Supplemental Session Abstracts

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VICC 2021
International Carotenoid Society
June 22, 23, 24, 25

VICC 2021
Supplemental Session

Hosting a Conference with attendees from across the Globe in time zones differing by as much as 16 hours presents a whole new set of problems. The issues of travel delays disappear only to be replaced by the complexities of internet and software reliability.

In our planning we envisioned that a few of our presenters would have the fates align to foil their well-planned talks and we decided to add a Supplemental Session to make certain that it would be possible for each talk to be presented. In the Supplemental Session we also have invited a small number of talks from presenters who missed our initial deadlines but whose contributions we felt would add to the overall quality of the Conference.

In the pages that follow you will find the abstracts of the presenters for the Supplemental Program. In part, these represent a redundancy with the Abstract Book but knowing that working from two sources to look up and read abstracts would complicate matters for those logging in for the Session, all Abstracts for the speakers included in the Supplemental Session are included here.

The Supplemental Session will begin immediately after the conclusion of Session XB. We will give everyone a 4 minute break and then move directly into the talks. The Summary and Concluding Remarks will move to follow the Supplementary Session Discussion.

VICC 2021
International Carotenoid Society
June 22, 23, 24, 25

11: 16:00 AM EST Questions/Discussion Session X
Neal Craft & Andre Duesterloh

11: 31:00 AM EST Break (4min)

11:35 am EST **Supplemental Session**

Moderators:

Professor John Landrum
Professor Emeritus, Florida International University, Miami, FL USA

Professor Elizabeth Johnson
Adjunct Professor, Friedman School of Nutrition and Science & Policy, Tufts University
Boston, Mass., USA

11: 35: 00 AM EST Supplement Session Begins

(30 sec intro)

1S. CAROTENOID PRODUCTION BY MIXED PURPLE PHOTOTROPHIC BACTERIA

Abstract 1S

Presenter: MARÍA GRASSINO (8 min)

11: 43: 30 AM EST

(30 sec intro)

2S. DISCOVERY OF A NEW SIPHONAXANTHIN BIOSYNTHETIC PRECURSOR: STRUCTURE AND ITS COUPLING TO PHOTOSYNTHETIC ANTENNA

Abstract 2S

Presenter: SOICHIRO SEKI (8 min)

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11: 52: 00 AM EST

(30 sec intro)

3S. PLASMA CAROTENOID CONCENTRATIONS ARE INFLUENCED BY CHEMOTHERAPY TREATMENT IN BREAST CANCER PATIENTS*

Abstract 3S (Identical to Abtract 8)

Presenter: RACHEL KOPEC (8 min)
**this presentation has been moved into the supplemental due to a power interruption during Session IB*

12: 00: 30 AM EST

(30 sec intro)

4S. A PLANT LIPOCALIN IS REQUIRED FOR RETINALDEHYDE-MEDIATED ROOT GROWTH

Abstract 4S

Presenter: ALEXANDRA J. DICKINSON

12: 09: 00 AM EST **Questions/Discussion (15 min)**
Moderators: Prof. Elizabeth Johnson and Prof. John Landrum

12: 24: 00 AM EST **VICC 2021 Summary and Concluding Remarks**

Abstract 1S

Carotenoid production by mixed purple phototrophic bacteria

María Grassino[1*], Ken Yong[2], Tim Hülsen[1] and Damien J. Batstone[1]

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1. Introduction

The agry-industry, specifically animal feeds, is a prime market for immune-nutritional additives such as carotenoids. Due to their anti-oxidant and disease-preventing properties and their colouring capacity the world demand is increasing, particularly for naturally sourced products. In this context, purple phototrophic bacteria (PPB) are an interesting source for natural immune-nutritional pigments. PPB have recently been proposed as mediator for resource recovery [1] and as an alternative protein source e.g. to substitute fishmeal [2], see **Figure 1.** The PPB biomass is generally characterised by very high protein contents as well as an arsenal of carotenoids and bacteriochlorophylls (BChls), which are synthesised to harvest photons to perform anoxygenic photosynthesis [3]. PPB have the potential to produce significant amounts of carotenoids and can be grown on cost free substrates in very effective reactor set- ups, which promises low overall production costs. However, the carotenoid contents reported in literature have mainly been expressed in the form of total carotenoids, which gives an incomplete picture of the actual capacities. In addition, the identification of the range of carotenoids is rudimentary. In this study, we developed a method to characterise and quantify the individual carotenoids produced by mixed cultures of PPB.

Figure 1. PPB photobioreactor (left), PPB formulated into fish after harvesting biomass (centre) and barramundi as potential target species for feed (right).

2. Experimental

In order to characterize and quantify the carotenoids generated by PPB, we have optimized an extraction method suitable for this type of biomass. The detection and quantification were carried out using an ultrahigh-pressure liquid chromatograph coupled to an ultra violet detector and a high resolution APCI mass spectrometer (UHPLC-UV-HRMS).

3. Results and Discussion

As shown in **Figure 2**, preliminary results confirm that mixed culture of PPB are able to produce lycopene, a carotenoid with relevant commercial interest. 3 *mgLycopene gVS^{-1}* and 50 *mgBchl a gVS^{-1}* were observed from our investigation.

In addition, the proportion of lycopene isomers present in the bacterial samples were also different to the isomers composition generated by the analytical standard and tomato sources.

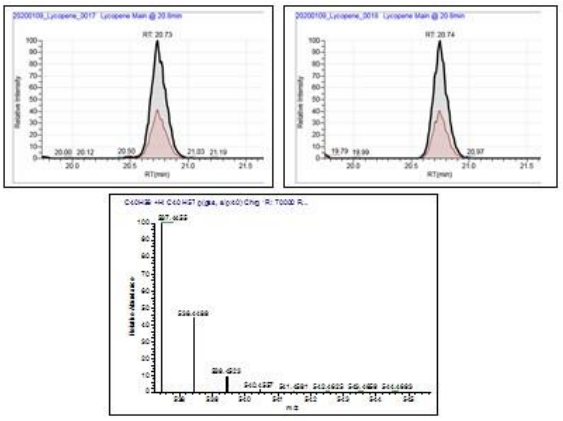

Figure 2. Comparison of the detected lycopene (Retention time: 20.7 min) in a lycopene standard (top left) and a PPB sample (top right). Isotropic profile of lycopene (M+H) ($C_{40}H_{56}$) (bottom).

This method has been used to study how different conditions such as light source, light intensity or growth stage affect the PPB carotenoid composition allowing better control over the biotechnological production.

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[1] T. Hülsen, D. J. Batstone and J. Keller, *Water Res.*, 2014, **50**, 18–26.

[2] J. Delamare-Deboutteville, D. J. Batstone, M. Kawasaki, S. Stegman, M. Salini, S. Tabrett, R. Smullen, A. C. Barnes and T. Hülsen, *Water Res.* X, 2019, **4**, 100031.

[3] M. T. Madigan and D. O. Jung, in T*he Purple Phototrophic Bacteria*, 2009.

Abstract 2S

DISCOVERY OF A NEW SIPHONAXANTHIN BIOSYNTHETIC PRECURSOR: STRUCTURE AND ITS COUPLING TO PHOTOSYNTHETIC ANTENNA

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Introduction: Siphonaxanthin (S) is the major light-harvesting pigment in Siphonous green algae to absorb green light under the sea [1]. In those algae, like xanthophyll cycle, irradiance-dependent interconversion of S and lutein (L) accumulation has been observed in naturally occurring condition [2]. However, the L-S conversion must include both carbonylation at C8 and hydroxylation at C9. Therefore, three questions arise: Is this exchange controlled only by light? Also, does L-S conversion occur directly without a precursor? And are both interconversion products bound to the photosynthetic antenna? In this work, we accurately tracked the pigment composition of *Codium fragile* in various light conditions that mimic the natural environment. Finally, the distribution of pigments in photosynthetic proteins under light conditions where the conversion occurs was investigated.

Materials & Methods: *C. fragile* (KU-065, KU-MACC, Kobe, Japan) was cultivated in laboratory. A home-made black box system with solid color LEDs (CCS Inc., Kyoto, Japan) was used to control the irradiance to each cultivation bottle. Pigment composition was determined using a binary HPLC system equipped with a photodiode-array detector. Total synthesis was performed for 19-deoxysiphonaxanthin (dS) to determine the unidentified peak. Pigment-protein complexes were isolated by sucrose density gradient followed by solubilization of thylakoid membranes. Action spectrum was measured by irradiating the culture bottles with monochromatic light of 10 wavelengths (fwhm = 8 nm) provided by the Okazaki Large Spectrograph facility (Aichi, Japan).

Results & Discussions: The S-to-L conversion was certainly observed only under intense blue light, but the conversion from S to unidentified carotenoid (UID) was also observed to the same extent. The structure of the unidentified carotenoid was determined to be dS by comparing the optical spectra including ^{1}H-NMR with those of synthesized dS. Both L and dS were strongly associated with the major photosynthetic antenna, LHCII complex. A strong correlation between the occurrence of dS and the decrease of S in the action spectrum indicates the presence of an S-to-dS interconversion. In contrast, the action spectra of L and S were both increased, and the correlation was unclear. The plausible molecular mechanism of the pigment conversion in the siphonous LHCII will be discussed. A modified biosynthetic pathway of algal carotenoids were proposed.

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PLASMA CAROTENOID CONCENTRATIONS ARE INFLUENCED BY CHEMOTHERAPY TREATMENT IN BREAST CANCER PATIENTS

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**Corresponding Author*

Introduction: Chemotherapy upregulates inflammatory processes, as measured by circulating concentrations of pro-inflammatory cytokines and their signaling lipids.[1] Previous studies in breast cancer survivors have reported an inverse correlation between dietary and total plasma carotenoid concentration and oxidative stress (as determined via urinary 8-hydroxy-2′-deoxyguanosine concentrations),[2] and a reduced risk of breast cancer reoccurrence.[3] However it is not known whether breast cancer treatment itself influences plasma carotenoid concentrations. Objective was to measure the change in circulating concentrations of carotenoids and fat-soluble vitamins in free-living breast cancer patients both before and after treatment.

Research & Methods: Serum samples were collected from patients (n=34) immediately prior to standard adjuvant and neo-adjuvant chemotherapy for breast cancer, and 4 months following chemotherapy commencement. Patient multivitamin and non-steroidal anti-inflammatory (NSAID) drug use was noted at both visits. Lipophilic extracts were analyzed using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) to quantify α- and β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin, retinol, α-tocopherol and phylloquinone. Linear mixed models were developed to assess the relationship between the main factors (i.e. chemotherapy, multivitamin, and NSAID use), and their interaction effects, on serum carotenoid and fat-soluble vitamin concentrations. Random effects included a fixed intercept for each subject.

Results and Discussion: Chemotherapy was significantly associated with reduced serum concentrations of α-carotene ($P = 0.053$) and retinol ($P = 0.042$), with a trend observed for reduced β-carotene ($P = 0.076$) and phylloquinone ($P = 0.082$). There was no main effect of multivitamin or NSAID use on any analytes investigated. An interaction effect was observed for chemotherapy*multivitamin use, with increased concentrations of serum retinol ($P = 0.004$) and lycopene ($P = 0.004$), and a trend observed for zeaxanthin ($P = 0.087$) for those who took multivitamins. Chemotherapy*NSAID use was also significantly associated with a trend in increased serum lutein ($P = 0.061$) for those who consumed NSAIDS. Our results suggest randomized, controlled trials of multivitamin use and/or provitamin A carotenoid-rich food consumption merit further investigation in patients undergoing chemotherapy treatment.

Acknowledgements
This research was supported by The Ohio State University Stefanie Spielman Breast Cancer Center Kroger Fund, a Pelotonia Fellowship awarded to D.B., NIH R01CA189947, and sample analyses partially supported by NIH P30 CA016058, OSU, and OSUCCC.

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[2] Thompson et al., *Epidemiology, Biomarkers, & Prevention*, **25(3)**, 94-102 (2007)
[3] Rock et al., *Journal of Clinical Oncology*, **23**, 6631-6638 (2005)

Abstract ПS

A PLANT LIPOCALIN IS REQUIRED FOR RETINALDEHYDE-MEDIATED ROOT GROWTH

ALEXANDRA J. DICKINSON[1, 2, 3,5,6 *], Jingyuan Zhang[1], Michael Luciano[4], Guy Wachsmann[1,5], Evan Sandoval-Bautista[6], Rupak Timilsina[6], Martin Schnermann[4], José R. Dinneny[2, 3], Philip N. Benfey[1, 5, *].

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Introduction: Branching of root systems to form lateral roots is essential for exploration and colonization of soil. In Arabidopsis roots, *de novo* organogenesis of lateral roots is patterned by an oscillatory mechanism called the root clock, which is dependent on unknown metabolites derived from the β-carotene pathway[1]. Retinoids are β-carotene-derived regulators of organogenesis in the animal kingdom. Furthermore, there are retinoid binding proteins (opsins) present in evolutionarily divergent lifeforms, including algae. Therefore, we hypothesized that retinoids might play a role in lateral root organogenesis.

Research and Methods: To determine if retinoids function in plant development, we measured apocarotenals in Arabidopsis plants treated with and without an inhibitor of carotenoid metabolism. Additionally, we treated plants with retinal and monitored its effect on lateral root organogenesis using *pDR5:LUC*, a root clock reporter. Furthermore, we conducted time-lapse imaging of a chemical reporter for retinaldehyde binding proteins.

Results and Discussion: We found that retinaldehyde binding precedes the root clock and accurately predicts sites of lateral root organogenesis. Exogenous application of retinaldehyde is sufficient to increase root clock oscillations and promote lateral root organogenesis. We identified TEMPERATURE INDUCED LIPOCALIN (TIL), an Arabidopsis homologue to vertebrate retinoid binding proteins. Genetic analysis indicates that TIL is necessary for normal lateral root development and a *til* mutant has decreased sensitivity to retinaldehyde. TIL expression in a heterologous system conferred retinaldehyde binding activity, suggesting a direct protein-ligand interaction. Together, these results demonstrate an essential role for retinal and for plant retinaldehyde binding proteins in lateral root organogenesis.

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