Growth, yield, quality and microbial diversity in hydroponic vertical farming – effect of phycocyanin-rich Spirulina extract

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Abstract

Vertical farming (VF) is a potential solution for the production of high-quality, accessible, and climate-friendly nutrition for growing urban populations. However, to realize VF's potential as a sustainable food source, innovative technologies are required to ensure that VF can be industrialized on a massive scale and extended beyond leafy greens and fruits into the production of food staples or row crops. A major obstacle to the economic and environmental sustainability of VF is the lighting energy consumed. While technological advances have improved the energy efficiency of VF lighting systems, there has been insufficient research into biostimulation as an approach to reduce energy needs. We conducted a controlled trial to investigate the application of a phycocyanin-rich Spirulina extract (PRSE) as a biostimulant in hydroponically grown, vertically farmed lettuce (*Lactuca sativa* and *Salanova®*). PRSE application reduced the time from seeding to harvest by 6 days, increased yield by 12.5%, and improved quality including color, taste, texture, antioxidant flavonoid levels and shelf life. Metagenomic analysis of the microbial community of the nutrient solution indicated that PRSE increased the overall bacterial diversity including raising the abundance of Actinobacteria and Firmicutes and reducing the abundance of potentially pathogenic bacteria. This preliminary study demonstrates that microalgae-derived biostimulants may play an important role in improving the economic and environmental sustainability of VF.

Keywords

Vertical farming, hydroponics, biostimulant, microalgae, Spirulina, phycocyanin, lettuce, metagenomics

Introduction

Climate change, food security challenges and environmental degradation due to large-scale outdoor industrial farming, make it vital to explore moving food production closer to large, urban populations (Benke & Tomkins, 2017). There has been a surge of public, government, and investor interest in controlled environment agriculture (CEA) (Petrovics & Giezen, 2021) and multi-layer plant production, generally known as vertical farming (VF) (Despommier, 2009) (Kozai, 2018). Vertical farms can be operated using high levels of automation including phenotype-driven, artificial intelligence (AI)-based management tools for production inputs, including lighting, environmental conditions, and nutrient delivery (Jung et al., 2021).

VF has rapidly transitioned from a promising food production concept into an accepted technology for providing fresh leafy greens to our cities (Petrovics & Giezen, 2021). To date, leading VF companies have raised billions of dollars (De Oliveira et al., 2021). VF offers a promising primary food production option (Despommier, 2009) reducing the need for valuable farmland and decreasing the use of synthetic agrochemicals such as pesticides and fertilizers (Benke & Tomkins, 2017). However, the economic viability of VF remains debatable due to high capital expenditure and energy costs, and it is still unclear as to whether VF is indeed a truly economically and environmentally sustainable solution as the prime source of vegetables for large cities (Goodman & Minner, 2019).

To date, VF optimization research has overwhelmingly focused on photobiology including improving plant physiological parameters with lighting technology, photomorphogenesis (light-induced plant development), and photosynthesis (Sharath Kumar et al., 2020). Current growing systems using soilless technologies include aeroponic and hydroponic systems (Lee & Lee, 2015) and aim to ensure optimal nutrient access and careful cultivar selection, that both increase the likelihood of reaching close to optimal production levels in the presence of appropriate lighting conditions. Much of the focus on increasing the efficiency of VF relates to incremental improvements in lighting to ensure lower energy consumption and appropriate plant light exposure (Nicole et al., 2016).

Further challenges in VF include enhancing post-harvest quality (shelf-life, color, flavor, and organoleptic properties), and increasing the density of primary nutrients and phytochemicals that have nutraceutical (antioxidant and anti-inflammatory) properties (Prakash et al., 2012) (Moreno-Escamilla et al., 2020). Phytochemicals include phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, and polyphenols such flavonoids, isoflavonoids, and anthocyanins (Prakash et al., 2012). Dietary intake of phytochemicals has been shown to have health benefits, with claims of protection against chronic disorders including cancer, and cardiovascular and neurodegenerative diseases (Zhang et al., 2015).

Conventional agriculture relies on agrochemicals including synthetic fertilizers, plant-protection chemicals or pesticides, and plant-growth hormones for efficient and economical food production to address the growing food demand (Mandal et al., 2020). However, agrochemical overuse comes with a negative impact on the environment, and on human health. To alleviate the problems associated with synthetic agrochemicals and reduce their application, attention has recently turned to ecologically benign solutions, including plant biostimulants (Chiaiese et al., 2018) (Yakhin et al., 2017). Although the term biostimulant is poorly defined (Yakhin et al., 2017), biostimulants can be broadly described as non-nutrient based, formulated biological products and bioactive compounds applied in low doses to enhance crop performance, increase resistance to stress, and optimize nutrient utilization efficiency (Yakhin et al., 2017) (Du Jardin, 2015). Their mode of action on plant metabolism can be directly on the plant or through the stimulation of the plant microbiome at the rhizosphere, phyllosphere, and endosphere (Compant et al., 2019). Bioactive molecules including phytohormones, transport regulators, signaling molecules, and modulators of stomatal opening, are responsible for direct biostimulation (Yakhin et al., 2017).

Current examples of biostimulants include live or viable microbial mixtures or non-viable biological amendments including humic substances and seaweed extracts (Hamza & Suggars, 2001) (Rouphael & Colla, 2020) (Frioni et al., 2018). The application of viable microbial biostimulants in hydro- and aeroponic systems has shown some promise (Lee & Lee, 2015), but scale-up leads to problems including nozzle blockages and general contamination (Dong et al., 2020). Humic substances have been shown to promote nutrient uptake and plant growth, but they are currently derived from non-renewable resources like coal and peat, therefore up-scaled applications require the development of new sustainable sources (Canellas et al., 2015). The application of seaweed (macroalgae) in agriculture goes back to ancient times and seaweed is particularly rich in phytohormones, complex organic compounds, vitamins, simple and complex sugars, enzymes, proteins, and amino acids (Craigie, 2011). However, large scale agriculture, seaweed extract application may be unsustainable due to the adverse impact of cultivation on the local marine environments (Campbell et al., 2019). Extracts from eukaryotic microalgae (including prokaryotic cyanobacteria) have been highlighted as high potential agricultural inputs (Alvarez et al., 2021) and microalgae are increasingly viewed as a renewable biological resource as part of a bio-refinery paradigm to foster the "bioeconomy of the future" (Orejuela-Escobar et al., 2021). Microalgae extracts have biostimulant properties, improving germination, growth, photosynthetic activity, and yield and acting at the level of the phyllosphere and rhizosphere of the plant microbiome (Chiaiese et al., 2018). Barone et. al. demonstrated that the application of microalgal extracts of *Chlorella vulgaris* and *Scenedesmus quadricauda* upregulated genetic pathways associated with increased growth and yield in hydroponic sugar beet (Barone et al., 2018) and for tomato plant cultivation (Barone et al., 2019).

Arthrospira platensis (Spirulina), a blue-green cyanobacterium is widely cultivated and used for nutraceuticals and food ingredients (Belay, 2013). It is a rich source of micronutrients and phytohormones (gibberellins, auxins, and cytokinins) and other functional biomolecules, such as phenolics, and polysaccharides (Finamore et al., 2017). Spirulina filtrates and homogenates have also been shown to improve growth and nutritional quality in radish plants following seed soaking (Godlewska et al., 2019) and to mitigate the harmful effects of the herbicides on *Vicia faba* (broad bean plant) (Osman et al., 2016).

This study is the first controlled trial to explore the utility of a phycocyanin-rich Spirulina extract (PRSE) for biostimulation in VF. Phycocyanin is a water-soluble phycobiliprotein extracted from Spirulina and is generally used as an FDA-approved blue food colorant and nutraceutical (Fernández-Rojas et al., 2014). Phycocyanin has antioxidant activity (Pleonsil et al., 2013) (Fernández-Rojas et al., 2014) and may also have soil bioremediation properties and potential as an agricultural input (Decesaro et al., 2017) (Castro et al., 2013).

Biostimulation properties of PRSE were tested in hydroponic systems, using two common lettuce species (*Lactuca sativa*, *Salanova®*). The primary focus of this work was to explore and quantify the impact of PRSE on plant growth velocity and yield. In addition, this study also examined the effect of PRSE on photosynthetic efficiency and lettuce quality (nutritional, and organoleptic properties, and shelf life). We also analyzed flavonoid antioxidant (quercetin and luteolin) levels, reported to be abundant in soil-farmed red butterhead, red leaf, and red romaine lettuces (Di Gioia et al., 2020), and undertook scouting metagenomic analysis of microbial community dynamics in the nutrient medium to explore the hypothesis that PRSE may enhance the hydroponic microbiome.

Methods

1.1 PRSE Extraction and Characterization

PRSE was produced using a proprietary aqueous, solvent-free extraction method (Lerer, 2020) from commercially available organic Spirulina powder (BlueTec, Inner Mongolia, China). The protein structure of PRSE was characterized and compared with a C-phycocyanin reference (Sigma-Aldrich, St. Louis, MO, USA) and Spirulina powder (Nutrex, Hawaii, USA) by Capillary Electrophoresis - Sodium Dodecyl Sulfate (CE-SDS) with a LabChip GXII analyzer (Caliper Life Sciences, Waltham, MA, USA). Before analysis, pulverized extracts were homogenized using a TissueLyser II (Qiagen, Hilden, Germany) in tissue lysis buffer (BioRad Laboratories, Hercules, CA, USA) followed by acetone precipitation. The protein precipitates were resuspended in 0.5 M triethylammonium bicarbonate (Sigma-Aldrich, St. Louis, MO, USA), 1 M urea, and 0.1% SDS (Sigma-Aldrich, St. Louis, MO, USA).

1.2 Hydroponic Lettuce Cultivation and Treatment With PRSE

All experiments were conducted indoors in a monitored and controlled environment. The grow rooms were sanitized with a 5% sodium hypochlorite (Sigma-Aldrich, St. Louis, MO, USA) solution. Sampling devices were autoclaved (HiClave Sterilizer, Hirayama Manufacturing Corporation, Japan) before use. Lettuce plant models selected were Salanova® Red Sweet Crisp for growth and quality studies and *Lactuca Sativa* (Kuting, USA) for microbiome analysis of the hydroponic nutrient medium. Seeds were propagated within Rockwool soaked in a deionized water solution containing Liquid Grow 7-9-5 (Dyna-Gro®, USA). A dynamic, 100 L tray-reservoir, shallow water culture hydroponic system holding 50 plants was used. Two independent systems consisting of PRSE treatment and non-treatment (control) groups, filled with deionized water, were constructed to ensure similar light exposure (photoperiod of 16 hours at 18000 lux) and environmental conditions. All plants were nourished with a standard hydroponic 8-15-36 FloraGro® (General Hydroponics, USA) NPK nutrient solution (600-700 mg/L with regular adjustment to a pH of 6) with PRSE applied weekly to maintain a 250 mg/L concentration within the nutrient solution. This concentration was measured using a UV-Vis Spectrophotometer (PerkinElmer®, Waltham MA, USA)on a weekly basis (Bennett & Bogorad, 1973).

1.3 Growth, Phenotype Analysis Quality Analysis

The growth period was assessed as the time from planting to harvest. Harvesting was undertaken when two blinded, experienced hydroponic growers reached the consensus that the plants were at the most optimal stage of growth and marketable. Total biomass (lettuce suitable for packaging), leaf length and basal stem width of 10 randomly selected plants were measured at the end of the trial period.

Chlorophyll fluorescence is a widely used measuring technique in plant physiological studies (Schreiber, 1998). Fluorescence emission measurements were performed just before harvest on 10 leaves from 10 randomly selected plants from respective groups using a FluorPen FP100max fluorometer (Photon System Instruments, Brno, Czech Republic). The fluorometer automatically calculates various geometric parameters of Kautsky curves using the OJIP protocol (Pantazi et al., 2013). The measured data were analyzed by FluorCam software 7.0 to determine the maximum quantum yield (QY_{MAX}) and performance index (PI_{ABS}). The QY_{MAX} is defined as the maximum quantum efficiency (F_v/F_m) of PSII photochemistry and is a sensitive indicator of plant photosynthetic performance, and lower values may also indicate stress (Maxwell & Johnson, 2000). PI_{ABS} is a multi-parametric parameter that combines the three main functional steps taking place in PSII (light energy absorption, excitation energy trapping, and conversion of excitation energy to electron transport) and is used to compare primary photochemical reactions (Strauss et al., 2006).

At the end of the trial, 10 plants were randomly selected from both control and treatment groups and 10 leaves were randomly selected from each group to assess CIELAB (expressed as three values: *L** for the lightness from black (0) to white (100), *a** from green (−) to red (+), and *b** from blue (−) to yellow (+) (Post & Schlautman, 2020). CIELAB measurement was performed with a Nix™ Pro color sensor (Nix Sensor Ltd., Hamilton, Ontario, Canada).

At harvest, six random samples were sourced from the treatment and control groups and packed in supermarket-style, transparent clamshells and stored at 16°C at 70% humidity in a controlled environment chamber. Daily blinded assessment was undertaken for wilting and color loss. In addition, blinded organoleptic testing was also conducted by an independent standards laboratory) (Intertek, New Orleans, LA, USA) of 6 harvested samples from the control and treatment groups assessing aroma, taste, and texture (Csajbokne & Gilingerne, 2011).

1.4 Flavonoid Analysis

Flavonoid analysis was conducted using a Flexar HPLC system (Perkin Elmer, Waltham, MA, USA) coupled with an Expression compact mass spectrometer (CMS) (Advion, Ithaca, NY, USA) using a standard method (Seal, 2016) (Wang et al., 2014). After harvesting, fresh leaves were homogenized in deionized-H2O and the solution was microfiltered for analysis. Separation was done on a Brownlee SPP C18 column (2.7 µm x 150 mm x 3.0 mm) (Perkin Elmer, Waltham, MA, USA) with a mobile phase flow rate of 0.2 mL/min. The first mobile phase was 10% methanol, 85.5% water, and 4.5% formic acid (v/v), and the second, was 80% methanol, 19% water, and 1% formic acid (v/v). The sample injection volume was 20 µL, and the separation was run at 25°C. The flavonoids investigated in this study were quercetin and luteolin with standards obtained from Sigma Aldrich (St. Louis, MO, USA).

1.5 Statistical Analysis

Treatment and control group phenotype were compared using a standard t-test with a significance level of 0.05.

1.6 Nutrient Solution Sampling of Microbial Biomass

Samples of growth media (60 mL) were taken from *Lactuca Sativa* control and treatment groups using 100 mL sterile syringes and collected in sterile Erlenmeyer flasks. The first sample was taken after 3 days to allow enough time for the PRSE to equilibrate within the system and another sample was taken at the end of the growth cycle.

1.7 DNA Extraction and Sequencing

Samples were passed through a 0.22 μm syringe filter (Millipore Corp., Bedford, MA, USA), and DNA was extracted from the solid residue on the filter using a Metagenom SOX Fluid Filtration and DNA Isolation Kit (Metagenom, Waterloo, ON, Canada) according to the manufacturer's instructions. The DNA samples were then stored at -80˚C and subsequently shipped on dry ice to Metagenom (Waterloo, ON, Canada) for targeted metagenomic library preparation and sequencing.

Results

1.8 PRSE Analysis

Figure 1 displays the LabChip molecular weight profiles of the phycocyanin laboratory reference standard (C-phycocyanin), Spirulina powder and PRSE (BYEXP1). All three showed the presence of bands 18-20 kDa molecular mass, indicating the characteristic *α-β* subunit assembly of phycocyanin (Chaiklahan et al., 2011) (Patel et al., 2006). The PRSE and phycocyanin laboratory reference specimens had fewer higher molecular weight proteins than the natural Spirulina powder specimen, indicating a higher level of protein purity. PRSE contained several lower molecular weight proteins that were absent in the phycocyanin laboratory reference, representing differences in the extraction and purification processes and the presence of additional low molecular weight (<16 kDa) bioactive molecules.

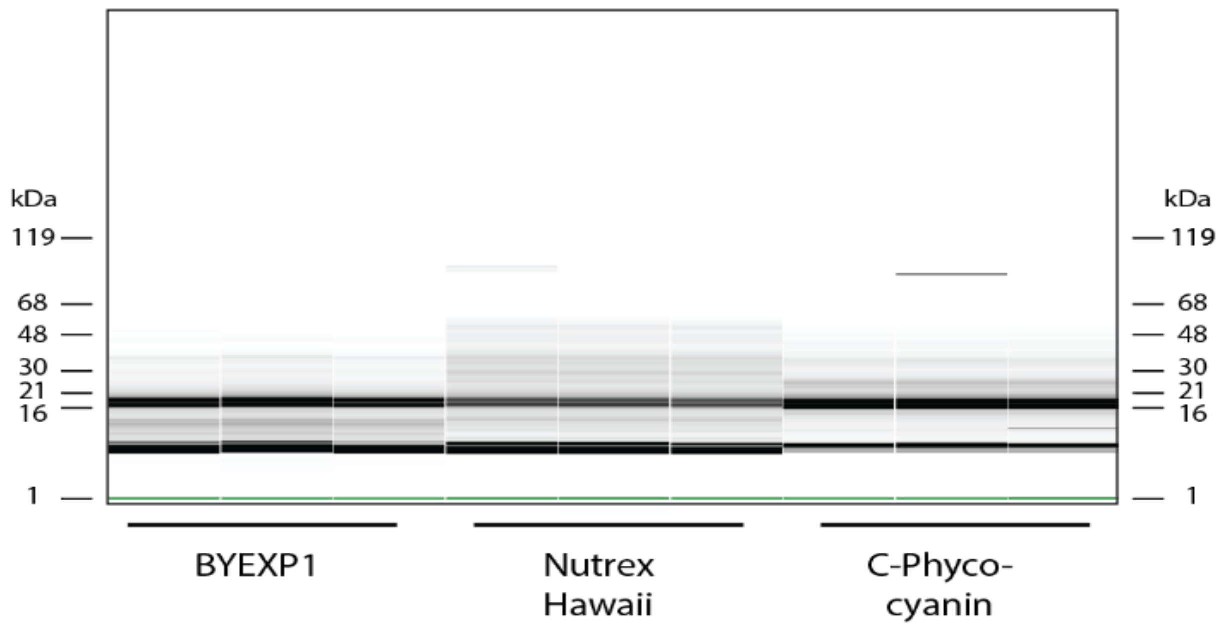

***Figure 1**: LabChip molecular weight profiles of phycocyanin laboratory reference (C-Phycocyanin), Spirulina powder (Nutrex Hawaii) and PRSE (BYEXP1*).

1.9 Growth, Phenotype, Yield, Photometry, and Photosynthesis

The PRSE-treated Salanova® group reached maturity and was harvested at 22 days, which was 6 days before the harvest of the untreated group. In comparison with the untreated lettuce, the treatment group showed an increase of 2.6 cm and 2.2 cm for leaf length and basal stem diameter respectively. The accelerated growth of PRSE-treated lettuce was accompanied by a 12.5% increase in yield. The PRSE-treated lettuce was also 17% brighter (L*) and 75% greener (a*) than the control group with a 65% improvement in QY_{MAX} and a 22% improvement in PI_{ABS}.

Table 1: *Mean leaf length and stem diameter at harvest (22 days for treatment and 28 days for the control group), values are presented as mean ± SD (n = 10).*

	Treatment	Control	P-value
Leaf Length (cm)	12.8 ±1.2	10.2 ± 1.5	<0.05
Basal stem diameter (cm)	6.5 ± 1.3	4.3 ± 0.75	<0.05

Table 2: *CIELAB, QY_{MAX}, and PI_{ABS} at harvest (22 days for treated and 28 days for the control group), values are presented as mean ± SD (n = 10).*

	Treatment Mean (SD)	Control	P value
CIELAB (L*), (a*), (b*)	(42 ± 3.9), (-3 ± 1.1), (22 ± 2.7)	(35 ± 2.6), (-12 ± 3.4), (2 ± .9)	<0.05
QY_{MAX}	6.5 ± 1.9	2.3 ± 0.9	<0.05
PI_{ABS}	1.6 ± 0.7	1.4 ± 0.5	<0.05

1.10 Quality and Flavonoid Analysis

Twelve clamshells of treated (harvested at 22 days) and control (harvested at 28 days) group lettuce were tested for shelf life. Wilting and loss of color were seen 2-3 days earlier in the untreated group. Three samples from the PRSE-treated and untreated groups were tested for the flavonoids and showed a mean increase of 30% in quercetin and an 8% increase in luteolin. Blinded visual inspection and organoleptic evaluation indicated that post-harvest, PRSE-treated lettuce had better texture, stronger aroma, more intense flavor, and better mouthfeel than the untreated group.

1.11 Metagenomic DNA Sequencing of the Nutrient Medium

Metagenomic DNA sequencing of the PRSE treatment and control group nutrient solutions was conducted on samples taken at 3 days and at the end of the trial (35 days). For the control group, 36,681 (3 days) and 34,116 (35 days) operational taxonomic units (OTU) were identified representing a 0.017% increase. For the PRSE treatment group, 36,942 OTU (3 days) and 14,035 OTU (35 days) were identified representing a 62% reduction of the bacterial population. To quantify changes in microbial diversity, the Richness (S), Shannon Diversity Index (H´) and Shannon Evenness Index (E´) were used to determine the class level of bacterial taxonomy (Hill et al., 2003) (Table 3). An increase of H´ and E´ was found in the PRSE treatment group as compared to a decrease in these parameters for the control group (Table 3).

Table 3: *Changes in S, H´ and E´ of the bacterial population in hydroponics nutrient medium for control and PRSE treatment group.*

Taxonomy level	Parameters	Control		Treatment	
		3 days	35 days	3 days	35 days
Class	S	7	9	7	11
	H´	0.55	0.17	0.55	1.61
	E´	0.28	0.08	0.28	0.67

Figure 2 illustrates the phylum level taxonomic abundance of bacteria (excluding Proteobacteria). Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant phyla in both groups. An abundance of Firmicutes (7%) was observed in the PRSE treatment group after 35 days. Similar microbial community composition has been reported for lettuce growth in aquaponic systems and soil (Kasozi et al., 2021) (Schreiter et al., 2014) and for cucumber growth in ebb-and-flow systems (Dong et al., 2020). The dominant classes identified in the Proteobacteria phylum included Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria and this is comparable with previous findings (Janssen, 2006) (Spain et al., 2009). A 40% decrease in the abundance of Gammaproteobacteria was found in the PRSE treated group and both groups displayed a decrease in the abundance of Bacteroidetes over time. It is also noteworthy that in the control group, there was also a 9% decrease in Alphaproteobacteria while in the treatment group there was a 38% increase in Alphaproteobacteria.

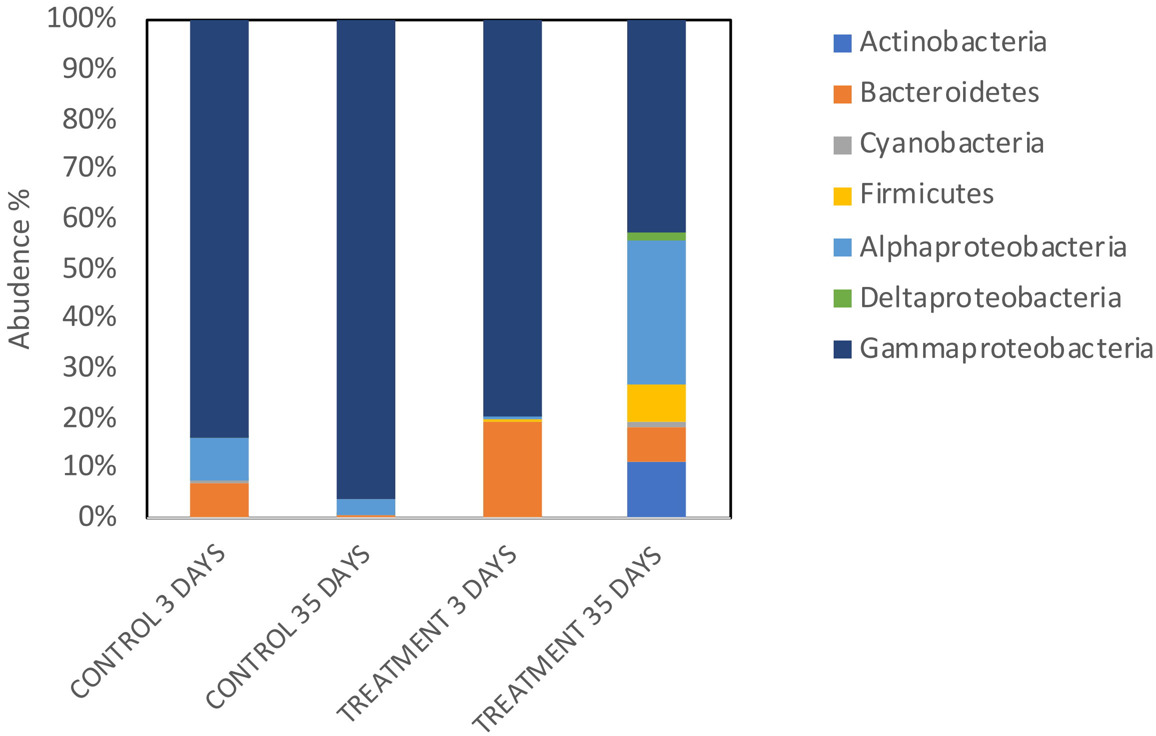

***Figure 2**: Abundance of bacteria phyla. Values reported represent the most abundant phyla.*

Discussion

This scoping, controlled trial yielded initial evidence that PRSE has a biostimulant effect, improving growth, yield, and quality in hydroponically cultivated lettuce. The PRSE-treated lettuce was more vigorous and reached maturity 21% (6 days) more rapidly than the untreated group. Shortening the time between planting and harvest reduces energy requirements and labor costs in VF. While current large-scale VF operations operate with an optimized growing environment, a shortened growing time (even less than 24 hours) may have important implications for profitability. A critical consideration remains energy consumption, where, in most geographies, outdoor farming may be a more economically (and possibly environmentally) sustainable food production option as compared to VF. Given that outdoor-farmed leafy greens are 3-5 times less expensive to grow than similar vertically farmed crops (Tasgal, 2019), reduced growing periods may have important implications for the economic viability of VF. While PRSE did improve yield, the economic impact of this finding on VF may be secondary to improved growth velocity, as VF yield is substantially influenced by grower skill, lighting, and environmental conditions.

The availability of biostimulants such as PRSE may assist in extending VF into the production of food staples such as wheat, corn, and rice. While excellent yields can be obtained in experimental, indoor wheat vertical farms, there is an urgent need to reduce energy consumption (Asseng et. al. 2020). Improved color, vigor, organoleptic and nutritional properties, and the longer preservation of the PRSE-treated lettuce may play a vital role in ensuring better selling prices, thereby also improving the economics of VF. As flavonoids are an important group of polyphenol antioxidants, increased levels in the PRSE-treated group may be helpful in ensuring that indoor cultivated lettuce offers similar or better nutritional quality when compared to outdoor-grown lettuce (Kim et al., 2016).

There is still considerable uncertainty as to the mechanism of action of natural biostimulants on plant growth, yield, and nutritional quality (Francesca et al., 2020). Microbial diversity is believed to be vital for plant and human health (Mahnert et al., 2018) and the increase in the diversity and evenness of microbial communities in the PRSE treatment group supports the contention that biostimulants have a positive impact on plant growth and performance through microbiome effects (Mahnert et al., 2018). A decrease within the abundance of Gammaproteobacteria and an increase in abundance of Actinobacteria and Firmicutes in the

PRSE treatment group is also noteworthy. Gammaproteobacteria includes several important pathogens such as *Salmonella*, *Yersinia*, *Vibrio,* and *Pseudomonas aeruginosa* (Erlacher et al., 2014). Alphaproteobacteria are reported to be an important and abundant class of Proteobacteria found within the rhizosphere of lettuce (Kröber et al., 2014) and Actinobacteria and Firmicutes are important plant growth-promoting bacteria (PGPB) (Strap, 2011)(Hamedi & Mohammadipanah, 2015) (Yadav et al., 2017) (Lee et al., 2021).

A key concern and challenge in soilless VF are the rapid dispersion, colonization, and domination of pathogenic microorganisms in the recirculating nutrient medium (Dong et al., 2020). To avoid the spread of pathogenic bacteria in hydroponic irrigation systems, commercial greenhouse growers routinely use disinfection methods such as ozone and UV-radiation (Lee & Lee, 2015). Such strategies have two key drawbacks. First, they require relatively high capital investment (Lee & Lee, 2015), and secondly these methods eliminate non-pathogenic and PGPB from within the nutrient solution which constitutes the plant-medium microbiome. Our preliminary metagenomic analysis indicates that biostimulants such as PRSE could be applied as a pre-biotic or post-biotic (Żółkiewicz et al., 2020) or symbiotic (Chandel et al., 2017) to improve (increase, diversify and stabilize) PGPB and reduce pathogens within the recirculating nutrient irrigation system. This is a possible route for reducing dependency on physicochemical disinfection in VF.

The clear effect of PRSE on the photosynthetic parameters and its activity at extremely low doses may also support the hypothesis that there is some cellular level activity, through the glycolate pathway (Eisenhut et al., 2008) and it is also possible that phycobiliproteins play some role linked to a core photosynthetic process, fluorescence resonant energy transfer (Matamala et al., 2007). Some support for this hypothesis can be derived from the finding that the addition of functional cyanobacterial components into plant chloroplasts improves photosynthetic efficiency including through ribulose bisphosphate carboxylase-oxygenase (RuBisCO) suppression (South et al., 2019) (Price et al., 2013). Furthermore, PRSE proteins have emulsifying properties similar to the biosurfactants produced by many rhizosphere and plant-associated microbes (Decesaro et al., 2017) (Sachdev & Cameotra, 2013). These biomolecules have been implicated in motility, signaling, and biofilm formation at the plant-microbe interface (Sachdev & Cameotra, 2013).

We are undertaking further studies to validate several observations, such as the substantial biomass increase and the shift in microbial richness, evenness, and diversity. Additional research is also required to fully elucidate the molecular mechanism of action of PRSE especially pertaining to its role in improving crop nutritional quality (Kim et al., 2016). It is also important to consider whether the growth velocity, yield, and quality benefits derived from using biostimulants such as PRSE justify their price, given that PRSE constitutes less than 15% of the algae biomass and that extraction and purification steps are required. Further analysis is also required, especially in vertical farms that are operating at near-optimal photosynthetic and nutritional efficiency, where the small, incremental increases in growth velocity and yield may be small. However, improved nutritional quality and shelf life may be of growing importance to large-scale growers, especially as the market for VF-grown leafy greens becomes more competitive.

Conclusions

The long-term economic, environmental, and social impact of VF will largely be determined by its economic sustainability (Goodman & Minner, 2019). This preliminary study showed that the application of PRSE enhanced growth velocity, yield, and quality in hydroponically grown lettuce. Metagenomic analysis of the nutrient medium also indicated that PRSE influences the microbial community, increasing its diversity, promoting PGPB such as Actinobacteria and Firmicutes, and reducing potentially pathogenic gammaproteobacteria.

While further research is required, the results indicate that PRSE may be an important and innovative production input contributing to the economic sustainability of VF. Besides showing the potential of PRSE to reduce growing time thereby saving energy, this study provides initial evidence that PRSE improves product quality (appearance, nutritional density, shelf life, and organoleptic properties). The availability of effective biostimulants will support deploying VF to enhance food security in areas with limited farmland and this could include the cultivation of food staples such as wheat and corn. Finally, this study of the application of PRSE in VF also provides some early support for the broader consideration of the role of combinations of microorganism extracts including bacteria, mycelia, and mycorrhizae as biostimulants in VF.

Declaration of interests

LL, JV, and CK are employees of Back of the Yards Algae Sciences LLC.

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