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Ketocarotenoids in chlorosomes of the acidobacterium *Candidatus* Chloracidobacterium thermophilum

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ABSTRACT

Candidatus Chloracidobacterium thermophilum is a recently discovered aerobic chlorophototroph that belongs to the phylum *Acidobacteria*. It grows in a highly enriched coculture with *Anoxybacillus* sp. and was initially isolated from Octopus Spring in Yellowstone National Park. It has unique properties in that it synthesizes type 1 reaction centers, the bacteriochlorophyll (BChl) a-binding protein FMO, BChl *c* and chlorosomes, but it does so in the presence of oxygen. Here we report the first isolation of chlorosomes from Cab. thermophilum and an initial analysis of their carotenoid content. Unlike the chlorosomes of *Chlorobi* (green sulfur bacteria) and *Chloroflexi*, the chlorosomes of Cab. thermophilum contain large amounts of ketocarotenoids, including echinenone and *cis* and *trans* isomers of canthoxanthin, which are typically found in aerobic phototrophs. We hypothesize that these carotenoids play an important photoprotective role in the chlorosomes of Cab. thermophilum.

INTRODUCTION

The phylum *Acidobacteria* is a poorly characterized bacterial division that contains members that are ubiquitous in soils worldwide (Ludwig *et al,* 1997). The phylum was first defined in 1997 and was named after its first cultured representative, *Acidobacterium capsulatum*, an isolate from an acid mine drainage site (Kishimoto, 1991). Since then, 16S sequence studies have revealed the acidobacteria to be a phylogenetically diverse group, with as many as 11 subdivisions (Zimmermann *et al*, 2005) and with some reports suggesting even 26 subdivisions (Barns *et al,*2007). Little or nothing is known, however, about the role these microorganisms play in

these habitats and only a few examples have been isolated in pure culture (Eichorst *et al*, 2007).

Recently, an acidobacterium capable of phototrophic growth was discovered (Bryant *et al,* 2007). Similar to the green sulfur bacteria, this acidobacterium, *Candidatus* Chloracidobacterium (Cab.) thermophilum, has a type 1 reaction centers, the FMO protein and synthesizes BChl *c* and chlorosomes as antenna structures. However, Cab. thermophilum differs from all known green sulfur bacteria in that it grows aerobically. Here we report that the carotenoids present in the chlorosomes of Cab. thermophilum are unique among all other chlorosome-containing phototrophs. They are more similar to the carotenoids synthesized by other aerobic phototrophs, such as the cyanobacteria and the aerobic anoxygenic phototrophs, than to those produced by anaerobic anoxygenic chlorophototrophs.

MATERIALS AND METHODS

Growth of bacterial cultures. Cab. thermophilum was grown in a highly enriched culture with *Anoxybacillus* sp. as previously described (Bryant *et al,* 2007).

Isolation and electron microscopy of chlorosomes. Cab. thermophilum chlorosomes were isolated as previously described (Vassilieva *et al,* 2002) with the following modifications. After the ultracentrifugation step, both supernatant and pellet fractions contained significant amounts of chlorosomes as verified spectrospically. The pellet fraction was loaded onto a 7-47% (w/v) sucrose gradient in chlorosome isolation buffer (CIB: 2M sodium thiocyanate, 10 mM Tris-HCl, 5 mM EDTA, 1 mM PMSF and 2 mM DTT) and centrifuged for 18 h at 220,000 x *g*. The supernatant fraction was brought to 20% (w/v) sucrose and overlaid with a 5% (w/v) sucrose solution in CIB. This gradient was also centrifuged for 18 h at 220,000 x *g*. The chlorosome fractions obtained from both gradients were resuspended in phosphate-buffered saline (10 mM potassium phosphate buffer, pH 7.2, 150 mM NaCl) and pelleted by centrifugation (2 hours at 240,000 x *g*). The pelleted chlorosomes were resuspended in a small volume of phosphate-buffered saline, aliquoted, and stored at –80ºC until required. Isolated chlorosomes were negatively stained with 1% (w/v) uranyl acetate and visualized in a JEOL 1200 EXII Transmission electron microscope (Peabody, MA).

HPLC analyses. Pigments from chlorosomes, whole cells and a canthaxanthin standard were extracted with a 7:2 (v/v) acetone:methanol mixture. Whole cells were subject to sonication. Prior to injection into the HPLC column, samples were filtered and adjusted with 0.1 volume of 1 M ammonium acetate. The column, analyzing software and buffer system used to separate the pigments have been described before (Bryant *et al*, 2007, Frigaard *et al*, 1997).

RESULTS

Chlorosome isolation and electron microscopy

Following a protocol previously described for the isolation of chlorosomes from green sulfur bacteria, we were able to isolate chlorosomes from Cab. thermophilum. A significant fraction (about 66%) of the chlorosomes of Cab. thermophilum were "lighter" than those of the green sulfur bacteria and remained in the supernatant fraction following ultracentrifugation. These chlorosomes were termed "light chlorosomes," while "heavy chlorosomes " (~1/3 of the total) were found in the pellet fraction. The heavy chlorosomes appeared as a dark brownish-green band on top of a 7-47% continuous sucrose gradient following ultracentrifugation at 220,000 x *g* for 18 h. The light chlorosome fraction was found as a broad green band on the top of a 5-20% step sucrose gradient. The presence of chlorosomes in all fractions was verified by BChl *c* absorption, which was maximal at approximately 745 nm for both light and heavy chlorosome fractions.

Electron microscopy of the final chlorosome fractions revealed that these structures closely resemble those of the green sulfur bacteria and the *Chloroflexi* (see figure 1). They are oval-shaped and, in general, about 200 nm in length and 70 nm wide.

HPLC analysis

The HPLC elution profile of isolated chlorosomes revealed 8 compounds that showed strong absortion at 491 nm (see Figure 2). We were able to positively identify peak 8 as β, β-carotene, since its two absorbance maxima of 452 nm and 478 nm in acetone/methanol match the known absorbance maxima for β, β-carotene, and its retention time corresponded to the retention time of β, β-carotene in *Synechococcus* sp. PCC 7002 using the same HPLC elution protocol. Peaks 6 and 7 have tentatively been identified as lycopene and γ-carotene, respectively. These are the precursors to β, β-carotene, and their elution times correspond to species slightly less hydrophobic than β, β-carotene.

The absorption spectra of peaks 1, 2, 3 and 5 were typical of ketocarotenoids, in which a keto group has been added to either the ring or the open chain. The addition of a keto group causes loss of fine structure in the absorption spectra and results in a single broad peak (see Figure 2). The absorption maximum of peak 5 corresponds to the absorption maximum of echinenone in acetone/methanol (465 nm), and this compound eluted at the same time as echinenone from *Synechococcus* sp. PCC 7002 (data not shown). Peak 1 had an

absorption maximum of 477 nm, a value that closely matches the maximum for canthaxanthin in ethanol (474 nm). This compound eluted at the same as an authentic canthaxanthin standard. Peak 2 corresponded to a slightly more hydrophobic species and had an absorption maximum at 466 nm. The presence of a "*cis*-peak" in the absorption spectrum at 365 nm, and its similarity to a compound in the canthaxanthin standard, allow us to tentatively identify this compound as *cis*-canthaxanthin.

DISCUSSION

Three phyla of the *Bacteria*, *Chlorobi*, *Chloroflexi*, and now *Acidobacteria*, have chlorophototrophic members that can synthesize chlorosomes. The isolated chlorosomes of the newly described acidobacterium, Cab. thermophilum are similar and size and overall appearance to those of *C. tepidum* (Frigaard *et al*, 2005). However, Cab. thermophilum is presently the only organism that has been shown to synthesize and to utilize these structures under oxic conditions. Cab. thermophilum was discovered in the microbial mats of alkaline, siliceous hot springs in Yellowstone National Park that are subject to very high irradiances. Because of both the high light intensity and the presence of oxygen, we anticipated that Cab. thermophilum might have unique photoprotection mechanisms to minimize the deleterious effects of reactive oxygen species and triplet states of BChl *c* due to excessive excitation. This study shows that the chlorosomes of *Cab. thermophilum* contain high levels of carotenoids that have not been reported to occur in the chlorosomes of organisms that grow in anoxic environments. This observation suggests that the ketocarotenoids may play an important role in photoprotection in this microorganism. Interestingly, we have identified a divergent, CrtO-type carotenoid ketolase, which likely derives from Cab. thermophilum, in the metagenomic sequence library created for the mats of Octopus Spring. This type of ketolase, which requires oxygen as a co-substrate, would presumably oxidize β, β-carotene to echinenone and subsequently convert echinenone to canthaxanthin. Prior to this study, ketocarotenoids had only been found in phototrophs belonging to the cyanobacteria, algae, higher plants, as well as the aerobic Bradyrhizobia. Our study shows that ketocarotenoids are also present in another aerobic anoxygenic phototroph, Cab. thermophilum.

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FIGURE LEGENDS

Figure 1. Electron micrograph of negatively stained Cab. thermophilum chlorosomes.

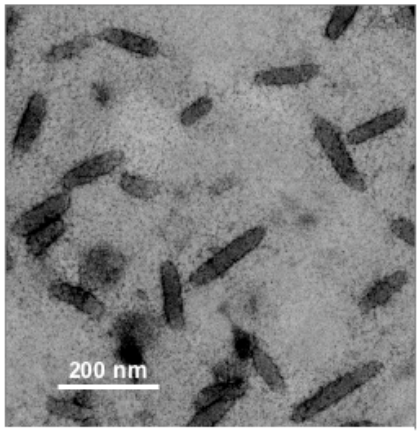

Figure 2. A. Elution profile monitored at 491 nm of pigments extracted from *Cab. thermophilum*'s chlorosomes. B. Absorption spectra of peak 1. C. Absorption spectra of peak 5.