# Characterization of Pigments consisting of Melanin and Carotenoids from a *Planococcus* Species

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## ABSTRACT

*Planococcus* sp. CAU13 produces a dark pigment, the identity of which is the focus of this study. Initial extraction methods utilized known procedures for carotenoid extraction, as *Planococcus* members are known to produce yellow to orange pigmented carotenoids. Extractions were followed by melanin isolation techniques, as the carotenoid methods yielded a yellow, but not the expected dark color. Ultraviolet–visible (UV-Vis) spectroscopy and infrared (IR) spectroscopy were used to characterize the extractions. Analysis found that both carotenoids and melanin are likely present. Genetic analysis supports the presence of both carotenoids and melanin. Portions of a known C-30 carotenoid biosynthetic gene cluster (BGC) are present in CAU13. which produces a type III polyketide synthase (PKSIII); this is a class of enzyme known to produce 1,3,6,8-tetrahydroxynaphthalene (T4HN), the first product in the biosynthetic pathway for DHN-type allomelanin. Multiple experiments verify the hypothesis that a mixture of a carotenoid and melanin are produced by the bacterium *Planococcus* sp. CAU13.

**Keywords**: *Planococcus*, Melanin, Carotenoid, Pigment, Extraction, CAU13, Polyketide Synthase

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## INTRODUCTION

*Planococcus* sp. CAU13 was first isolated and cultured from a soil sample extracted from the desert sands of Hashemite Kingdom of Jordan. Isolation revealed spherical smooth colonies presenting a dark pigment morphology after 10-20 days of growth (Fig 1). The bacterium's genome was sequenced and it was determined to be a member of the *Planococcus* genus (1). Other members in the genus are known to produce orange and yellow pigmentation due to the presence of carotenoids (2; 3). Carotenoids are a class of biomolecules produced by fungi, microorganisms, and plants (4). These biomolecules serve as pigments and give rise to orange, yellow, and red colors due to their absorbance in the visible spectrum via their conjugated system of single and double bonds, created by their origin as tetraterpene derivatives (5). Carotenoids can be divided into two types, xanthophylls and carotenes. Xanthophylls contain oxygen and are typically the type of carotenoids that *Planococcus* members produce, while carotenes are pure hydrocarbons. Microorganisms can produce a variety of carotenoids, for example *Arthrobacter* sp. NamB2 produces a pink pigment, the heterothallic zygomycetous fungus, *Blakeslea trispora* produces $\beta$-carotene, a yeast *Phaffia rhodozyma* (also known as *Xanthophyllomyces dendrorhous*) produces $\beta$-carotene and astaxanthin, bacteria such as *Croceibacterium* produces zeaxanthin, *Micrococcus luteus* produces yellow pigment (5)

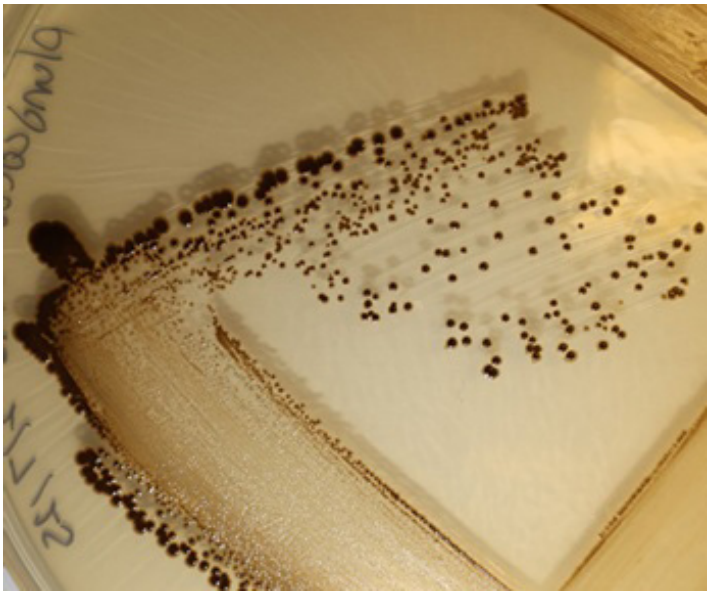
Carotenoids primarily have two functions, absorbing light energy to aid in photosynthesis, and providing photoprotection while acting as an antioxidant (3). Due to the seemingly conserved nature of carotenoids throughout the genus, methods for carotenoid extraction were utilized to determine whether they play a role in the pigmentation of the focus of this study *Planococcus* sp. CAU13. Previous unpublished studies from this laboratory have shown the possible presence of carotenoids, but have not yet elucidated the identity of the black pigment or investigated the genetic origin of carotenoid production.

While no other member of the genus is known to exhibit black pigmentation one member, *Planococcus maritimus*, produces 1,8-dihydroxynapthalene (DHN) which is a precursor to allomelanin (5). Melanin is another biomolecule which is ubiquitous in all forms of life responsible for a brown to black range of pigmentation. Melanin's primary function is for protection against environmental factors including DNA damage from UV radiation, high temperatures, and chemical stressors like oxidation. Allomelanin, the type that *P. maritimus* is hypothesized to produce, is most common in fungi (6) but is present in other bacteria (7; 8; 9; 10). Production of allomelanin through the DHN pathway involves the use of a class of enzyme called a type III polyketide synthase (PKSIII). PKSIII catalyzes the formation of (1,3,6,8)-tetrahydroxynapthalene (T4HN) utilizing malonyl-CoA as a substrate, which is followed by enzymatic reduction and dehydration steps to form 1,8-dihydroxynaphthalene (1,8-DHN) (7). PKSIII enzymes exist as homodimer proteins which produce a wide variety of products (11). A PKSIII involved in bacterial T4HN production as a melanin precursor has been described in both its structure and its active site which allows for the comparison to possible homologous enzymes in CAU13 (11).

The goal of this study is to isolate and characterize the black pigment and determine if *Planococcus* sp. CAU13 produces either or both melanin and carotenoids. As previous work suggested the presence of carotenoids, initial methods for preliminary extraction included established procedures for carotenoid extraction. This was followed by a procedure for melanin extraction, as carotenoid methods did not yield the desired black pigmentation. Both methods were followed by IR and UV-Vis spectroscopy to characterize the extract, and genetic analysis was performed to understand the pathways involved.

## FIGURE 1

Planococcus *sp. CAU13 grown on marine agar plate with 5% NaCl.*



Colonies exhibited dark brown glossy color.

## MATERIALS & METHODS

### CULTURE GROWTH

*Planococcus* sp. CAU13 was streaked onto plates of Zobell Marine Broth 2216 supplemented with 5% NaCl and 7.5% agar. Cultures were grown in the dark at room temperature (23 ℃) for two weeks until the dark pigment appeared. The dark pigmented colonies were scraped into 15 mL conical screw cap tubes for extraction of the pigment.

### CAROTENOID BASED EXTRACTION

Preliminary extraction utilizing carotenoid methods dissolved a collected plate of CAU13 in 2.0 mL of a 7:3 mixture of acetone: methanol containing 0.1% BHT as an antioxidant (3). The mixture was incubated at 50 °C for 50 minutes and sonicated for 15 seconds before being placed in a centrifuge at 11,000 xg rcf for 5 minutes and the supernatant was collected. Diethyl ether was used as replacement solvent in a secondary extraction following the same procedure to adjust for nonpolar factors and ease of residue collection.

### MELANIN BASED EXTRACTION

Preliminary extraction utilizing melanin methods (3; 13) followed the same method as carotenoid extraction (5; 12). A collected plate of CAU13 was washed with 2 mL of hexane then the remaining residue was dissolved in 2 mL of 2M NaOH (12). The mixture was incubated at 50°C for 50 minutes and sonicated for 15 seconds before being placed in a centrifuge at 11,000 xg for 5 minutes and the supernatant was collected.

### IR-SPECTROSCOPY

Carotenoid-based diethyl ether extracted supernatant was air dried in fume hood and the solid residue was collected. This residue was scanned directly on a JASCO FT/IR-4X1typeA IR spectrometer from 4000-500 $cm^{-1}$. Melanin extract was precipitated using 2M HCl dried and collected. This extract was scanned directly on a JASCO FT/IR-4X1typeA IR spectrometer from 4000-500 $cm^{-1}$.

### UV-VIS SPECTROSCOPY

The supernatant of the melanin and carotenoid extracts were scanned on a Perkin Elmer Lambda 35 UV-Vis spectrometer from 300-800 nm; melanin-based extract required a 40x dilution with 7:3 v/v acetone:methanol from the original concentration prior to analysis.

### PROTEOGENOMIC ANALYSIS

The online genomic database collection website BioCyc (14) was used for investigating the genome of CAU13 to identify hypothetical polyketide synthase. T-Coffee "Expresso" alignment from T-Coffee (15) was used for BLAST analysis comparing specific polyketide synthase in CAU13 with other organisms.

## RESULTS

### CAROTENOID EXTRACTION

Carotenoid extraction method that used 7:3 acetone:-methanol yielded a yellow-orange supernatant, Figure 2. UV-Vis full spectrum scan yielded three distinct peaks at 440.5, 462.6, and 484.1 nm, Figure 3. IR spectra exhibited bands at 3300 $cm^{-1}$, 1535 $cm^{-1}$, 1400 $cm^{-1}$, 1340 $cm^{-1}$ Figure 4.

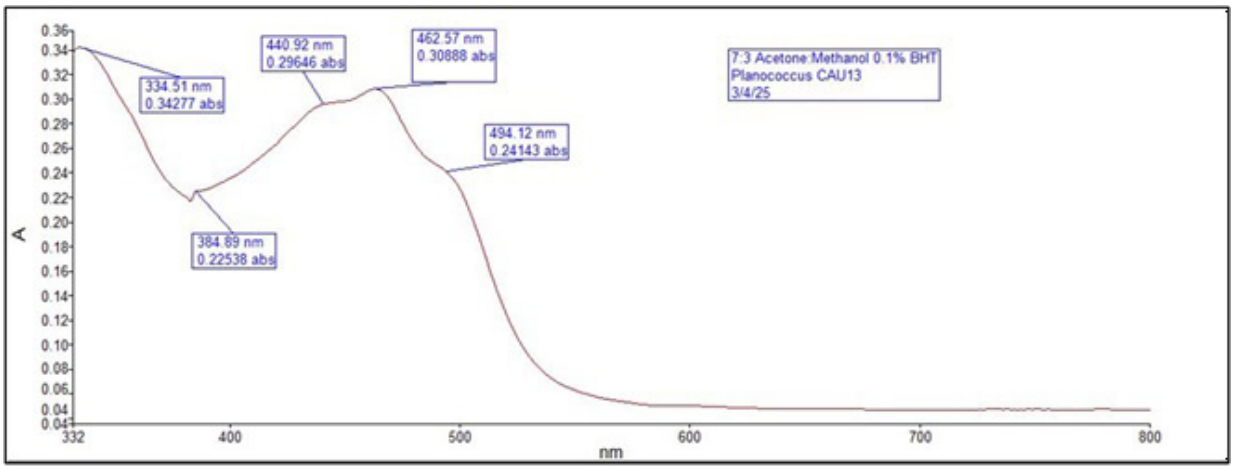
## FIGURE 2

*Carotenoid extraction with 7:3 v/v acetone:methanol.*

Color of the extract appears yellow to light brown.

## FIGURE 3

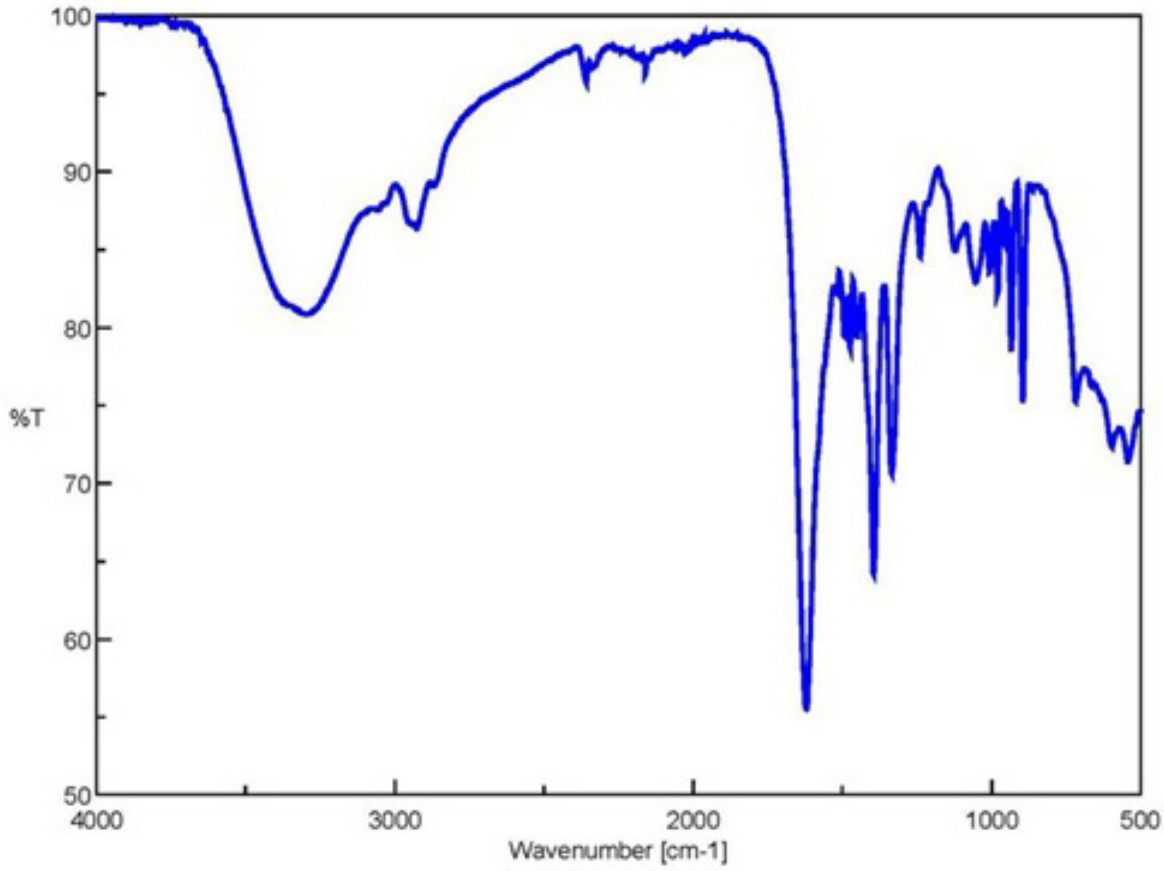
*UV-Vis spectrum of the carotenoid extraction with 7:3 v/v acetone:methanol.*




Prominent spectral features are 334 nm peak, 385 nm valley, 463 nm peak with 441 nm and 494 shoulders.

## FIGURE 4

*IR spectrum of the carotenoid extraction with 7:3 v/v acetone:methanol.*




Prominent spectral bands are broad absorbance between 3300 and 3500 $cm^{-1}$, and sharp bands at 1535 $cm^{-1}$, 1400 $cm^{-1}$, and 1340 $cm^{-1}$.

## MELANIN EXTRACTION

Melanin extraction methods using 2 M NaOH yielded a dark brown supernatant liquid, Figure 5. UV-Vis analysis of the liquid showed peaks and maximum at 223.31 nm and 244.88 nm, Figure 6. IR spectral analysis showed broad absorbance at 3300-3500 $cm^{-1}$, sharp bands at 1627 $cm^{-1}$, 1540 $cm^{-1}$, and 1051 $cm^{-1}$, Figure 7.

## FIGURE 5

*Melanin extraction using 2 M NaOH.*

Color of the extract appears brown.

## FIGURE 6

*UV-Vis spectrum of melanin extraction using 2 M NaOH.*

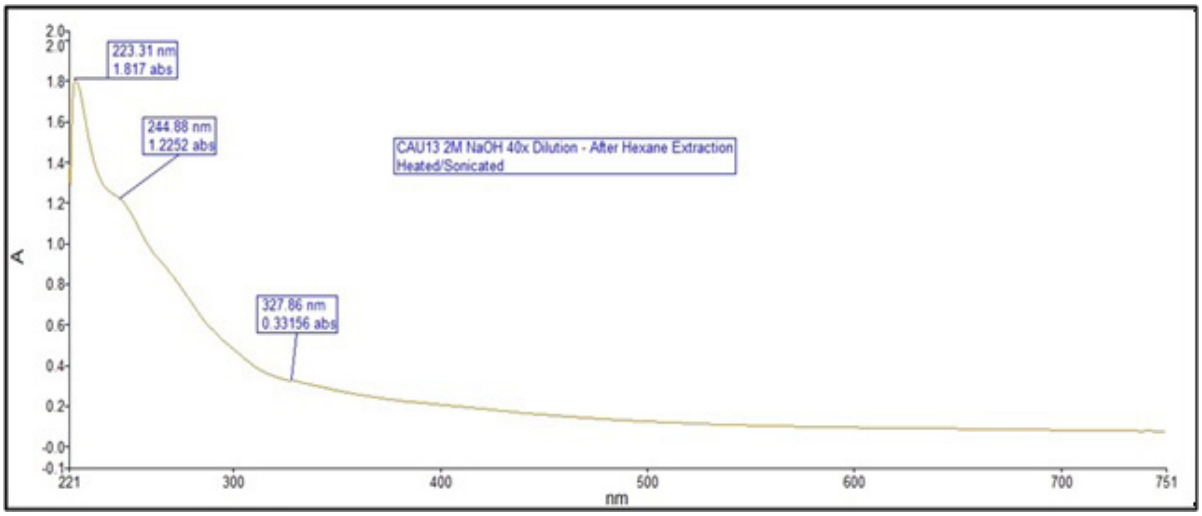
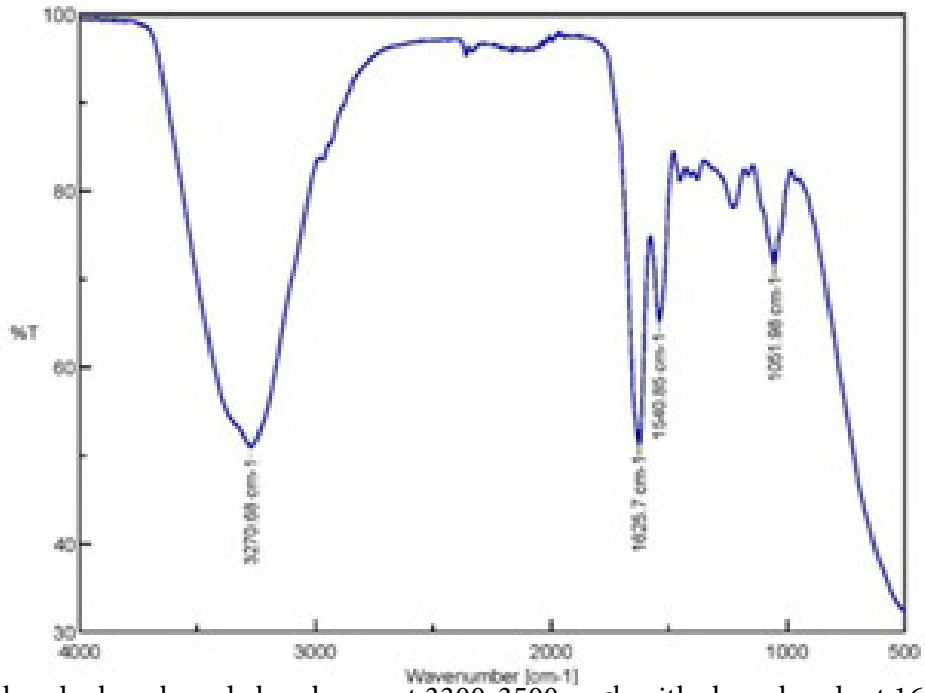



Figure 6: UV-Vis spectrum of melanin extraction using 2 M NaOH.
Prominent spectral features peaks at 223 nm with shoulders at 245 and 327 nm.

## FIGURE 7

*IR spectrum of melanin extraction using 2 M NaOH.*




Prominent spectral bands show broad absorbance at 3300-3500 $cm^{-1}$, with sharp bands at 1627 $cm^{-1}$, 1540 $cm^{-1}$, and 1051 $cm^{-1}$,

## DNA AND PROTEIN SEQUENCE ANALYSIS

### FIGURE 8

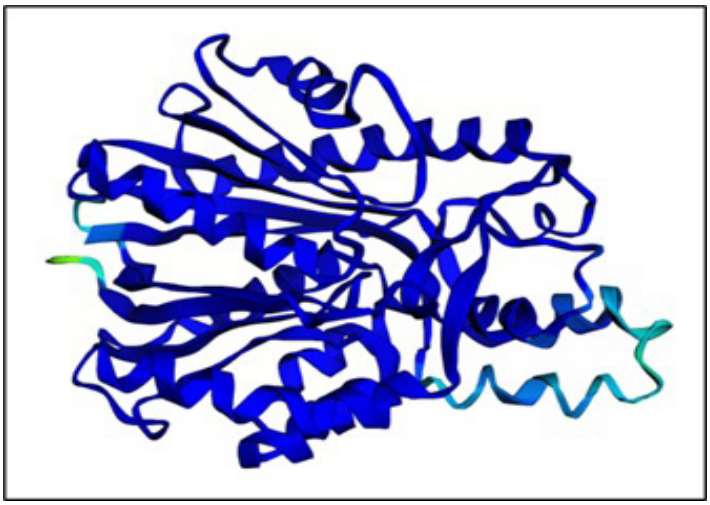
*Multiple sequence alignment using T-COFFEE (13).*

```
T-COFFEE, Version_11.00 (Version_11.00)
Cedric Notredame
SCORE=92
*
 BAD AVG GOOD
*
AUX21439.1        :  91
WP_033543849.1    :  93
WP_152152970.1    :  92
WP_394120825.1    :  93
WP_088007427.1    :  92
cons              :   9

AUX21439.1        MSTGHHEMPFAPMIAAVGTALPPHYATQEETCAGLRRLWAGKGDALALVERVQRSIEVRGRHLAMP
WP_033543849.1    M----------SIIRKVVTENAPYHVDQKEIVQVVRNLFGDHFSDIERLLRVFGNGQIEGRYFAAP
WP_152152970.1    M----------PFIRSVVTENPPYRFDQQDIVEIVRNLFGGYYDDIERLLRVFGNGQIENRHFAAP
WP_394120825.1    M----------SMIRKVVTENAPYYVDQKEIVSVVRNLFGGHYTDIERLLKVFGNGQIEGRYFAAP
WP_088007427.1    M----------PYIRNVATANAPYKVDQQQTVEVVRNLFGSQFADIERLLKVFDNGEIESRYFAAP

cons              *          . *  * *  .*:   *::   :*.*:..    :  : :*  . :::.*::* *

AUX21439.1        VEAYHAASTFAEQNRRWNACALELGERACRAALERAGLLPEDIDQIVFVSVTGISAPSLDARLVNR
WP_033543849.1    LEWFEKERGLEEKNRLYIEEAVRMGSRAVERCLEESGTAKEEVDAFIFVSSSGMATPTIDARIMNN
WP_152152970.1    LSWFEKEHGLEEKNQKYIEEAVRLGSRAVANCLKKADVNKEEIDAFVFVSSSGMATPTIDVRIMNE
WP_394120825.1    LVWFEEERGLEEKNRLYVEEAVKMGSLAVSRCLEEAQVDKEDVDAFIFVSSSGMSTPTIDARIMNV
WP_088007427.1    LDWFEQERGLEEKNGRYVDQAVEMGSDAVMRSLAKARLSPDDIDAFIFVSSSGMATPTIDARIMNK

cons              :  :.   : *:*  :   *:.:*. *   .* .:    :::* ::*** :*:::*::*.*::*

AUX21439.1        LGFRRDVKRTPIFGLGCVAGVAGVARVTDTLRAFPAHTSLLLSVELCSLTLQRDDTSAANVIAAQL
WP_033543849.1    LKMPAHIKRLPIWGLGCAGGAAGLSRAHDYCRAYPDAKVLVLCLELCSLTFQRSDLTKSNLIGTSL
WP_152152970.1    LSLPSHIKRMPLWGLGCAGGAAGISRAHDYCLAYPNAKVLVLCIELCSLTFQRSDTSKSNLIGTSL
WP_394120825.1    LQMPQHVKRIPLWGLGCAGGASGISRADDYCRAYPEAVVVVLCLELCSLTFQRSDTSKSNLIGTSL
WP_088007427.1    LAFPPHVKRIPLWGLGCAGGAAGISRAHDFCLAYPEATVLVLCIELCSLTFQRQDLSKSNLIGTSL

cons              * :  .:** *::****..*.:*::*. *   *:*    ::*.:******:**.* : :*:*.:.*

AUX21439.1        FADGAAAVVARGAQAARKSGRAGGAAVLATHAVFYPDTEHIMGWDVVDSGFRVVLSAGVPALVREH
WP_033543849.1    FADGSACALVTGERAA---GDDAGFFIHDTQSTLMPESEDVMGWDVKDEGLHVVFSRDIPKIIESW
WP_152152970.1    FADGAACALVTGEEGQ---TAGAGFFTKDTQSTLMSNSEDVMGWDVKDEGLHVIFSRDIPNIVESW
WP_394120825.1    FADGAACALVTGDSVR---LPGEGFHIKETQSTLMRDSENVMGWDVKDEGLHVVFSRDIPKIIEKW
WP_088007427.1    FADGAACALVSGDRTA---GAD-GFFIRDTQSTLMPESEEVMGWDVKDGGLHVVFSRDIPSIIENW

cons              ****:*..::. *      *    *::.:  ::*.:***** * *::*::* .:* ::..

AUX21439.1        VRRDVDAFLARHGLSRRDVRHWIVHTGGPKVLEAVAETLELPGDALDRSWRSLREIGNVSSASVLF
WP_033543849.1    LKPNLDQFLEKLDRKPEDIEHFIAHPGGKKVLSAYEKSLSIGTGKTENPRNVLRDFGNMSSPTVLY
WP_152152970.1    LKPNVDLFLDKLGKKSSEIDHFIAHPGGKKVLEAYEKSLGFSAEKTAVSRKVLASHGNMSSPTVLY
WP_394120825.1    LKPNVDLFLGGISRTSADITHFIAHPGGKKVLAAYEKSLGLATGKTAISRAVLAKYGNMSSPTVLY
WP_088007427.1    LKPNVDGFLDSIGKTGADIDHFIAHPGGKKVLAAYEKALDISREKTAVSREVLTRFGNMSSPTVLY

cons              :: ::* **   . .  :: *:*.*.** *** *  ::* :       .   *   **:**.:**:

AUX21439.1        VLRDFLDE-PCARPGDHGVMMAFGPGFCSEMALLRW------
WP_033543849.1    VLKEFMEQQPNA--GEQGLLTALGPGFSSEMLWLEWR-EKSG
WP_152152970.1    VLKEIIENQPAA--REQGLLTALGPGFSSEMLWLEWG-EM-E
WP_394120825.1    VLKDFMEKKPRQ--GEEGLLTALGPGFSSEMLWLEWG-ESVL
WP_088007427.1    VLKEFMEQEPAD--GTNGLLTALGPGFSSELVWLEWEGLM-E

cons              **:::::: *    .*:: *:****.**:  *.*
```

NCBI reference sequence numbers represent the protein sequence for the respective organism. AUX21439.1 chalcone synthase *Sorangium cellulosum*, WP_033543849.1 type III polyketide synthase *Planococcus* sp. CAU13, WP_152152970.1 type III polyketide synthase *Bacillus aerolatus*, WP_394120825.1 type III polyketide synthase *Planococcus donghaensis*, WP_088007427.1 type III polyketide synthase *Indiicoccus explosivorum*.

### FIGURE 9

*ColabFold v1.5.5: AlphaFold2 using MMseqs2 Structure Prediction of CAU13 PKSIII production from KW93 (14).*



## DISCUSSION

### CAROTENOID

The carotenoid extraction method using 7:3 acetone:-methanol yielded a yellow-orange supernatant (Figure 2) UV-Vis of which (Figure 3) provided results consistent with carotenoids, three distinct peaks are present at 440.52, 462.57, and 484.12 nm which are common for *Planococcus* xanthophylls (2; 15; 16). These three major maxima suggest a chromophore which is composed of nine conjugated double bonds, the typical length of xanthophylls (17). IR spectra shown in (Figure 4) after collection from diethyl ether is consistent with *Planococcus* carotenoids with absorption at labeled values described in previous studies (18). The broad absorbance at ~3300 $cm^{-1}$ suggests the presence of hydroxyl groups which supports the xanthophyll identity of carotenoid(s) produced by CAU13. IR spectra obtained for acetone:methanol mixture provided inconclusive results, improved procedure for solvent removal may provide a higher quality spectrum. As diethyl ether is a nonpolar solvent in comparison to the acetone:-methanol mixture, analyzed extract may include a higher ratio of nonpolar carotenoid precursor molecules. Both spectroscopic techniques therefore support the previous conclusion that carotenoids are present in CAU13 and reasonably would be responsible for a yellow/orange pigmentation.

IR spectra provide overlapping results that match both carotenoids and melanin within the fingerprint region, <1500 $cm^{-1}$, indicating a possible incomplete separation/extraction between the components in solution. This is expected with preliminary solvent extraction, and can be mitigated by chromatographic techniques such as gas chromatography or multiple combined washings of varying polarities

### MELANIN

Melanin extraction methods using 2 M NaOH yielded a dark brown supernatant shown in Figure 5. UV-Vis analysis (Figure 6) of the liquid showed peaks and maximum at 223.31 nm, and 244.88 nm, absorption values consistent with those present in melanin (8). Known absorption spectra of melanin produced by *Bacillus* bacteria show peaks at approximately 220 and 260 nm preceding a gentle slope towards larger wavelengths. The gentle slope shown in Figure 6, is a key characteristic for melanin, as absorbance present over the entire visible region is what provides the dark brown/black pigmentation. Due to the varying nature of its structure and the different types, UV-Vis spectra for melanin come in a variety of forms. The spectrum therefore allows for some limited distinguishability between these types.

The IR spectra obtained for the melanin extract (Figure 7) is consistent with , with absorption at labeled values described in the literature (19). The broad absorbance at 3300-3500 $cm^{-1}$ corresponds to -OH stretching. Other characteristic bands include the 1627 $cm^{-1}$ which is attributed to C=C, COO, and C=O, 1540 $cm^{-1}$ bands which are aromatic C=C, and 1051 $cm^{-1}$, which is C-O in phenols or carboxylic groups (19). The 1540 $cm^{-1}$ absorbance has been stated to correspond to peptide like structures (20) as well as stretches from quinoid structures present in allomelanin (21).

## PROTEOGENOMIC ANALYSIS

### CAROTENOID

The genome of *Planococcus* sp. CAU13 was analyzed using the BioCyc SmartTables function. Seven genes predicted to code for carotenoid biosynthesis pathway enzymes were found, including four distinct phytoene desaturase (*crtI*), two squalene synthase family enzymes, and a carotenoid biosynthesis enzyme. Furthermore, Moyo *et al.* compared the genetic sequences of 33 *Planococcus* strains known to produce carotenoids (2). Six genes (*crtM, crtN, crtNc, crtNb, gtf2,* and *agpat*) make up the 30-carotenoid biosynthetic gene cluster (BCG). In this study all six genes are present in all but two strains, with CAU13 being one of them that only showed *crtM, crtNc,* and *crtN* in its gene cluster.

### MELANIN

As *P. maritimus* is a tentative melanin producing bacterium with the 1,8-DHN pathway for allomelanin production, this specific pathway for melanin production was investigated (6). *P. maritimus* was determined to experimentally produce melanin through the 1,8-DHN pathway, however the published genome was not available at the time of this publication.

The enzyme described previously as PKSIII is the first step in this pathway. Using BioCyc's Whole Genome Blast tool, a preliminary BLAST was computed against the entire genome of CAU13 to determine if a homologous PKSIII enzyme is present. The PKSIII sequence used in the analysis was from *Sorangium cellulosum* produced from the soceCHS1 gene (7). The CAU13 locus_tag KW93_RS14965 translated to protein_id WP_018395371.1 produced significant alignment with the *S. cellulosum* chalcone synthase PKSIII with an E value of 0.0 and 90.17 percent identity through a BLAST search in the NCBI database.

The software predicts the function of the protein WP_018395371.1 as a chalcone synthase, a similar PKSIII

typically involved in producing the precursor to flavonoids (22). BLAST analysis using the PKSIII sequence from CAU13 revealed significant sequence similarty to the following enzymes: T4HN producing PKSIII enzymes from four other bacterial species, AUX21439.1 chalcone synthase *Sorangium cellulosum*, WP_033543849.1 type III polyketide synthase *Planococcus* sp. CAU13, WP_152152970.1 type III polyketide synthase *Bacillus aerolatus*, WP_394120825.1 type III polyketide synthase *Planococcus donghaensis*, WP_088007427.1 type III polyketide synthase *Indiicoccus explosivorum*. The protein sequences were then aligned using T-COFFEE as shown in Figure 8. (15)

The protein sequence alignment shown in Figure 8 shows a score of 95% similarity between the sequences, supporting the previous alignment identification as a T4HN producing PKSIII. The active site and 3D structure of the *Streptomyces coelicolor* A3 PKSIII have previously been determined (10). This active site is described to operate via Cys-His-Asn catalytic triad. All enzymes aligned show this consensus sequence at the positions described in the literature, C138, H270, and N303. To confirm that the active site residues are positioned at the correct orientation in space, AlphaFold software was used to predict the 3D structure of CAU13 PKSIII (Figure 9). This predicted model consists of the same general structure as the single monomer form of S. coelicolor A3.These three factors, the primary sequence similarity, the conserved active site, and the predicted 3D structure indicate that KW93_RS14965 codes for a T4HN producing PKSIII enzyme. Therefore CAU13 likely produces T4HN the first compound in the biosynthetic pathway for allomelanin.

## CONCLUSION

The spectra of the yellow carotenoid-based extract and the presence of a predicted phytoene desaturase within the potential C-30 BGC suggest that CAU13 produces xanthophyll type carotenoids consistent with other members of the genus. The dark pigmentation of CAU13 is likely due to the presence of allomelanin, a type of melanin produced in the 1,8-DHN pathway. Genetic and molecular analysis supports that a T4HN producing PKSIII is likely present due to a conserved active site and high protein homology to other characterized enzymes. UV-Vis and IR characterization of the dark extract support the determination of melanin, though full characterization of the pigment compounds will require analysis of a pure extract with the use of chromatographic techniques, GS-MS, or NMR spectroscopy. Carotenoids and melanin both provide potential fitness advantages by protecting against certain environmental factors, characterization of these compounds can help determine the specific role they play in CAU13 and better understand the organism's place within its ecosystem. Furthermore, biological pigments offer a wide array of industrial, medical, and research applications which should be explored.

## ACKNOWLEDGEMENTS

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## REFERENCES


1. Unverferth CA, Santisteban IC, Setterdahl AT. *Draft Genome Sequence of the Novel Black-Pigmented Planococcus sp. Strain CAU13.* 6, 2014, Genome Announcements, Vol. 2. doi.org/10.1128/genomeA.01160-14
2. Anesu Conrad Moyo, Laurent Dufossé, Daniele Giuffrida, Leonardo Joaquim van Zyl, Marla Trindade. *Structure and biosynthesis of carotenoids produced by a novel Planococcus sp. isolated from South Africa.* 43, 2022, Microb Cell Fact , Vol. 21. doi.org/10.1186/s12934-022-01752-1
3. López, G. D., Álvarez-Rivera, G., Carazzone, C., Ibáñez, E., Leidy, C., & Cifuentes, A. *Bacterial Carotenoids: Extraction, Characterization, and Applications.* 6, 2021, Critical Reviews in Analytical Chemistry, Vol. 53, pp. 1239–1262. doi.org/10.1080/10408347.2021.2016366
4. Nancy A. Moran, Tyler Jarvik. *Lateral Transfer of Genes from Fungi Underlies Carotenoid Production in Aphids.* 2010, Science, Vol. 328, pp. 624-627. doi.org/10.1126/science.1187113
5. Vijayan, V., Jasmin, C., Anas, A., Parakkaparambil Kuttan, S., Vinothkumar, S., Perunninakulath Subrayan, P., & Nair, S. *Sponge-associated bacteria produce non-cytotoxic melanin which protects animal cells from photo-toxicity.* 1, 2017, Applied Biochemistry and Biotechnology, Vol. 183, pp. 396-411. doi.org/10.1007/s12010-017-2453-0
6. Valeria Lino, Paola Manini. *Dihydroxynaphthalene-Based Allomelanins: A Source of Inspiration for Innovative Technological Materials.* 18, 2022, ACS Omega, Vol. 7, pp. 15308-15314. doi.org/10.1021/acsomega.2c00641
7. Sone Y, Nakamura S, Sasaki M, Hasebe F, Kim S, Funa N. *Bacterial Enzymes Catalyzing the Synthesis of 1,8-Dihydroxynaphthalene, a Key Precursor of Dihydroxynaphthalene Melanin, from Sorangium cellulosum.* 2018, Appl Environ Microbiol, Vol. 84. doi.org/10.1128/AEM.00258-18
8. Vishal Ghadge, Pankaj Kumar, Sanju Singh, Doniya Elze Mathew, Sourish Bhattacharya, Satish Balasaheb Nimse, and Pramod B. Shinde. *Natural Melanin Produced by the Endophytic Bacillus subtilis 4NP-BL Associated with the Halophyte Salicornia brachiata.* 25, 2020, Journal of Agricultural and Food Chemistry, Vol. 68, pp. 6854-6863. doi.org/10.1021/acs.jafc.0c01997
9. Funa, Nobutaka & Funabashi, Masanori & Ohnishi, Yasuo & Horinouchi, Sueharu. *Biosynthesis of Hexahydroxyperylenequinone Melanin via Oxidative Aryl Coupling by Cytochrome P-450 in Streptomyces griseus.* 2005, Journal of Bacteriology, Vol. 187, pp. 8149-55. doi.org/10.1128/JB.187.23.8149-8155.2005
10. Michael B. Austin, Miho Izumikawa, Marianne E. Bowman, Daniel W. Udwary, Jean-Luc Ferrerl, Bradley S. Moore¶, Joseph P. Noel. *Crystal Structure of a Bacterial Type III Polyketide Synthase and Enzymatic Control of Reactive Polyketide Intermediates.* 43, 2004, Journal of Biological Chemistry, Vol. 279, pp. 45162-45174. doi.org/10.1074/jbc.M406567200
11. Pfeifer BA, Khosla C. *Biosynthesis of polyketides in heterologous hosts.* 1, Mar 2001, Microbiol Mol Biol Rev, Vol. 65, pp. 106-18. doi.org/10.1128/MMBR.65.1.106-118.2001
12. Sanju Singh, Satish B. Nimse, Doniya Elze Mathew, Asmita Dhimmar, Harshal Sahastrabudhe, Apexa Gajjar, Vishal A. Ghadge, Pankaj Kumar, Pramod B. Shinde. *Microbial melanin: Recent advances in biosynthesis, extraction, characterization, and applications.* 2021, Biotechnology Advances, Vol. 53, p. 107773. doi.org/10.1016/j.biotechadv.2021.107773
13. Paolo Di Tommaso, Sebastien Moretti, Ioannis Xenarios, Miquel Orobitg, Alberto Montanyola, Jia-Ming Chang, Jean-François Taly, Cedric Notredame. *T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension.* Jul 2011, Nucleic Acids Res, Vol. 39. doi.org/10.1093/nar/gkr245
14. John Jumper, Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger, Kathryn Tunyasuvunakool, Russ Bates, Augustin Žídek, Anna Potapenko, Alex Bridgland, Clemens Meyer, Simon A. A. Kohl, Andrew J. Ballard, Andrew Cowie, Bernardino. *Highly accurate protein structure prediction with AlphaFold.* 2021, Nature, Vol. 596, pp. 583-589. doi.org/10.1038/s41586-021-03819-2
15. Takemura M, Takagi C, Aikawa M, Araki K, Choi SK, Itaya M, Shindo K, Misawa N. *Heterologous production of novel and rare C30-carotenoids using Planococcus carotenoid biosynthesis genes.* 1, Oct 9, 2021, Microb Cell Fact, Vol. 20, p. 194. doi.org/10.1186/s12934-021-01683-3

16. Eugenia Vila, Dámaso Hornero-Méndez, Gastón Azziz, Claudia Lareo, Verónica Saravia. *Carotenoids from heterotrophic bacteria isolated from Fildes Peninsula, King George Island, Antarctica.* 2019, Biotechnology Reports, Vol. 21. doi.org/10.1016/j.btre.2019.e00306
17. Marquardt, Julian, Dieter, Hanelt. *Carotenoid composition of Delesseria lancifolia and other marine red algae from polar and temperate habitats.* 2004, European Journal of Phycology, Vol. 39, pp. 285-292. doi.org/10.1080/09670260410001712572
18. Nisha Pallath, Bismimol Francis, Sandhanasamy Devanesan, Karim Farhat, Muthuraj Balakrishnan. *Isolation and characterization of novel carotenoid pigment from marine Planococcus maritimus MBP-2 and their biological applications.* 8, 2023, Journal of King Saud University - Science, Vol. 35, p. 102872. doi.org/10.1016/j.jksus.2023.102872
19. Shujing Sun, Xiaojuan Zhang, Shiwei Sun, Liaoyuan Zhang, Shukai Shan, Hu Zhu.*Production of natural melanin by Auricularia auricula and study on its molecular structure.* 2016, Food Chemistry, Vol. 190, pp. 801-807. doi.org/10.1016/j.foodchem.2015.06.042
20. Z. Filip, K. Haider, H. Beutelspacher, J.P. Martin. *Comparisons of IR-spectra from melanins of microscopic soil fungi, humic acids and model phenol polymers.* 1, 1974, Geoderma, Vol. 11, pp. 37-52. doi.org/10.1016/0016-7061(74)90005-6
21. Saranshu Singla, K. Zin Htut, Runyao Zhu, Amara Davis, Jiayang Ma, Qing Zhe Ni, Michael D. Burkart, Christopher Maurer, Toshikazu Miyoshi, and Ali Dhinojwala. *Isolation and Characterization of Allomelanin from Pathogenic Black Knot Fungus-a Sustainable Source of Melanin.* 51, 2021, ACS Omega, Vol. 6, pp. 35514-35522. doi.org/10.1021/acsomega.1c05030
22. Hongjiao Zhang, Zixin Li, Shuang Zhou, Shu-Ming Li, Huomiao Ran, Zili Song, Tao Yu & Wen-Bing Yin. *A fungal NRPS-PKS enzyme catalyses the formation of the flavonoid naringenin.* 2022, Nature Communications, Vol. 13. doi.org/10.1038/s41467-022-34150-7
23. Ana Antunes, Francisca Carmo, Sara Pinto, Nelson Andrade, Fátima Martel. *The anti-proliferative effect of β-carotene against a triple-negative breast cancer cell line is cancer cell-specific and JNK-dependent.* 2022, Pharma Nutrition, Vol. 22, p. 100320. doi.org/10.1016/j.phanu.2022.100320
24. Armougom, F., Moretti, S., Poirot, O., Audic, S., Dumas, P., Schaeli, B., Keduas, V., Notredame. C. *Expresso: automatic incorporation of structural information in multiple sequence alignments using 3D-Coffee.* 2006, Nucleic Acids Res, Vol. 34, pp. 604-608. doi.org/10.1093/nar/gkl092
25. Cristian Tapia, Bárbara López, Allisson Astuya, José Becerra, Concetta Gugliandolo, Boris Parra, Miguel Martínez. *Antiproliferative activity of carotenoid pigments produced by extremophile bacteria.* 22, 2021, Natural Product Research, Vol. 35, pp. 4638-4642. doi.org/10.1080/14786419.2019.1698574
26. Engel, S.R., Wong,E.D., Nash,R.S., Aleksander,S., Alexander,M., Douglass,E., Karra,K., Miyasato,S.R., Simison,M., Skrzypek,M.S., Weng,S. and Cherry,J.M. *New data and collaborations at the Saccharomyces Genome Database: updated reference genome, alleles, and the Alliance of Genome Resources.* 4, 2022, Genetics , Vol. 220. doi.org/10.1093/genetics/iyab224
27. Iwata, S., Kamata, K., Yoshida, S., Minowa, T., Ohta, T. *T and R states in the crystals of bacterial L-lactate dehydrogenase reveal the mechanism for allosteric control.* 1994, Nat Struct Biol, pp. 176-185. doi.org/10.1038/nsb0394-176
28. Karp PD, Billington R, Caspi R, Fulcher CA, Latendresse M, Kothari A, Keseler IM, Krummenacker M, Midford PE, Ong Q, Ong WK, Paley SM, Subhraveti P. *The BioCyc collection of microbial genomes and metabolic pathways.* 4, Jul 19, 2019, Brief Bioinform, Vol. 20, pp. 1085-1093. doi.org/10.1093/bib/bbx085
29. Kwang Hyun Cha, Song Yi Koo, and Dong-Un Lee. *Antiproliferative Effects of Carotenoids Extracted from Chlorella ellipsoidea and Chlorella vulgaris on Human Colon Cancer Cells.* 22, 2008, Journal of Agricultural and Food Chemistry, Vol. 56, pp. 10521-10526. doi.org/10.1021/jf802111x
30. Liu W, Li L, Ye H, Chen H, Shen W, Zhong Y, Tian T, He H. *From Saccharomyces cerevisiae to human: The important gene co-expression modules.* 2, Aug 2017, Biomed Rep., Vol. 7, pp. 153-158. doi.org/10.3892/br.2017.941
31. Nosanchuk JD, Casadevall A. *Impact of melanin on microbial virulence and clinical resistance to antimicrobial compounds.* 11, Nov 2006, Antimicrob Agents Chemother., Vol. 50, pp. 3519-28. doi.org/10.1128/AAC.00545-06
32. Rassbina, A., Khabibrakhmanova, V., Babaev, V., Daminova, A., & Minibayeva, F. *Melanins from the Lichens Lobaria pulmonaria and Lobaria retigera as Eco-Friendly Adsorbents of Synthetic Dyes.* 24, 2022, International Journal of Molecular Sciences, Vol. 23, p. 15605. doi.org/10.3390/ijms232415605

33. Tran-Ly AN, Reyes C, Schwarze FWMR, Ribera J.*Microbial production of melanin and its various applications.* 11, Oct 12, 2020, World J Microbiol Biotechnol, Vol. 36, p. 170. doi.org/10.1007/s11274-020-02941-z
34. Zhadin, N., Gulotta, M. and Callender, R. *Probing the role of dynamics in hydride transfer catalyzed by lactate dehydrogenase.* 2008, Biophys. J., pp. 1974-1984. doi.org/10.1529/biophysj.108.132464