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Creating a synthetic lichen: Mutualistic *co*-culture of fungi and extracellular polysaccharide-secreting cyanobacterium *Nostoc* PCC 7413

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ABSTRACT

In order to create synthetic lichens, extracellular polysaccharide (EPS)-secreting cyanobacterium *Nostoc* PCC 7413 was cultured together with fungal *Aspergillus nidulans* and *Aspergillus niger* species without extraneous organic carbon. Cyanobacterial supernatants and harvested EPS both supported fungal growth, while fungal supernatants slightly enhanced *Nostoc* growth. Furthermore, when *Nostoc* and *A. nidulans* were co-cultured together, total biomass was approximately 3-fold higher than the axenic *Nostoc* cultures in pH 6 buffered BG-11 media. The spectrum of fatty acids generated in co-culture differed from those of the individual cyanobacterial and fungal species. The fatty acid fractions of C18:0 and C18:1 were reduced or intermediate in co-cultures compared to mono-cultures while fractions of C16:1 and C18:3 fatty acids increased in co-culture, suggesting a shift in the fatty acid biosynthesis following co-cultivation. Our study establishes a low-cost mutualistic co-culture platform composed of cyanobacteria and filamentous fungi for producing biomass and biofuel precursors with potential commercial applications.

1. Introduction

Organisms in natural environments typically do not live or function optimally in isolation. Most microbes live in complex interacting heterogeneous populations and communities. Lichens represent one of the most widespread stable and self-supporting symbioses composed of fungal heterotrophs (mycobionts) and photoautotrophs represented by cyanobacteria and/or green algae (photobionts) [1,2]. The carbon needed for biosynthesis is provided by the photobionts and supplied to the heterotrophic mycobionts. In turn, the mycobionts provide shelter, protection, and metabolites to support the health of the partner species [3–6]. The phototroph-heterotroph co-culture systems utilize the capabilities of two or more species simultaneously, providing a larger array of metabolite pathways and metabolite exchange, which can mean less reliance on exogenous nutrient sources compared to single cell culture systems. Thus, this natural symbiotic relationship represents a significant biotechnology opportunity to sustainably transform CO_2 and sunlight into bio-based products. While natural lichens are stable, self-supporting, mutualistic symbionts, they are among the slowest growing species; a growth rate of 1 cm/year is considered to be high [7]. Furthermore, cultivation of natural lichens *in vitro* is typically challenging, and thus most current research efforts focus on exploration of their secondary metabolites and interactions as defined in 'omics analysis [8,9]. Therefore, utilization and exploitation of natural lichens is minimal. However, co-culture systems represent a potentially innovative platform for the generation of biofuels, biochemical, and medical products and as a methodology for enhanced sustainability and environmental applications.

While natural lichen partnerships can include either eukaryotic green algae and/or cyanobacterial photobionts, cyanobacterial species (or cyanobionts) are observed in numerous natural lichens species found worldwide [10]. *Nostoc* species (sp.) are by far the most widely observed cyanobiont genus within these cyanolichens, including *Nephroma arcticum*, *Peltigera aphthosa*, *P. membranacea*, and *P. canina*

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among others [11]. Interestingly, the *Nostoc* species observed in lichens are closely related to free-living forms of the same genus [10].

Likewise, numerous lichen mycobionts are closely related to commercially important free-living filamentous fungi such as *Aspergillus*, some of which may have originally evolved as partners in lichens and subsequently became free-living independent species [12]. Indeed, mutualism in lichens may represent the original evolutionary state for certain ascomycetes. Thus, current production workhorses of *Aspergillus* may have resulted from a gradual transition to a non-lichenous state. These species may be capable of existing in both an independent and mutualistic state, or even in a transitional condition [12] representing the gradual evolution from lichenous to non-lichenous form.

In this study, we explored the potential to create a mutualistic bioproduction platform based on lichen systems composed of cyanobacteria *Nostoc* and fungal *Aspergilli* species. One of the critical requirements for generating a synthetic lichen is the capacity of a photobiont to provide consumable organic carbon to its mycobiont fungal partner. *Nostoc* strains can be prolific secretors of organic carbon, in the form of polysaccharides and potentially other metabolites [13–15]. In particular, *Nostoc* PCC 7413 is one of the most prolific producer strains releasing polysaccharides into the surrounding medium [15,16]. The polysaccharides from *Nostoc* and other cyanobacterial strains are typically composed of different monosaccharides including hexoses (glucoses, galactose, and mannose), pentoses (ribose, xylose, arabinose) deoxyhoses (fucose, rhamnose), and acidic hexoses (glucuronic and galacturonic acid) [13,14]. Furthermore, the secreted products from *Nostoc* may also include peptides and amino acids [15]. However, to the best of our knowledge, *Nostoc* species have never been explored previously as co-culture partners together with heterotrophic microbes. Alternatively, *Aspergillus* species are among the most effective heterotrophic degraders and consumers of polymeric substrates [17–19], with the capacity to break down polysaccharides and utilize the resulting mono-sugars released. Fungal species *Aspergillus nidulans* and *Aspergillus niger* used in this study are both important model organisms as well as commercial producers of food products, biochemicals, antibiotics, and enzymes [20–22]. In order to take advantage of the capabilities of these autotrophic and heterotrophic microbes, we constructed an artificial lichen containing polysaccharides-secreting cyanobacteria together with commercially important fungi and observed the effects of their interactions on growth and lipid production in different pH buffered media. This study evaluates for the first time the capacity to cultivate *Nostoc* PCC 7413 and two *Aspergillus* species in each partner's supernatants, and also their behavior together in physically integrated co-cultures. We have successfully established a synthetic lichen system composed of *Nostoc* PCC 7413 and two *Aspergillus* species. As *Nostoc* PCC 7413 and the two *Aspergillus* species were not isolated from the same lichen, the synthetic lichens we have established may not be able to explore all the mutualistic interactions that exist among members in a natural lichen. However, by appropriately manipulating the culture conditions as described in this study, we have constructed a synthetic lichen partnership that represents a potential low-cost platform for producing biomass and biofuel precursors with commercial applications.

2. Material and methods

2.1. Strains and growth conditions

The *Nostoc* strain PCC 7413 was purchased from the Pasteur Culture Collection, and was cultured in 250 mL Erlenmeyer flasks containing 100 mL BG-11 medium. Flasks were incubated at 28 °C agitated with filter-sterilized air that was enriched with CO_2 to (*v*/v). For experimental cultures, exponentially growing *Nostoc* PCC 7413 was inoculated into each flask to make the initial inoculum of *Nostoc* PCC 7413 as 0.1 OD_{750} for axenic cultures as well as co-cultures of *Nostoc* PCC 7413 with fungi. Illumination was provided by cool-white fluorescent lamps to give a light intensity of 65 μmol m^{-2} s^{-1} with 16:8 h light/dark cycle.

Fungal strains *Aspergillus nidulans* (FGSC A991) and *Aspergillus niger* (FGSC A1121) were obtained from the Fungal Genetics Stock Center. Strains were maintained as spore suspension in 25% glycerol at −80 °C. A spore inoculum was prepared by growing *A. nidulans* and *A. niger* on potato dextrose agar at 28 °C for 14 days. Spores were washed from agar plate with sterile water, filtered and maintained as suspension at 4 °C up to 30 days. The initial inoculum of fungal spores was 5–6 × 10^5 spores/mL for both axenic culture and co-culture with *Nostoc* PCC 7413. Cultures were maintained at 28 °C, agitated with air enriched with CO_2 to 1% (*v*/v), and illuminated at light intensity of 100 μmol m^{-2} s^{-1} with 16:8 h light/dark cycle.

Co-culture medium BG-$11_{[co]}$ consists of BG-11 supplemented with 1 mM NH_4Cl. To prepare supernatant of fungal cultures, suspensions of fungal spores were inoculated into BG-$11_{[co]}$ added with 10 g/L glucose, with initial concentration of 5–6 × 10^5 spores/mL. After growing at 28 °C and 180 rpm under illumination of 100 μmol m^{-2} s^{-1} with 16:8 h light/dark cycle for 3 days, supernatants were collected by filtration with cellulose filter paper (Whatman, Piscataway, NJ), followed by sterilized filtration through a 0.22-μm filter. The supernatant of cyanobacteria was collected by centrifugation of PCC 7413 cells cultured for 2 weeks, followed by sterilization through autoclave at 121 °C for 20 min. For experimental cultures, flasks were weighed at the setup of the time course experiments, and water was added prior to each sampling to correct for evaporation rates.

2.2. Quantification of cyanobacteria and fungi

For axenic culture of *Nostoc* PCC 7413, growth was monitored by spectrophotometrically measuring optical density at 750 nm (OD_{750}). The OD_{750} correlated well with biomass dry weight (mg/L) by a regression equation:

$$\text{Biomass dry weight} = 398.61 * OD_{750} - 7.8496,\ R^2 = 0.996 \quad (1)$$

Cell biomass of *Nostoc* PCC 7413 in the final axenic culture was harvested by centrifugation, and washed with distilled water three times. For axenic culture of fungi and co-culture of fungi with *Nostoc* PCC 7413, biomass was collected and filtered with cellulose filter paper (Whatman, Piscataway, NJ), and washed with distilled water three times. The biomass pellets were then lyophilized by freeze dry vacuum (LGJ-25, Xiangyi, China). The dried biomass was weighed and used later for lipid analysis.

2.3. EPS production

The axenic culture of *Nostoc* PCC 7413 was centrifuged at 3000 × *g* for 10 min. The supernatant was then treated with 4 volumes of cool ethanol at 4 °C for 24 h and the precipitate was freeze-dried in order to obtain the crude extracellular polysaccharides (EPS). The extracted EPS was dissolved in distilled water and autoclaved for 20 min. The total EPS in the supernatant was quantified by colorimetric assay on 96-plate in a Multiskan FC (Multiskan FC, Thermo, USA) at 620 nm wavelength. The process of the color reaction was carried out according to Yemm & Willis [23], and pure glucose was used as the standard.

2.4. Measurements and analysis

The pH of the samples was determined using a pH meter (Accumet Model 15, Fisher Scientific) using a combination pH electrode with silver/silver chloride reference (Fisher Scientific), which had been previously standardized to pH 4, 7, and 10. Cell images were taken using an Axiovert 200 fluorescence microscope (Carl Zeiss). Total lipids and lipid profile were analyzed with gas chromatography, using heptadecanoic acid (C17:0) as an internal standard. The corresponding chromatographic conditions and the extraction/transesterification

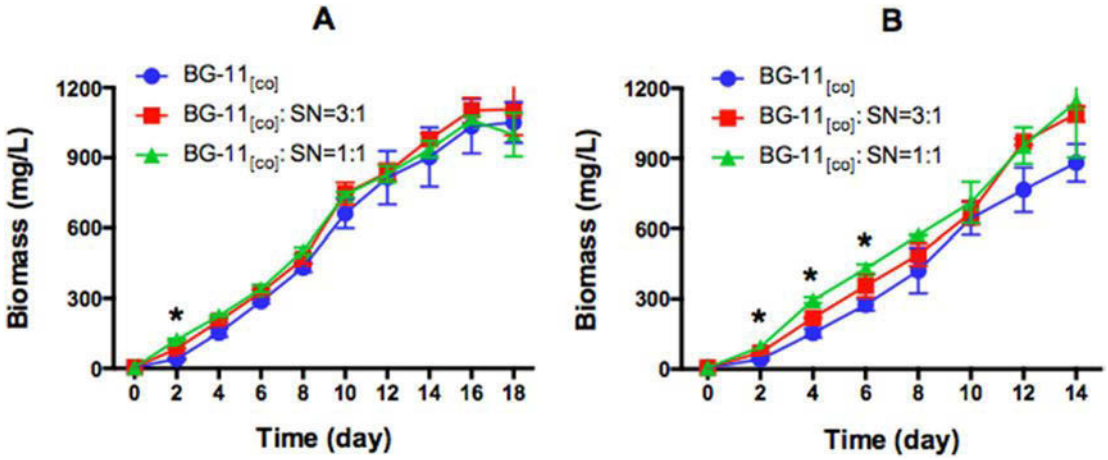

Fig. 1. Growth of *Nostoc* PCC 7413 in BG-11$_{[co]}$ and BG-11$_{[co]}$ mixed with supernatant (SN) from fungal culture. **A**) SN from *A. nidulans*; **B**) SN from *A. niger*. Data are presented as the mean of biological triplicates ± standard deviation. The * represents significant difference (p-value ≤0.05) between BG-11$_{[co]}$ and BG-11$_{[co]}$:SN = 1:1 samples within the same time period.

method are described in Dong et al. [24].

2.5. Statistics

Three biological replicates were performed for all data collection, and the statistical tests for significance were determined *via* a two-tailed *t*-test, with a significance level of 0.05, unless stated otherwise.

3. Results and discussion

3.1. Effect of fungal supernatant on growth of Nostoc PCC 7413

To examine whether there's any effects of fungal strains on cyanobacterial growth, we first evaluated the growth of *Nostoc* PCC 7413 in BG-11$_{[co]}$ alone and in BG-11$_{[co]}$ supplemented with fungal supernatants. Fungal supernatants were collected after cultivation of *A. nidulans* and *A. niger* for 3 days in BG-11$_{[co]}$ supplemented with 2 g/L of glucose. As shown in Fig. 1, cyanobacterium *Nostoc* PCC 7413 grew well both in BG-11$_{[co]}$ and in BG-11$_{[co]}$ mixed with fungal supernatants. When mixed with fungal supernatants particularly at a ratio of 1:1, the growth of PCC 7413 was slightly enhanced with significant differences (t-test, n = 3, p-value ≤0.05) at the start of the cultures, especially for the BG-11$_{[co]}$ media mixed with supernatant of *A. niger* (Fig. 1B).

3.2. Effect of cyanobacterial supernatant on growth of fungi A. nidulans/A. niger

To examine if fungi could consume EPS and/or other metabolites secreted from *Nostoc* PCC 7413, cultivations of *A. nidulans* and *A. niger* were carried out in BG-11$_{[co]}$ mixed with cyanobacterial supernatants (BG-11$_{[co]}$ + SN) or BG-11$_{[co]}$ mixed with crude EPS extracted from cyanobacterial supernatants (BG-11$_{[co]}$ + EPS), respectively. Here BG-11$_{[co]}$ was prepared as a 5-fold concentrate and mixed with supernatant (SN) or crude EPS at the ratio of 1 part of concentrate to 4 parts of SN. Our results showed that fungi *A. nidulans* and *A. niger* could grow and accumulate cell biomass in these two culture conditions, but the accumulated fungal biomass was significantly higher in BG-11$_{[co]}$ + SN than in BG-11$_{[co]}$ + EPS (t-test, n = 3, p-value ≤0.05), especially for *A. nidulans* (Fig. 2A). The initial total EPS content in BG-11$_{[co]}$ + SN was lower than that in BG-11$_{[co]}$ + EPS (Fig. 2B). For both types of cultivation, the EPS level declined after fungal growth compared with its initial content for both the crude EPS-containing and the SN-containing cultures (Fig. 2B). The relative decline in EPS was largest in the SN-containing cultures, with 32% and 42% of the initial EPS consumed by *A. nidulans* and *A. niger*, respectively, as compared to 15% and 12% of the EPS consumed for the crude EPS supplemented cultures. Therefore, EPS in cyanobacterial supernatants were more efficiently consumed, but more than half still remained at the end of the cultures. Nonetheless, cell growth was clearly more robust in the SN supplements as compared to the EPS supplements.

In terms of overall conversion yield, up to 45% of the consumed EPS could be traced to *A. nidulans* in SN-containing cultures, assuming total consumption of all polysaccharides, which was much higher than 26% converted for the EPS-containing cultures. There are several possible reasons for the different growth performance in the two cultural conditions. Compared with cyanobacterial supernatants, the relatively less consumable characteristics of sugars from crude EPS could be attributed to its harvest process. Crude EPS was collected from sediments after precipitation with ethanol, which was mostly composed of EPS polymers. Furthermore, there are likely to be additional compounds or growth factors secreted or released from *Nostoc* cultures and then discarded after ethanol precipitation, such as monosaccharides, short oligosaccharides, amino acids, amino sugars and other metabolites that may be present in the SN and readily incorporated by filamentous fungi to facilitate growth. Indeed, Dembitsky and Rezanka found that *Nostoc* species will release a number of compounds, including lipids, lipid-like compounds, boron-containing macrocycles, arsenolipids, oligopeptides and amino acid derivatives [25], that can serve as potential substrates for *A. nidulans* and *A. niger*.

3.3. Evaluation of pH on axenic growth of fungi and Nostoc PCC 7413

The growth of most cyanobacteria is typically optimal at pHs between pH 7.5 and pH 10.0 [26]. In contrast, the optimal pH for fungal growth is often around 5.0, although fungi can grow at a relatively wide range of pHs, ranging from 3.0 to more than pH 8.0 in liquid media [27]. In order to identify a suitable pH environment for both strains in co-culture, we tested the effect of pH on axenic growth of *A. nidulans* and PCC 7413, respectively. Our results showed that fungal spores of *A. nidulans* could germinate and develop mycelia as fungal biomass in both buffered and unbuffered pH conditions (Additional file 1). As the pH value increased from 6.0 to 9.0 in the BG-11$_{[co]}$ medium, the amount of *A. nidulans* biomass accumulating gradually declined from 870 mg/L at pH 6.0 to 280 mg/L at pH 9.0 (Fig. 3A). The pH values in the final cultures declined even in buffered conditions, with the pH value falling to 3.15 for the culture starting with an initial pH of 6.0 (Fig. 3B). Without any pH buffering, *A. nidulans* acidified the culture environment to pH 2.5 and also accumulated the highest median amount of biomass dry weight (DW) compared with the pH buffered groups. Clearly, *A. nidulans* can acidify the culture media severely with an acidic

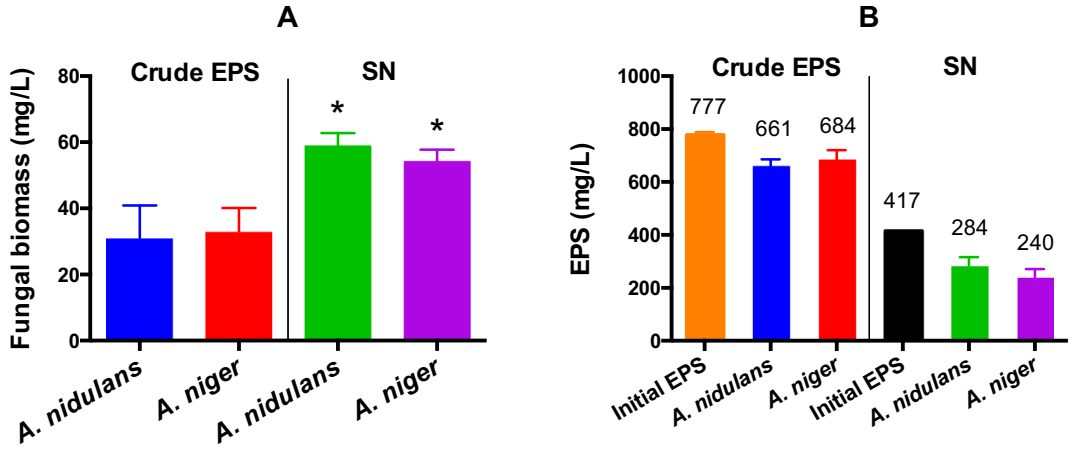

Fig. 2. Evaluation of fungal growth on carbon source from cyanobacterial culture. **A**) Dry weight of fungal biomass after growing for 2 weeks in crude EPS and supernatant (SN) of cyanobacterial culture; **B**) Measurement of EPS content before and after fungal growth. Data are presented as the mean of biological triplicates ± standard deviation. The * represents significant difference (p-value ≤0.05) between cultures in BG-11$_{[co]}$ + EPS and BG-11$_{[co]}$ + SN conditions within the same fungal species.

environment providing the best conditions for build-up of biomass.

Nostoc PCC 7413 exhibited proficient growth without pH control and accumulated 2216 mg/L of DW by 10 days, but it failed to grow at pH 9.0 (Fig. 3C). For the pH buffered groups, PCC 7413 grew best at the initial pH 7.0 condition, in which DW reached 1375 mg/L after 10 days' cultivation. PCC 7413 had similar reduced growth curves in initial pH buffered 6.0 and pH 8.0 conditions. Contrary to fungi, PCC 7413 basified its environment, changing from 7.2 to pH 8.9 in unbuffered pH condition, and reaching pH 6.7 and pH 7.9 in conditions with initial buffered pH 6.0 and 7.0 conditions, respectively. No significant pH change was observed in the initial pH 8.0 buffer condition (Fig. 3D). Generally, phototrophs drive biochemical reactions from proton

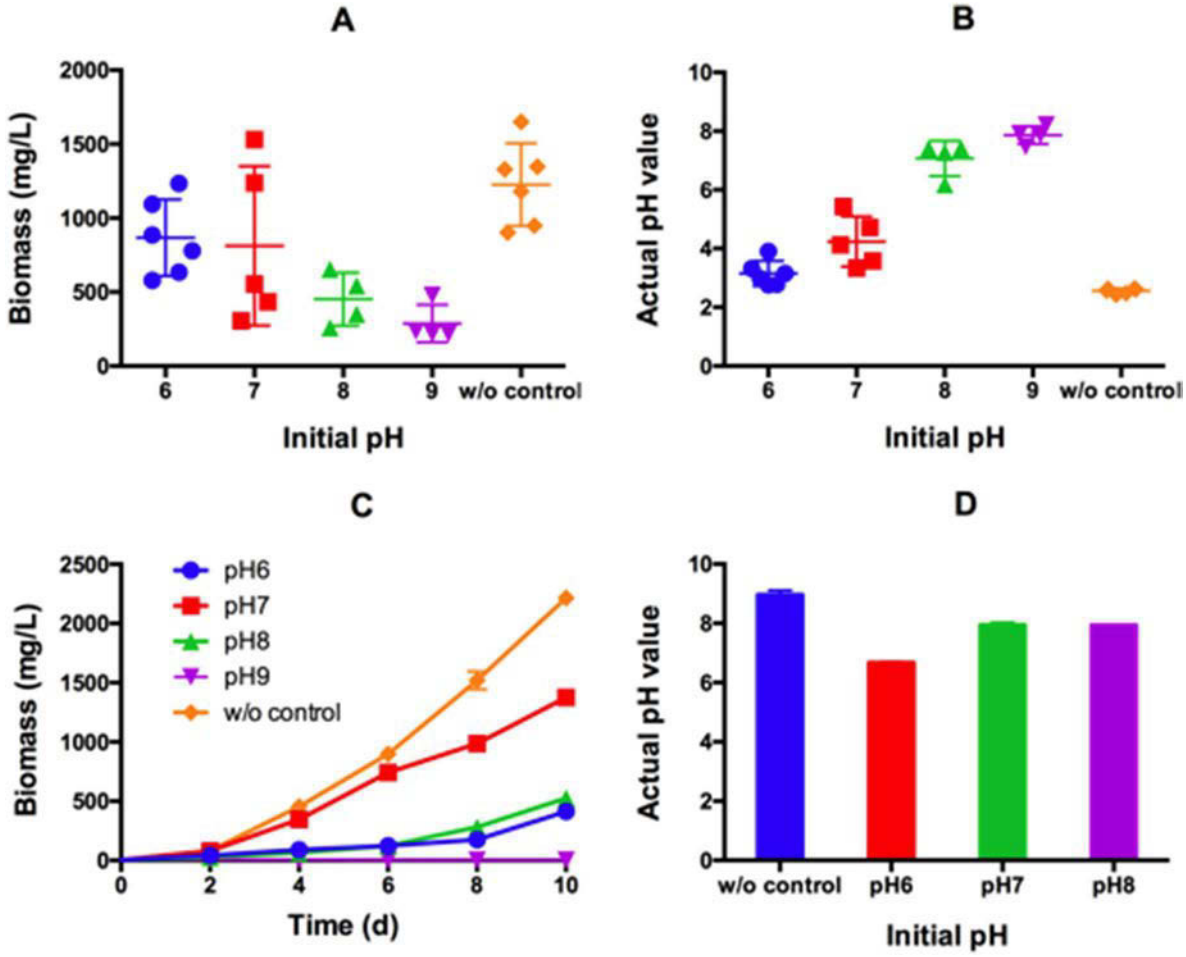

Fig. 3. Effects of pH on axenic growth of fungi *A. nidulans* and *Nostoc* PCC 7413. **A**) Dry weight of fungal biomass after 3 days' culture; **B**) Final pH value in the fungal culture; **C**) Growth curve of PCC 7413; **D**) Final pH value in the culture of PCC 7413.

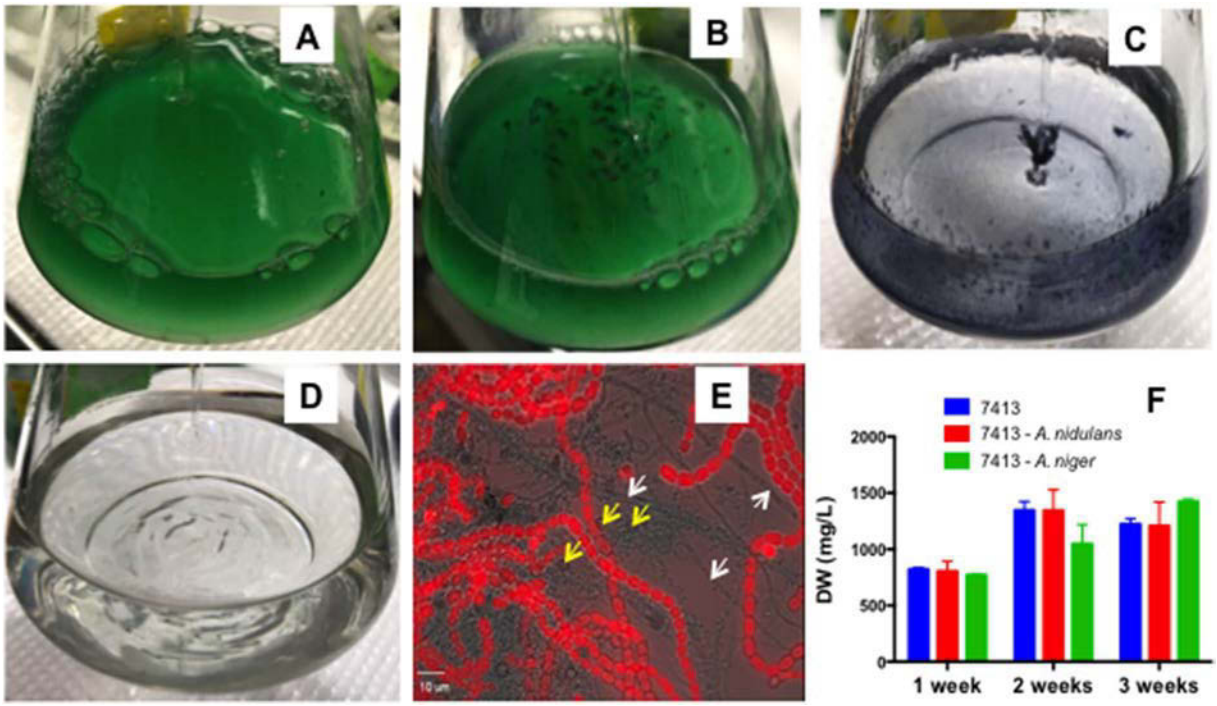

Fig. 4. Mono-culture of PCC 7413 and co-culture of PCC 7413 with fungi in BG-$11_{[co]}$ with unbuffered pH condition. **A**) Axenic culture of *Nostoc* PCC 7413 in BG-$11_{[co]}$ medium; and **B**) co-culture of *Nostoc* PCC 7413 with *A. nidulans* in BG-$11_{[co]}$ medium; **C**) and **D**) axenic culture of fungus *A. nidulans* in BG-$11_{[co]}$ supplied with 1 g/L glucose and BG-$11_{[co]}$, respectively; **E**) Fluorescence microscope image of edge parts of flocculants formed in *Nostoc* PCC 7413-*A. nidulans* co-cultures; **F**) DW of axenic culture of PCC 7413 and co-culture of *Nostoc* PCC 7413-*A. nidulans* and *Nostoc* PCC 7413-*A. niger*. In the fluorescence microscope image (**E**), red colored cells indicate *Nostoc* PCC 7413, white arrows indicate fungal hyphal fragments, and yellow arrows indicate fungal spores. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

gradients established across internal thylakoid membranes and naturally alkalinizes its environment, whereas most heterotrophs actively acidify their growth media.

3.4. Co-culture of Nostoc PCC 7413 and fungi A. nidulans/A. niger without pH buffering

Nostoc PCC 7413 and *A. nidulans* were axenically cultured and co-cultivated, respectively, in BG-$11_{[co]}$ medium bubbled with 1% CO_2. The PCC 7413 cells grew both in axenic condition (Fig. 4A) and in co-culture with the fungi in which some flocculants appeared in the co-culture after 3 days' cultivation (Fig. 4B). Flocculants were also observed in independent fungal cultures in BG-$11_{[co]}$ with glucose (Fig. 4C), yet no significant growth in lacking supplemented BG-$11_{[co]}$ medium (Fig. 4D). In order to investigate the fungal-cyanobacterial co-cultivation further, the PCC 7413-*A. nidulans* co-cultures at 3 days were examined under a fluorescence microscope (Fig. 4E). The PCC 7413 cells appeared as red colored beads in chains or in isolation (Fig. 4E), while *A. nidulans* hyphae (white arrows in Fig. 4E) and spores (yellow arrows in Fig. 4E) were also observed. After cultivation for 1, 2, and 3 weeks, the dry weight (DW) of PCC 7413-fungi co-cultures reached approximately 780, 1250, and 1280 mg/L, respectively, which were similar to the total amounts of DW for the corresponding PCC 7413 mono-cultures alone (Fig. 4F). These results indicate that *Nostoc* can grow well in axenic culture or co-cultured with fungi in unbuffered pH conditions. However, the fungal *A. nidulans* species could germinate but exhibited limited growth in co-culture with PCC 7413 as indicated by microscopy (Fig. 4E, Additional file 2). The limited growth exhibited by fungi fueled solely by the organic substrate from *Nostoc* in co-culture may be due to the lack of sufficient digestible sugars or other substrates provided by the phototrophic partner.

3.5. Co-culture of Nostoc PCC 7413 and fungi A. nidulans/A. niger in pH 6 buffered media

When lacking an additional pH buffer in the media, PCC 7413 grew well and accumulated a similar amount of biomass in both axenic culture and co-culture with fungi. Previously we observed very limited fungal biomass accumulation in the unbuffered co-cultures (see Fig. 4E and Additional file 2). Since the fungus *A. nidulans* could not grow well in higher pH conditions (Fig. 3A), the alkalinized environment in unbuffered *Nostoc* - fungi co-cultures may have contributed to the lack of fungal growth. To evaluate ways to facilitate growth of the filamentous fungi, we carried out the *Nostoc*-fungi co-cultures in media with a buffer set to an initial pH of 6.0.

One of the challenges of synthetic lichen co-cultures is determining the growth of the individual participants especially for flocculating systems such as this *Nostoc-Aspergillus* co-culture pairing. A microscopic observation indicated most of filamentous fungal cultures detected in the co-culture were present as a part of flocculants (data not shown). Therefore, an approximation of the non-flocculated expansion of PCC 7413 in co-culture with fungi was estimated by measuring OD_{750} after removal of the flocculants by filtration with Whatman cellulose filter paper. This was performed because the flocculation in the PCC 7413-*A. nidulans* co-cultures occurred due, at least in part, to the presence of filamentous fungi, which accumulated primarily in flocculants, as indicated in Fig. 4B. In the first 4 days, PCC 7413 in co-culture with *A. nidulans and A. niger* exhibited a similar growth trend compared with axenic culture of PCC 7413 (Fig. 5A). After 4 days, growth in the co-culture containing *A. nidulans* was significantly higher according to OD_{750} measurement and observation of the co-culture flask in Fig. 5B as compared to the axenic culture which slightly increased before declining after day 6 (Fig. 5A & B). Unlike the co-culture of PCC 7413 and *A nidulans*, which expanded in OD_{750} up through day 12, the PCC 7413-*A niger* co-culture grew slightly better than the control after 4 days and

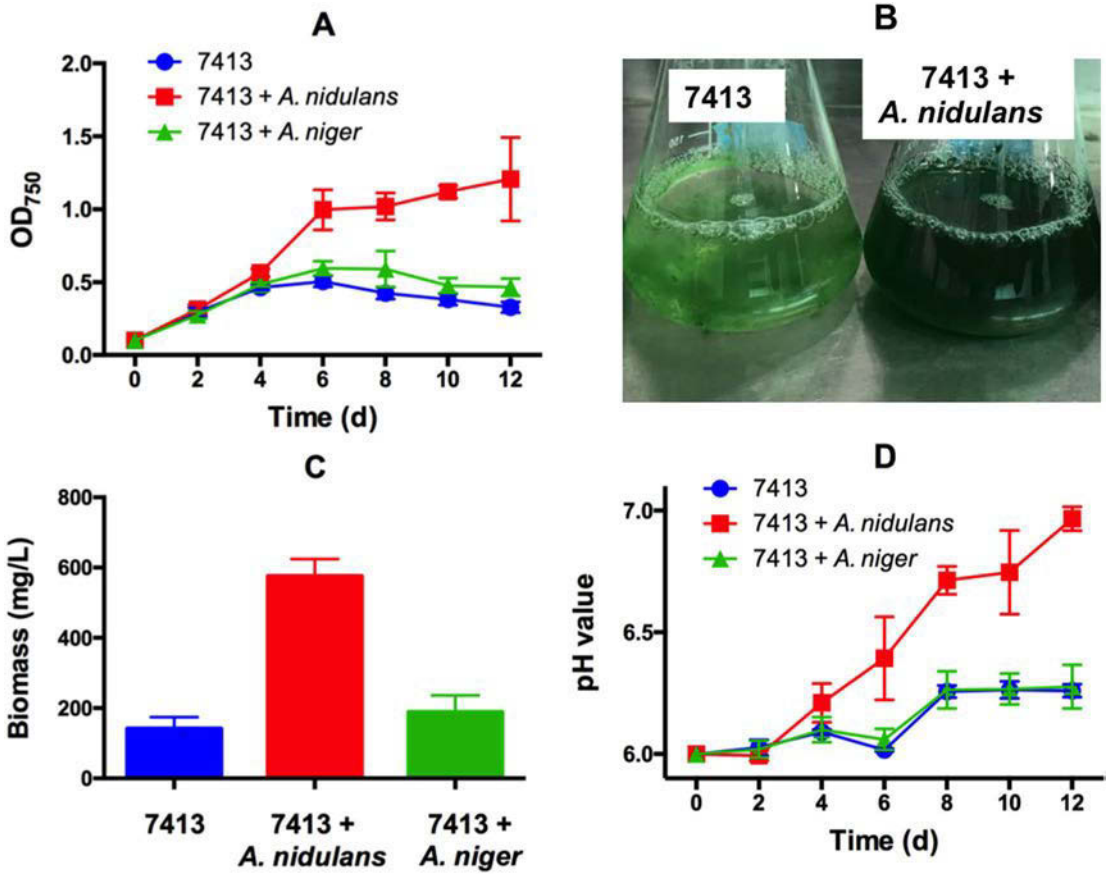

Fig. 5. Mono-culture of PCC 7413 and co-culture of PCC 7413 with fungi in BG-11$_{[co]}$ with buffered pH at 6. **A**) Growth curves over time up to 12 days ; **B**) Images of mono-culture of PCC 7413 and co-culture of PCC 7413-*A. nidulans* in culture flasks; **C**) Dry weight of final biomass; All data are averages of biological triplicates ± standard deviation;**D**) pH value of the cultures with time.

started to decline after 8 days. Indeed, the DW of final biomass obtained from the co-culture of PCC 7413-*A. nidulans*, was approximately 3-fold higher than the PCC 7413-*A. niger* co-cultures or PCC 7413 alone (Fig. 5C). Both the mono-culture of PCC 7413 and co-cultures of PCC 7413 with the two *Aspergillus* strains served to neutralize the culture environment, with co-culture of PCC 7413-*A. nidulans* gradually increasing the broth pH from 6.0 to 7.0, and the mono-culture of PCC 7413 and co-culture of PCC 7413-*A. niger* increasing the pH less extensively to 6.2 (Fig. 5D). Interestingly, fungal hyphae and spores in the flocculants formed in co-cultures were both observed under the fluorescence microscope, similar to those observed previously (Fig. 4E, Additional file 2); however, appreciable flocculants amounts were not formed upon inspection of the final culture. Clearly, the existence of fungi, especially *A. nidulans*, greatly facilitated the growth of *Nostoc* in a pH buffered and more acidic environment.

3.6. Lipid analysis

As noted above, estimating the fraction of biomass represented by fungal cells in co-culture can be challenging. Cellular fatty acid profiles have been utilized to rapidly characterize and differentiate fungal isolates in soil [28], and as biomarkers to analyze numbers of freshwater microalgae [29]. Therefore, we also performed fatty acid profile analysis in order to examine for the lipid contributions of the fungus and cyanobacterium to the co-culture and to determine the capacity of the co-cultures to produce biofuels precursors. The levels of different lipids were evaluated individually for PCC 7413 and the two *Aspergilli* strains and also in the co-cultures following growth in pH 6.0 buffered media.

A fatty acid profile analysis revealed that the principal fatty acids produced in the mono-culture of cyanobacterium PCC 7413 was distinctly different compared with mono-culture of fungi. Most fatty acids present in PCC 7413 were palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3),

Table 1
Fatty acid profiles of mono-cultures of *Nostoc* PCC 7413, *A. nidulans* and *A. niger*, and co-culture of *Nostoc* with fungi.

	7413	*A. nidulans*	*A. niger*	7413 + *A. nidulans*	7413 + *A. niger*
$C_{16:0}$	35.0 ± 1.4	17.8 ± 1.6	23.0 ± 2.0	26.6 ± 1.1	35.1 ± 3.0
$C_{16:1}$	12.9 ± 0.2	N/D	N/D	20.3 ± 0.6	14.3 ± 0.9
$C_{18:0}$	12.7 ± 0.8	7.7 ± 1.7	N/D	2.8 ± 0.6	6.7 ± 1.1
$C_{18:1}$	12.0 ± 0.9	22.8 ± 0.0	31.9 ± 0.5	11.4 ± 0.6	10.0 ± 0.9
$C_{18:2}$	15.7 ± 0.5	51.7 ± 3.3	45.1 ± 1.9	24.1 ± 0.5	16.8 ± 1.5
$C_{18:3}$	11.7 ± 1.0	N/D	N/D	14.9 ± 0.5	17.1 ± 2.4

All data are averages of biological triplicates ± standard deviation.
N/D: not detected.

however, C16:1 and C18:3 were not detected in either *A. nidulans* and *A. niger* cultures, and C18:0 was not observed in *A. niger* culture (Table 1). Furthermore, saturated fatty acids consisting of C16:0 structures represented the most abundant fraction among all fatty acids components in PCC 7413 at 35%; on the contrary, unsaturated fatty acids of the C18:1 and C18:2 type predominated in fungi, representing over 70% of the total fatty acid level. Thus, cyanobacterium PCC 7413 and fungi *A. nidulans* and *A. niger* contain disparate lipid compositional fractions.

Not surprisingly, the major fatty acid profile of the co-culture of 7413 and *Aspergillus* displayed components that were a combination of the PCC 7413 and fungal species, validating the accumulation of both cell types in the co-culture. However, the fractions varied in almost all cases from the axenic strains.

The highest fatty acid fractions in the co-culture of PCC 7413 and *A. nidulans*, C16:0 and C18:2, were the same as mono-culture of PCC 7413, but at greatly adjusted fractions. In both cases, their levels were intermediate between those of PCC 7413 and the fungal species reflecting the intermediate nature of a co-culture's composition. Alternatively, the fractions observed in the PCC 7413-*A. niger* co-cultures were more similar to the PCC 7413 fractions, perhaps due to the limited accumulation of fungal participants in these cultures. Interestingly, the next most abundant species, C16:1 at 20%, in the PCC 7413-*A. nidulans* co-culture was not detected at all in the *A. nidulans* mono-cultures and presented at only 13% in the PCC 7413 mono-culture. The same fatty acid fraction is also elevated in the PCC 7413-*A. niger* co-culture as well, but its fraction is not as abundant as detected in the PCC 7413-*A.nidulans* co-culture. Similarly, the next most abundant fraction in the co-cultures was C18:3, present at 15% and 17% in the PCC 7413-*A. nidulans* and PCC 7413-*A. niger* co-cultures, respectively. Once again, this fraction was not detected in either fungal mono-culture and presented at only 11.7% in the PCC 7413 mono-cultures. Consistent with the elevated levels of C16:1 and C18:3 in the co-cultures was the detection of low levels of the saturated C18:0 structures especially in the PCC 7413-*A. nidulans*. For the PCC 7413-*A. nidulans* co-culture, the C18:0 saturated structure fraction at approximately 3% was well below the levels observed in mono-cultures of either contributing species. Its level was more substantial at 6.7% in the PCC 7413-*A. niger* co-cultures albeit still reduced relative to the PCC 7413 mono-cultures. The remaining component, C18:1 was also lower, although slightly, than its observed value in the PCC 7413 and much lower than in *A. nidulans* and *A. niger*.

These findings are all consistent with a shift in the co-cultures from C18:0 and C18:1 structures to ones with higher levels of unsaturation at C16:1 and C18:3, which indicates metabolic interactions between *Nostoc* and *Aspergillus* species that may lead to the enhancement of pathways facilitating the desaturation of fatty acids into less saturated forms such as palmitoleic acid and linolenic acid.

4. Conclusions

In the present study, we evaluated the cultivation of filamentous fungi *Aspergillus* together with polysaccharide-secreting cyanobacterium *Nostoc* PCC 7413. The fungal supernatants supplied in culture medium could elevate cyanobacterial growth, while the cyanobacterial supernatants could support the biomass accumulation in axenic culture of fungi. When *Aspergillus* and *Nostoc* co-cultured together without pH buffered, *Nostoc* could grow well while *Aspergillus* showed subtle growth, present in the flocculants in culture suspension. In pH buffered *A. nidulans-Nostoc* co-culture at pH 6, the growth of *Nostoc* was dramatically boosted compared with its poor growth performance in axenic culture. Therefore, fungi present in the co-culture significantly facilitated the growth of *Nostoc* in a less favorable acidic environment. Meanwhile, contents of two major fatty acid components of C16:0 and C18:2 exhibited a combination of the PCC 7413 and *A. nidulans* attributes, validating the accumulation of both types of cells in the co-culture. In addition, a shift of fatty acids content in the co-culture from C18:0 and C18:1 to C16:1 and C18:3 also occurred, indicating metabolic interactions between *Nostoc* and *Aspergillus* species may occur and lead to adjustment of pathways benefiting fatty acid desaturation. Characterizing the metabolic interactions and the capacity of this synthetic lichen as a bioproduction platform represent a robust opportunity for future study of the intimate relationships of co-cultures of autotrophs and heterotrophs with potential for commercial applications.

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Authors' contribution

TL participated in the study design, data collection and analysis, and drafted the manuscript. LJ performed the fatty acid analysis and the manuscript revision. YH assisted the laboratory work and the manuscript revision; JTP contributed to cultivation of fungi strains; CZ and KZ contributed to experimental design and discussion of experimental results; MJB conceived the experimental design, participated and supervised manuscript preparation and editing. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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Statement of informed consent, human/animal rights.

No conflicts, informed consent, human or animal rights applicable.

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