I WENT TO THE BEACH AND BROUGHT BACK SAND: A STORY OF ANOXYGENIC PHOTOTROPHIC BACTERIA

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ABSTRACT

Anoxygenic phototrophic bacteria (APB) refers to prokaryotic microorganisms that undergo photosynthesis to create energy using chlorophyll pigments and do not release oxygen. These include purple sulfur, green sulfur and purple non-sulfur bacteria, all of which were enriched for in this study from a microbial mat found in Little Sippewissett salt marsh (Cape Cod, MA). After obtaining pure cultures only the purple non-sulfur bacteria, which were found to be most closely related to *Rhodovulum* sp. JA545, was explored further. Carbon sources, photoperiods and nitrogen sources were altered in all combinations to explore growth patterns and if the plant hormone indole-3-acetic acid could be produced. Indole-3-acetic acid is known to be produced by other phototrophic bacteria such as cyanobacteria that can be found in association with plants. Here, it was found that tryptophan is necessary for indole-like compounds to be produced under any photoperiod or carbon source. It was also found that propionate used as a sole carbon source allowed indole-like compounds to be produced only under full light conditions. Propionate also was the most quickly consumed carbon source at 24h while acetate was consumed most during 16h light periods. This brings up an ecological question as to whether APBs have an unknown interaction with plants or how/if the production of these indole-like compounds influences the surrounding environment.

INTRODUCTION

Anoxygenic phototrophic bacteria (APB) are prokaryotic microorganisms do not release oxygen and use photosynthesis to create energy using chlorophyll pigments (11). These bacteria include purple bacteria and aerobic bacteriochlorophyll *a*–containing bacteria, green sulfur bacteria, filamentous green non-sulfur bacteria, and heliobacteria. Based on their phenotypic characters, anoxygenic phototrophic bacteria had been divided into the five families Rhodospirillaceae (purple non-sulfur), Chromatiaceae (purple sulfur), Ectothiorhodospiraceae (purple sulfur), Chlorobiaceae (green sulfur), and Chloroflexaceae (green non-sulfur) (9). Many of these bacteria can often be found in association together in the infamous microbial mat. A fully developed microbial mats consist of a top aerobic green layer of diatoms and cyanobacteria, a green layer consisting mostly of cyanobacteria, a red-purple microaerophilic layer of purple non-sulfur bacteria, an anaerobic purple layer of BChl *a*-containing purple sulfur bacteria, a peach-colored layer formed by BChl *b*-containing purple sulfur bacteria, a green-grey layer of green sulfur bacteria and a grayish to blackish bottom layer of sediment containing sulfate reducers (9). Another location phototrophic bacteria are often noticed is in dense blooms which consist of green or purple sulfur bacteria in usually stagnant or shallow aquatic ecosystems. Purple non-sulfur bacteria are not known for this and do not form dense blooms in nature.

Green sulfur bacteria, which can be brown or green in color, form their own bacterial phylum, *Chlorobi*. This group uses free sulfide, usually found as hydrogen sulfide (H_2S) as an electron donor for CO_2 reduction using the reverse TCA cycle (6). During the oxidation of sulfide, the cells generate extracellular globules of elemental sulfur. Their antenna chlorophylls consist of bacteriochlorophyll (BChl) *c, d* or *e* and absorb light near 750-770nm.

Purple sulfur bacteria are found in the subphyla *Gammaproteobacteria*. They too use free sulfide as an electron donor for CO_2 reduction but use the Calvin cycle for autotrophy. They are not limited to this electron donor however and can use other reduced sulfur compounds like thiosulfate ($S_2O_3^{2-}$). These bacteria generate intercellular globules of elemental sulfur (6). They are able to photosynthesize using BChl *a* and absorb light from 800-900nm or use BChl *b* and absorb at around 1030nm.

Purple non-sulfur bacteria are found in the *Alphaproteobacteria* and *Betaproteobacteria* subphyla. They are not always purple but can be peach, violet or brown-yellow. Characteristically, members of these two groups exhibit a high metabolic versatility and are capable of photoorganotrophic, photolithoautotrophic and chemoorganotrophic growth (9). Being photoheterotrophic they are able to use light as the energy source and an organic compound as a carbon source. Photosynthetic pigments are BChl *a* or *b* and a variety of carotenoids.

APBs are known to live in shallow waters in both marine and freshwater environments where there are opportunities to be in contact with plant tissues. This brings up an ecological question as to whether APBs have an unknown interaction with plants. Plant associated microorganisms are often capable of synthesizing phytohormones and studying the ability of APBs to produce these may reveal an interaction that was not previously known. These bacteria would likely be producing plant-like hormones that encourage plant growth and provide nutritional exudates that may serve as a carbon source, especially purple non-sulfur bacteria as they are metabolically resourceful. It has been recently shown that the oxygenic phototrophic organisms known as cyanobacteria are able to produce the auxin indole-3-acetic acid (IAA) with and without the tryptophan precursor, indicating that these organisms may use more than one biosynthetic pathway for IAA production (12, 14). IAA biosynthesis in bacteria can occur via multiple pathways as has been observed in plants. There are at least 6 known pathways for IAA synthesis with 5 being tryptophan-dependent and 1 tryptophan-independent (10, 15). It was also seen that cyanobacteria under modulated light exposure varied IAA production, with IAA being produced most at a full 24h of light (10). This is interesting as it known that IAA is light sensitive, especially at wavelengths between 380-470nm, which is ideal for cyanobacteria growth (9, 18). At higher wavelengths, such as 620-760nm, IAA is still degraded but 40% less than at the aforementioned lower wavelengths (7, 18). It is at these higher wavelengths are what APBs thrive under, further supporting the hypothesis that these bacteria can produce IAA.

Here, purple non-sulfur bacteria were monitored for growth rates and IAA production. Carbon sources, tryptophan supplementation and varying light periods were tested. For this set of experiments there are 3 major hypotheses: 1) under complete dark conditions purple non-sulfur bacteria will not grow at rates comparable to “natural” or full light exposure, 2) purple non-sulfur bacteria will grow at a greater rate when provided a less complex carbon source and 3) purple non-sulfur bacteria have the ability to make indole-acetic acid.

METHODS

Enrichments for anoxygenic phototrophic bacteria

Microbial mats were sampled from Little Sippewissett march (Cape Cod, MA). A modular marine medium base was used for all enrichments (960ml Seawater base, 10ml 1M NH_4Cl, 10ml 100mM K_2HPO_4, 1ml 1M Na_2SO_4, 0.4g $NaHCO_3$ and autoclave, add sterile 20ml 1M MOPS buffer pH 7.2, 1ml HCl-dissolved trace elements, 1ml multivitamin solution after autoclaved solution has cooled). The sterile serum vials with added marine medium were bubbled with N_2 while the headspace was subsequently flushed with N_2 and sealed with butyl rubber stoppers to provide anoxic conditions. This was used for all 3 enrichments of various anoxygenic phototrophic bacteria. Appropriate light wavelengths were applied to support the growth of the organism of choice.

Purple non-sulfur bacteria

To select for purple non-sulfur bacteria, a small piece of the microbial mat was placed in a N_2 flushed vial containing 10ml marine phototrophic base. While continuing to bubble with N_2, the addition of 0.1ml of 1M sodium succinate ($C_4H_4Na_2O_4$) was added to the medium. Flushing with N_2 persisted for an additional 10m and the bottle was stopped and crimped to seal. After sealing, the bottle was flushed with 80% N_2 and 20% CO_2 mix using an additional needle to release gasses. It was then placed in light at 850nm at 30ºC.

Purple sulfur bacteria

Purple sulfur bacteria were selected for by adding a small piece of the microbial mat to a N_2 flushed vial with 10ml marine phototrophic base. While continuing to bubble with N_2, the addition of 0.1ml of 0.5M sodium thiosulfate ($Na_2S_2O_3$) was added to the medium. The bottle was stopped and crimped. The bottle was then flushed with 80% N_2 and 20% CO_2 mix using an additional needle to release gasses for another 10m. After flushing the headspace, 0.1ml of 0.1M Na_2S solution was added. These vials were stored in light at 850nm at 30ºC.

Green sulfur bacteria

A small piece of microbial mat was added to 10ml of marine phototroph base flushed with N_2 for selection of green sulfur bacteria. The bottle was stopped and crimped. It was then flushed with 80% N_2 and 20% CO_2 mix using an additional needle to release gasses for another 10m. After flushing the headspace, 0.3ml of 0.1M Na_2S solution was added. These vials were stored in light at 750nm at 30ºC.

Isolation for anoxygenic phototrophic bacteria

Shake tubes were made for isolation of the above organisms. Marine phototroph base was prepared as above but with 15g/L agar and the appropriate electron donor added to the media after autoclaving but before dispensing into shake tubes. The media was maintained in 42ºC water bath to prevent agar from solidifying. Sterile Balch tubes were placed in a water bath and flushed with N_2 for 10m before and after dispensing 10ml media in each. A dilution series was set up and 1ml of enrichment was added with a N_2 flushed syringe, inverted, and 1ml was taken from there to the next dilution. These tubes are sealed using Hungate technique and placed on ice immediately to solidify agar. After the tubes are solidified, they were flushed with 80% N_2 and 20% CO_2 mix for another 10m to flush out any oxygen. These were again sealed using Hungate technique and placed inverted in the appropriate light conditions at 30ºC. Colonies were seen after around 1 week.

Colony isolation and transfer

After colonies developed in the shake tubes, a single colony was transferred to a N_2 flushed vial for beginning a pure culture. To release the agar from the shake tube, a N_2 gassing probe was set to low and inserted into the tube to begin to push the agar plug out of the tube and into a sterile petri plate. Using a dissecting microscope (Discovery.V8, Zeiss) and sterile techniques, a single colony was obtained. This was done using a flamed Pasteur pipette broken at the end in such a way that the opening was narrowed greatly. This was attached to tubing and the colony was sucked out of the media and transferred to the flushed vial with the appropriate electron donor. The vials were then flushed with 80% N_2 and 20% CO_2 mix for another 10m to flush out any oxygen. These were incubated in the appropriate light at 30ºC. When pure cultures shown turbidity, a Cary60 UV-Vis spectrophotometer and phase contrast microscopy (Axio Scope.A1, Zeiss) was used to verify there were no contaminants and that the organism selected for were showing the appropriate maximum wavelength peaks for their given chlorophyll and showing characteristic morphologies under the scope.

Culture identification
Three chosen vials containing each of the selected organisms were chosen for identification. Using a N_2 flushed syringe, 50µl of culture was boiled at 100ºC for 5m to release DNA. This was then centrifuged and placed on ice. This was used as template for a subsequent PCR reaction. A Go-Taq Green MasterMix (Promega) was used with 16S_8F (AGAGTTTGATCCTGGCTCAG) and 16S_1492R (GGTTACCTTGTTACGACTT) universal primers at an annealing temperature of 46ºC for 30s and extension temperature of 72ºC for 1.5m. PCR product was cleaned-up using the Wizard PCR Preps DNA Purification Systems (Promega). The product was sent for sequencing at MBL facilities in Woods Hole, MA.

Experimental setup for purple non-sulfur bacteria
Light, carbon and nitrogen sources were used in all combinations to determine the effect on growth rate and indole-3-acetic acid production. For two different nitrogen sources, the marine phototroph base was used as described above or made without 1M NH_4Cl but instead with sterile 5mM tryptophan (final concentration) which was added after autoclaving. Three different carbon sources were used: 10mM sodium citrate ($Na_3C_6H_5O_7$), 10mM sodium acetate ($NaC_2H_3O_2$) and 10mM sodium propionate ($NaC_3H_5O_2$) (final concentration). To a centrifuge tube, 1ml of pure culture was added using a N_2 flushed syringe. The tubes were spun down and the media was removed to ensure no succinate remained as a carbon source. The cells were quickly resuspended in 1ml marine phototroph base (without a nitrogen source) and added to the fresh media. All 6 combinations were made in a total of 20ml. These 6 combinations of carbon and nitrogen were placed in 3 different light schedules all in 850nm at 30ºC. The light schedules included 24L, 16L:8D and 0L. Carbon consumption and indole production were measured every other day for 9 days.

Determination of growth for purple non-sulfur bacteria
Growth of purple non-sulfur bacteria was measured by carbon consumption using HPLC. A BioRad Aminex HPX-87H column with BioRad 125-0129 guard cartridge was used to measure citrate, acetate and propionate. The samples were prepared by extracting 900µl with a N_2 flushed syringe and adding 100µl of 5N H_2SO_4. This was centrifuged at 14,000rpm for 5m. From the supernatant, 900µl was drawn off and added to a HPLC vial. A known concentration of each carbon source was processed in the same way to address the accuracy of each run. HPLC was used starting at day 1 and continuing every other day until day 9. The EZstart 7.4 software was used to measure and process the data.

Determination of indole compounds
The Salkowski reagent was used to measure amounts of indole-3-acetic acid produced by the purple non-sulfur bacteria. The Salkowski reagent is specific for IAA, indolepyruvic acid, and indoleacetamide (1). Here, 88ml of reagent grade H_2SO_4 was added to 100ml diH_2O followed by the addition of 2.4g $FeCl_3$. From each of the treatments, 0.5ml was extracted using a N_2 flushed syringe. This was then centrifuged for 5m at 14,000rpm and 400µl of the supernatant was added to 400µl of Salkowski reagent. This was allowed to incubate in the dark for 30m for color to fully develop. A standard was made using 0, 10, 20, 40, 60, 80, 100 and 200 µM concentrations of IAA. The standard and all treatments were measured at OD_{540} and concentrations were determined.

RESULTS
Colony isolation
After around 1.5 weeks, the shake tubes had detectable colonies suspended in the agar plugs. The colonies must be large enough to visualize without a microscope to be able to transfer successfully

to fresh media for the start of a pure culture (Fig. 1). Once turbid cultures were acquired, UV-Visible spectrometry was performed. This method would give evidence that the culture was pure and the organism chosen for enrichment was selected. For purple non-sulfur bacteria, a peak was seen at 461nm and 750nm, indicating that BChl *c* was present which is found exclusively in green photosynthetic bacteria. The purple sulfur isolation shown peaks at 589nm, 805nm and 834nm (Fig. 2). The purple non-sulfur isolation shows peaks at 590nm, 804nm and 857nm (Fig. 2). Both purple phototrophs have evidence for possessing BChl *a*, a chlorin found in purple photosynthetic bacteria. Both sulfur oxidizing phototrophs were seen to have sulfur granules when observed in phase contrast at 100X objective (Fig. 3A, 3C). The purple non-sulfur bacteria had no such sulfur granules and the cells were larger than the other two isolates (Fig. 3B).

Culture identification

Sequencing results identified the purple non-sulfur bacteria to be most closely related to *Rhodovulum* sp. JA545 (GI: 304656617) with 81% coverage, 99% identity and an e-value of 5e-82. The green sulfur isolate was most closely related to *Prosthecochloris aestuarii* DSM 271 (GI:444303941) with 93% coverage, 95% identity and an e-value of 1e-160. The purple sulfur bacteria sequence was unable to be retrieved.

Determination of growth for purple non-sulfur bacteria

The growth of the purple non-sulfur bacteria was measured by its consumption of the given carbon source. This was performed using HPLC. Initial culture concentrations were 10mM for citrate, acetate and propionate. Starting at day 1 and continuing everyone other day over 9 days, carbon sources were measured. The cells incubated with propionate at 24h light exposure consumed this source more quickly than the cells in any other vial, reaching 0.333mM of propionate by day 9. By day 5 the propionate was nearly half consumed (Fig. 5C; Table 1). Cells incubated with acetate at 24h light exposure also consumed rapidly, reaching 1.255mM by day 9 (Fig. 5B; Table 1). Cells incubated with acetate for a 16h light exposure consumed more of this carbon source, reaching 7.2mM by day 9, than the same light period with propionate which only reached 9.8mM by day 9 (Fig. 4B). Citrate was not readily consumed by this organism at any light schedule. None of the cells with tryptophan consumed much of any carbon source over all 9 days at any light period (Fig. 4). Similarly, none of the cells incubated in complete dark consumed much of any carbon source (Fig. 4C).

Determination of indole compounds

The color indicating Salkowski assay was performed using supernatants from all treatments. There was a clear distinction between all cells incubated with tryptophan and those that were not, regardless of carbon source or light treatment. All cell supernatants incubated with tryptophan shown a color change when the Salkowski reagent was added (Fig. 7). When compared to the OD_{540} of the standard these tryptophan-supplemented cells produced an indole-like compound immediately at day 1 with increasing concentration of this compound accumulating through day 9 (Fig. 6). For all carbon sources with tryptophan, cells produced the most indole at 16h light treatment (Table 2). Cells incubated with propionate and no tryptophan also showed a color change for the 24h light treatment only with concentrations reading up to 18.3µM concentrations of indole-like compounds. The acetate treatment without tryptophan also showed a slight increase in indole-like compounds by day 9 (Fig. 6).

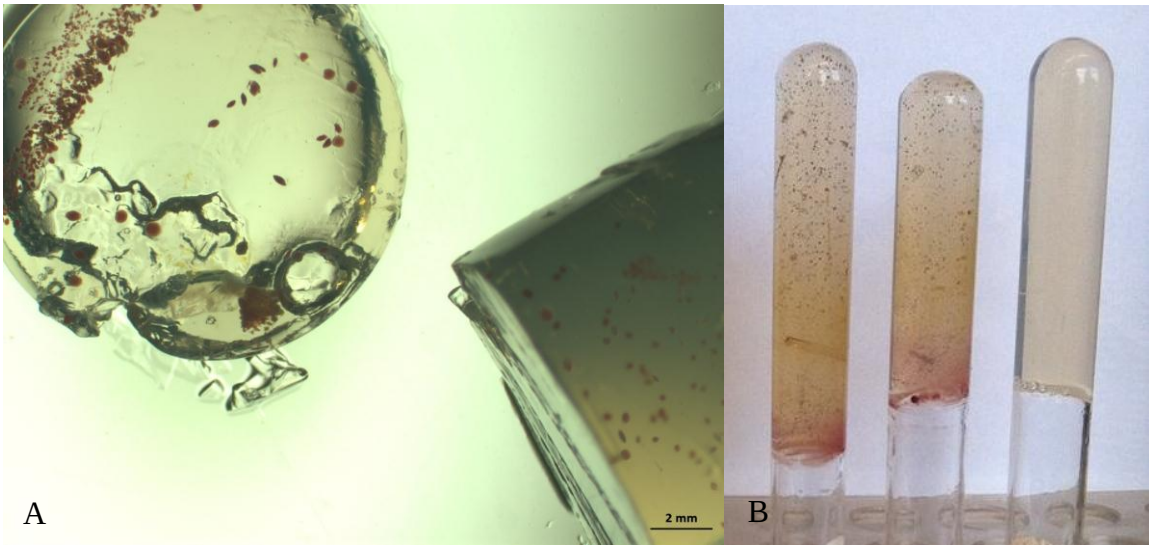

Figure 1. Purple non-sulfur bacteria shake tubes. Panel A shows purple non-sulfur bacteria colonies in an agar plug at a 1X objective. Panel B depicts shake tubes containing colonies that are ready for transfer.

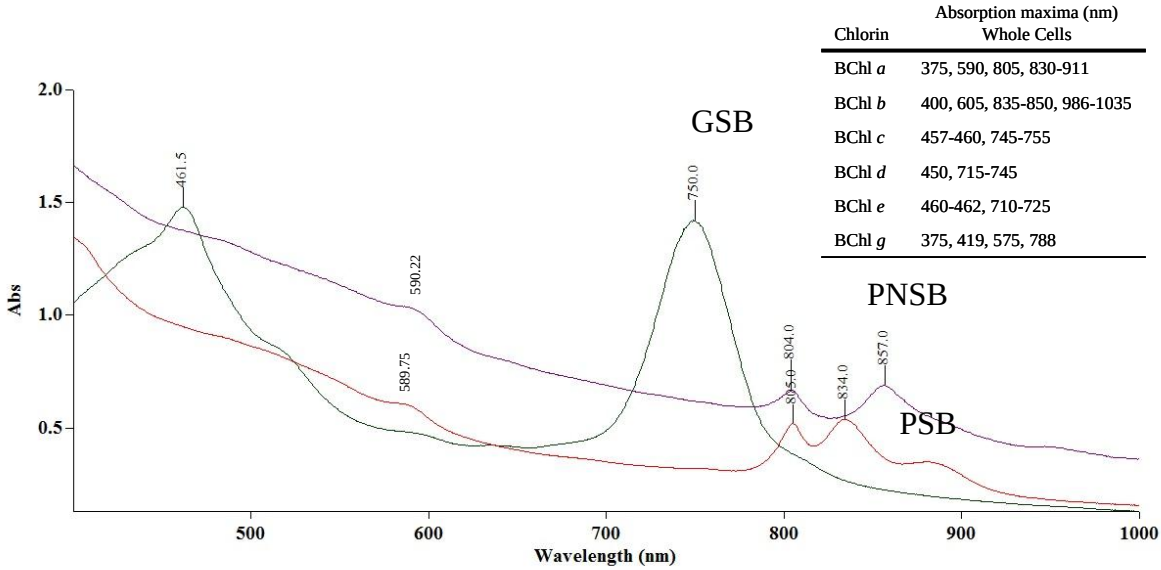

Figure 2. UV-Visible spectroscopy of purple non-sulfur, purple sulfur and green sulfur isolates. The purple non-sulfur and purple sulfur bacteria have the characteristic BChl *a* chlorin showing wavelengths around 590nm, 805nm and 830-857nm. The green sulfur isolate shows peaks around 460nm and 750nm, characteristic of BChl *c*. Table modified from Overmann & Garcia-Pichel 2006 (9).

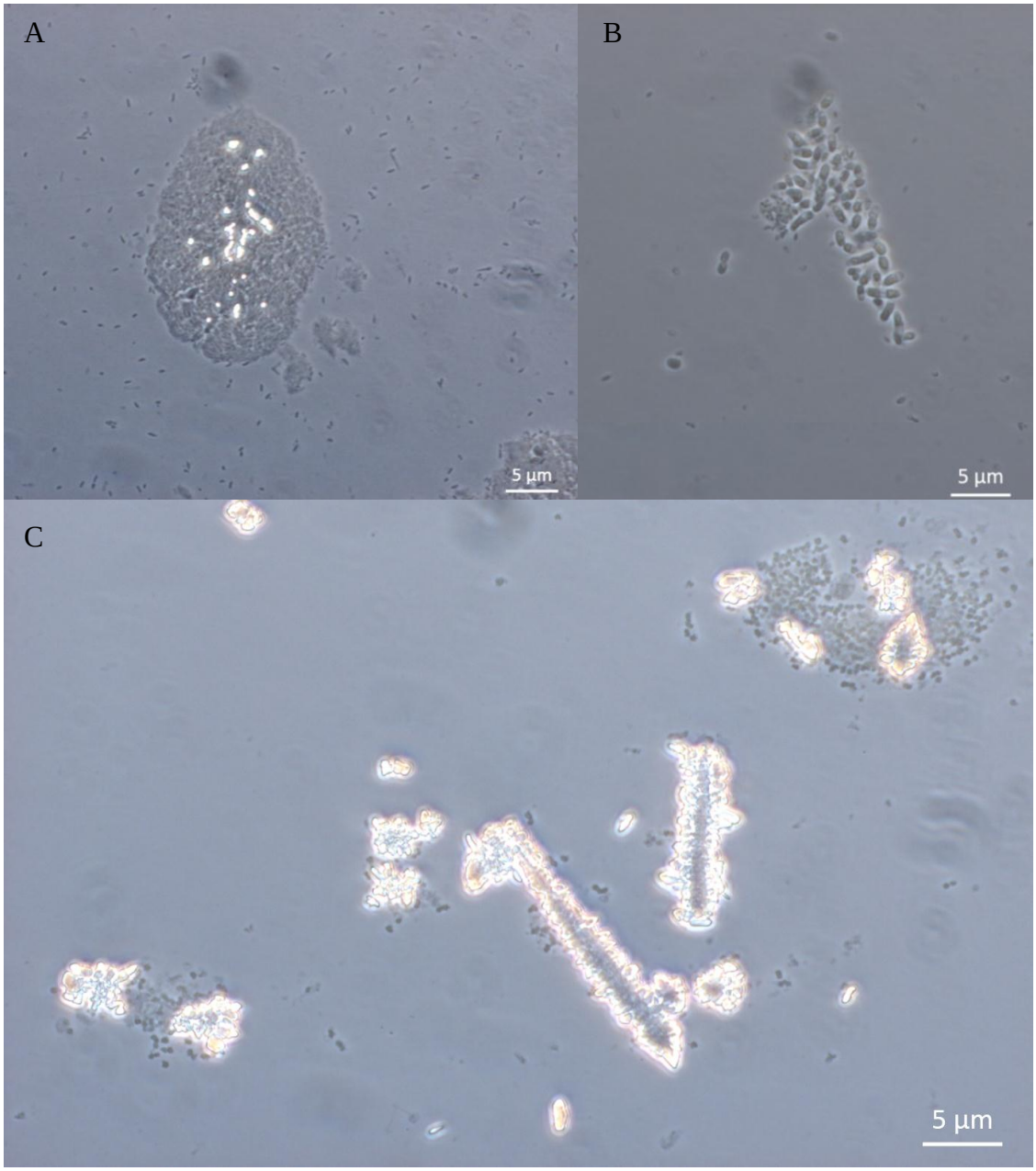

Figure 3. Phase contrast microscopy of anoxygenic phototrophic bacteria seen at 100X objective. Panel A shows the purple sulfur bacteria isolate. Internal sulfur granules can be observed, a characteristic of purple sulfur bacteria. Panel B shows the purple non-sulfur bacteria isolate. Panel C shows the green sulfur bacteria isolate.

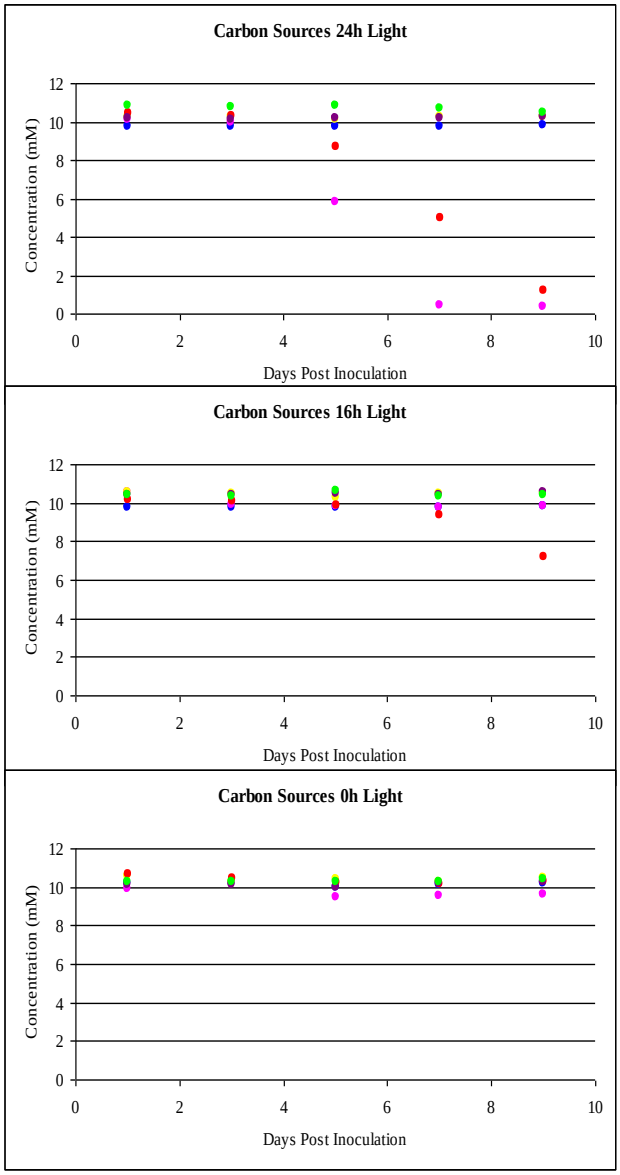

Figure 4. Concentrations of carbon sources at 24h, 16h and 0h light over time. Carbon sources include citrate ●, citrate+tryp ●, acetate ●, acetate+tryp ●, propionate ●, proptionate+tryp ●. Over light exposure periods, 24h light shown acetate and propionate both consumed and 16h light only acetate was consumed.

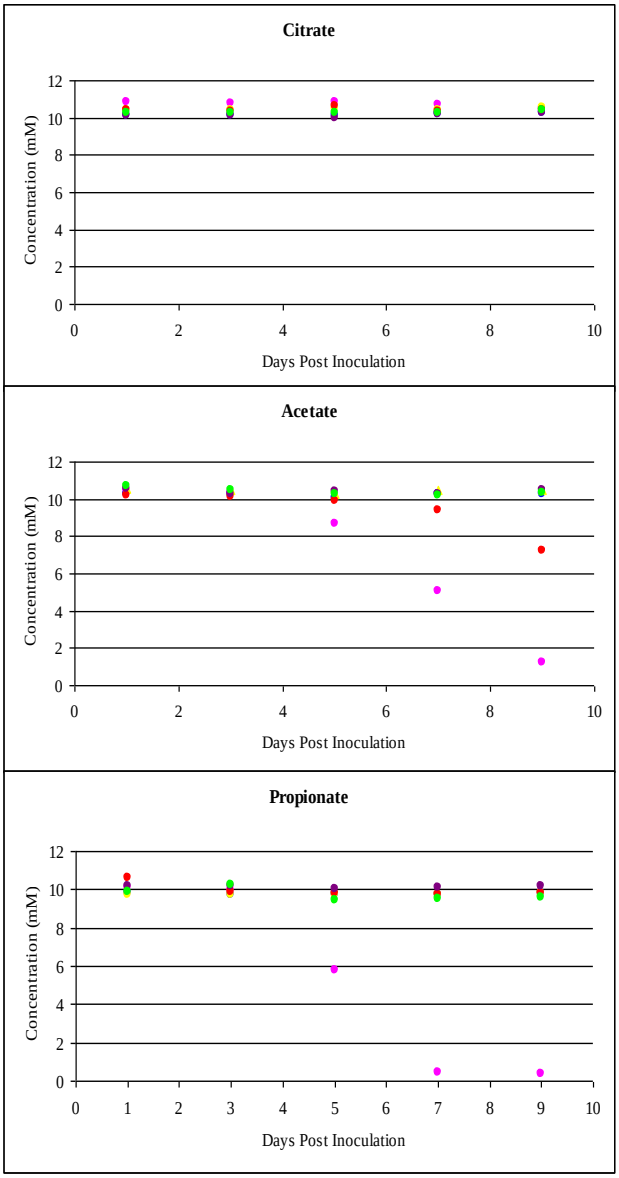

Figure 5. Concentrations of citrate, acetate and propionate shown independently with all light schedules. Each carbon source is at 24h ●, tryp+24h ●, 16h ●, tryp+16h ●, 0h ● and tryp+0h ●. Within carbon sources only acetate and propionate were consumed at 24h light and only acetate at 16h light

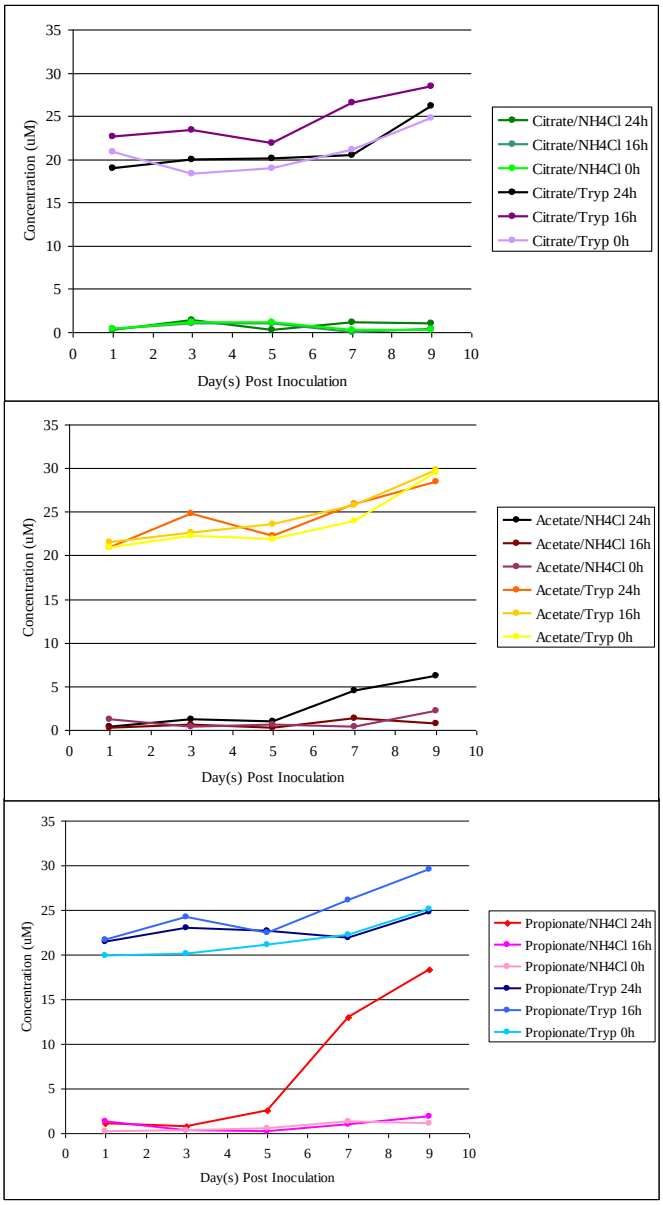

Figure 6. Concentrations of indole-like compounds found from purple non-sulfur bacteria using the Salkowski assay. Those cultures with tryptophan as a nitrogen source shown elevated levels of indole-like compounds compared to those cultures without tryptophan. There was little difference between production of indole-like compounds among cultures with tryptophan of varying light treatments or carbon sources.

Table 1. Values for concentrations (mM) from HPLC analysis of citrate, acetate and propionate over 9 days. Isolate abbreviations are designated by carbon source (C, citrate; A, acetate; P, propionate), with the addition of tryptophan (T) and light schedule exposure (24h, 16h, 0h). Initial concentrations for all vials was 10mM. Purple non-sulfur bacteria with propionate incubated at 24h light periods were consumed most reaching 0.333mM by day 9.

	DAY				
Isolate	1	3	5	7	9
PT24	9.776	9.735	9.741	9.767	9.789
P24	10.101	9.948	5.804	0.455	0.333
AT24	10.272	10.232	10.082	10.245	10.273
A24	10.463	10.315	8.697	5.053	1.255
CT24	10.150	10.107	10.154	10.180	10.277
C24	10.863	10.795	10.811	10.664	10.460
PT16	9.756	9.729	9.766	9.759	9.815
P16	10.585	9.879	9.827	9.754	9.813
AT16	10.585	10.447	10.278	10.468	10.513
A16	10.188	10.141	9.887	9.402	7.242
CT16	10.439	10.392	10.464	10.432	10.540
C16	10.382	10.359	10.601	10.351	10.429
PT0	10.168	10.198	9.997	10.087	10.171
P0	9.897	10.219	9.468	9.540	9.617
AT0	10.534	10.284	10.378	10.287	10.476
A0	10.669	10.456	10.264	10.186	10.312
CT0	10.103	10.117	10.003	10.191	10.295
C0	10.278	10.237	10.288	10.280	10.392

Table 2. Indole-like compound concentrations found in each treatment. Tryptophan supplemented cells produced much more indole-like compounds than those not supplemented with tryptophan. Propionate and acetate both produced indole-like compounds without tryptophan when incubated in 24h light periods but these concentrations were still lower than the same treatment with tryptophan.

		Concentration (uM)				
Carbon/Nitrogen Source	Light Exposure (h)	1	3	5	7	9
Citrate/NH4Cl	24	0.193	1.353	0.193	1.160	0.967
	16	0.387	0.967	0.967	0.000	0.387
	0	0.387	1.160	1.160	0.193	0.193
Citrate/Tryp	24	18.947	19.914	20.107	20.494	26.101
	16	22.621	23.394	21.847	26.488	28.421
	0	20.881	18.367	18.947	21.074	24.748
Acetate/NH4Cl	24	0.387	1.160	0.967	4.447	6.187
	16	0.193	0.580	0.193	1.353	0.773
	0	1.160	0.387	0.580	0.387	2.127
Acetate/Tryp	24	20.881	24.748	22.234	25.908	28.421
	16	21.461	22.621	23.587	25.714	29.774
	0	20.881	22.234	21.847	23.974	29.581
Propionate/NH4Cl	24	1.160	0.773	2.513	12.954	18.367
	16	1.353	0.387	0.193	0.967	1.933
	0	0.193	0.387	0.580	1.353	1.160
Propionate/Tryp	24	21.461	23.007	22.621	21.847	24.748
	16	21.654	24.168	22.427	26.101	29.581
	0	19.914	20.107	21.074	22.234	25.134

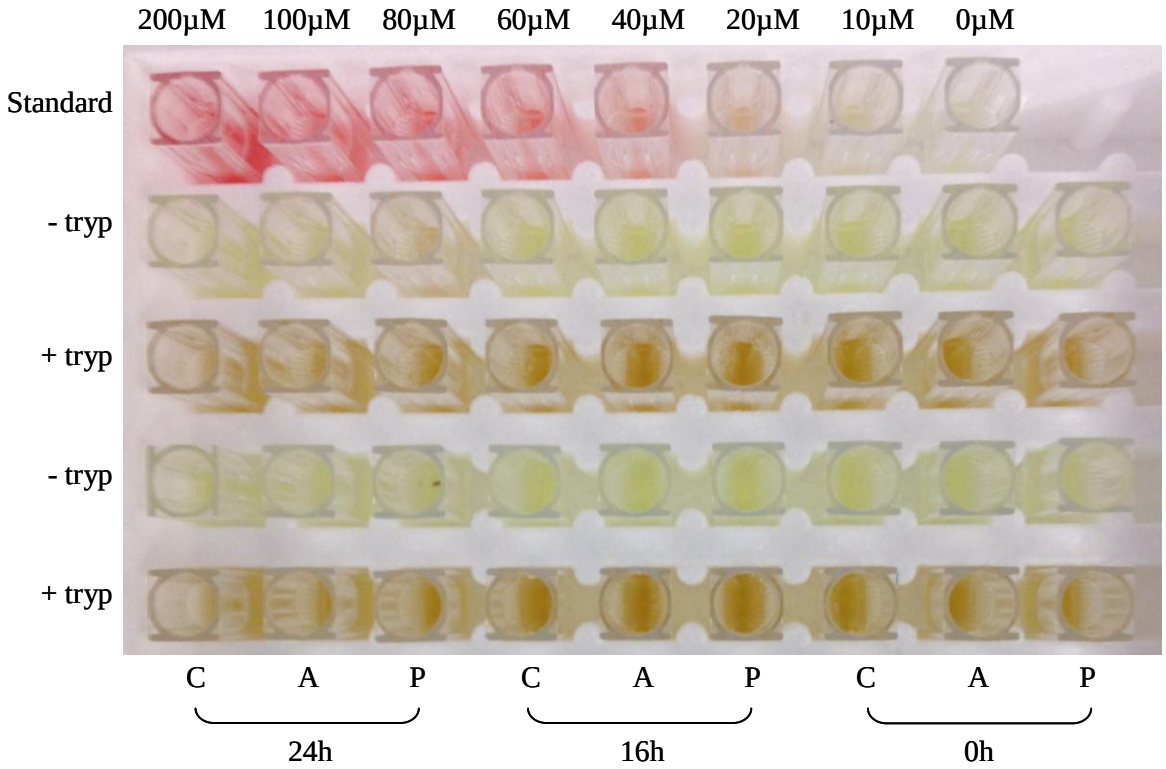

Figure 7. Visible color change between tryptophan supplemented treatments and non-tryptophan supplemented treatments. The top row depicts the IAA standard created with known concentrations of IAA, listed across the top of the photo. Below the photo shows the carbon sources (C, citrate; A, acetate; P, propionate) and the light periods (24h, 16h, 0h).

DISCUSSION

The purple non-sulfur bacteria isolated was most similar to *Rhodovulum* sp. JA545 based on 16S rDNA sequence. This genus was created recently when 16S homology found that *Rhodobacter* species were divided into 2 distinct groups (2). Those members of the *Rhodovulum* genus are the marine *Rhodobacter* species were found to be differentiated phenotypically from the freshwater species on the basis of salt requirement for optimal growth, sulfide tolerance, final oxidation product of sulfide, and polar lipid composition (2). *Rhodovulum* are Gram negative, motile, ovoid/rod-shaped and prefer to grow heterotrophically under anoxic conditions in the presence of light (6). Here, optimal conditions were used and the purple non-sulfur bacteria were given various carbon sources in order to observe optimal carbon metabolism.

When challenged with 3 different carbon sources, 2 nitrogen sources and under various light schedules in all combinations, growth was variable among treatments. Under complete darkness, none of the cultures utilized any carbon source. With more natural light conditions, 16L:8D, acetate was consumed greatest followed by propionate, while under 24h light conditions, propionate was consumed most. Citrate was never consumed under any treatment. This could be because it is a more complex molecule where the carbon is not immediately available. Carbon sources such as citrate and aromatic compounds are not often used by *Rhodovulum* spp. (3). This is not the only occurrence of *Rhodovulum* sp. unable to use citrate as a carbon/electron donor (5, 16, 17). Using citrate is variable for *Rhodovulum* as some of these organisms can use the TCA cycle, where citrate

is an intermediate, while others use derivatives of the TCA cycle, such as the glyoxylate cycle (3). This is used when 2- and 3- carbon acids are used as growth substrate and this is not enough to replenish the 4-carbon acid oxaloacetate to keep the TCA cycle operating (3, 6). Isocitrate lyase is 1 of 2 extra enzymes used to bypass the steps in the TCA cycle that release CO_2 and split isocitrate to succinate and glyoxylate. Gloxylate is then modified by malate synthase to yield malate which is easily converted to oxaloacetate and maintains the TCA cycle (6). If the main method of metabolizing carbon only uses small carbon molecules perhaps the ability of processing larger carbon molecules has been lost. Also notable was that no carbon source was greatly used at any light schedule in the presence of tryptophan as a sole nitrogen source, which leads to questions about how growth correlates to indole-like compounds produced.

Indole-3-acetic acid (IAA) synthesis is a known characteristic of bacteria that inhabit the plant phyllosphere. There is curiosity as to the part IAA synthesis plays in contributing to phytopathogenicity and stimulation of plant growth. There are 6 known pathways for its synthesis, with 5 requiring tryptophan as a precursor (15). Phototrophic bacteria have not been known to produce IAA until very recently (12, 14). Cyanobacteria, a type of oxygenic phototrophic bacteria, have been shown to produce IAA with and without the addition of exogenous tryptophan. This differs from what has been demonstrated here as the ecology of purple non-sulfur phototrophs differs completely from cyanobacteria as these organisms do not release O_2 during any point of their growth. The purple non-sulfur bacteria always produced indole-like compounds with the addition of tryptophan and also without tryptophan when propionate was the sole carbon source and under the 24h light schedule. The Salkowski method was used to screen for indole-like compounds. This method is not the most sensitive option to detect indole-like compounds and has been seen to produce false-negative results (14). Besides this draw-back, the Salkowski method also fails to distinguish between IAA and other indole-like compounds such as indolepyruvic acid, and indoleacetamide, all which stain red (1). Other color reactions have also been seen when the reagent is added to cell supernatant, with other indole compounds reacting olive, brown or purple in color (1). In this study, the cell supernatant of the purple non-sulfur bacteria stained rust-brown upon the addition of the Salkowski reagent. In the Glickmann and Dessaux study, a full spectrum analysis of known indole-like compounds was performed to compare peaks (1). This present study would have benefited from a similar experiment, comparing peaks of known indole-like compounds against the experimental cell supernatants that reacted upon the addition of the Salkowski reagent as any comment on the color-change is purely speculative.

The purple non-sulfur bacteria, most similar to *Rhodovulum* sp. JA545, showed color-positive indication of indole-like compounds present in the supernatant. Although this species does not have a full genome sequence available, a closely related species, *Rhodovulum* sp. PH10, has a draft genome accessible on GenBank (4). When a protein search was performed looking for enzymes utilized in any of the 6 known IAA biosynthetic pathways (15), every enzyme for the tryptophan-independent pathway was annotated in *Rhodovulum* sp. PH10. This pathway is best described in *Arabidopsis thaliana* but has also been seen in microbes, although it is less understood (8, 13, 15). The tryptophan-independent pathway has been illustrated in Figure 8 and the annotated enzymes from *Rhodovulum* sp. PH10 have been designated by their GI numbers. This provides good bioinformatic evidence for IAA production in *Rhodovulum* sp. PH10. The 16S rDNA gene of *Rhodovulum* sp. JA545 is 85% identical with 95% coverage and an e-value of 0.0 to *Rhodovulum* sp. PH10. Although this may not be a good indicator of functional similarity, it does give some rationale as to why these purple non-sulfur bacteria may be able to produce indole compounds. This is the first example of anoxygenic phototrophic bacteria producing indole-like molecules.

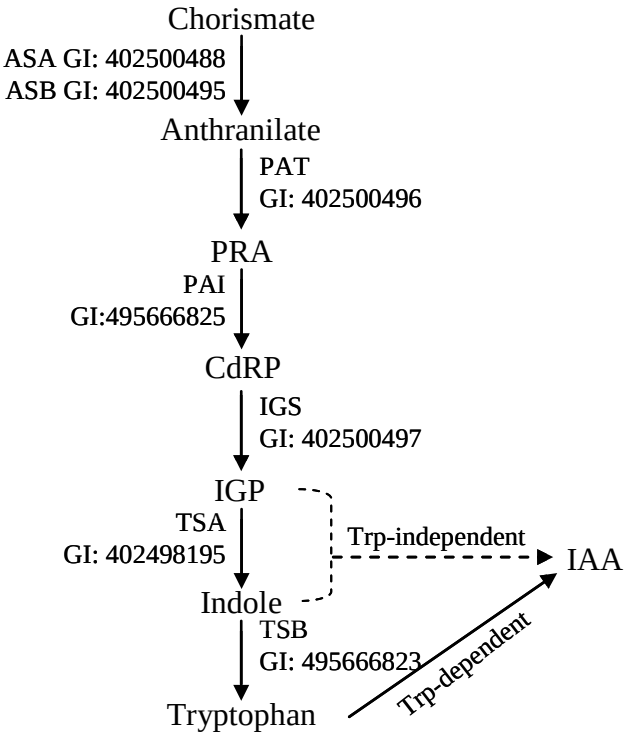

Figure 8. The IAA biosynthesis pathway shown is found in *Arabidopsis thaliana*. IAA can be made through the Trp-dependent and/or Trp-independent pathways in plants and bacteria. The enzymes for each step in the pathway are given with a corresponding *Rhodovulum* sp. PH10 protein GI number. ASA, anthranilate synthase alpha subunit; ASB, anthranilate synthase beta subunit; CdRP, 1-(O-carboxyphenylamino)-1-deoxyribulose-5-phosphate; IGP, indole-3-glycerol phosphate; IGS, indole-3-glycerol phosphate sythase; PAI, phosphoribosylanthranilate isomerase; PAT, phosphoribosylanthranilate transferase; PRA, 5-phosphoribosylanthranilate; TSA, tryptophan synthase alpha subunit; TSB, tryptophan synthase beta subunit. Adapted from Ouyang et al 2000 (8).

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