG9 with *Ct*CBM3a and YFP increased the endoglucanase activity and suggested the presence of microcrystalline cellulose in the cell wall. This opens the way to the development of a semi continuous process where *B. braunii* colonies are grown, treated with enzymes to separate the cells from ECM, making easier hydrocarbons extraction and allowing the cells to regenerate colonies. Further studies are needed to confirm that each of the released cells can regenerate a colony of *B. Braunii*.

Declaration of competing interest

None.

Author contributions

Amélie Saumonneau, conceptualization and interpretation of the experiments

Nathan Lagneau, performed the pull-down experiments and the cytometry analysis

Lydia Awuor Ogonda, cloning and overexpression of cellulases and their fusion

Catherine Dupré project administration for Gepea, investigation, reviewing

Stéphanie Dutertre investigation/ confocal colocalization microscopy

Dominique Grizeau, conceptualization, methodology, investigation

Charles Tellier, conceptualization, writing and draft preparation

Cyrille Grandjean, contributes to the final version and supervises the project

Franck Daligault project administration, writing, original draft preparation

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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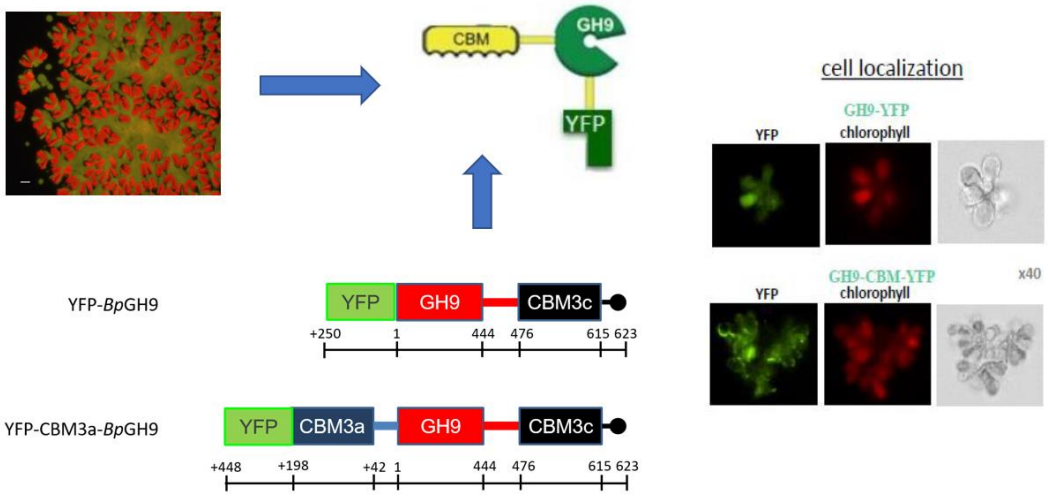
Graphical abstract



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Highlights

- glycosidases release *Botryococcus braunii* colonial microalga cells from their matrix
- probes are constructed by fusion of BpGH9 endoglucanase with CtCBM3a and YFP
- flow cytometry demonstrates the disruption efficiency of colonies by glycosidases

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