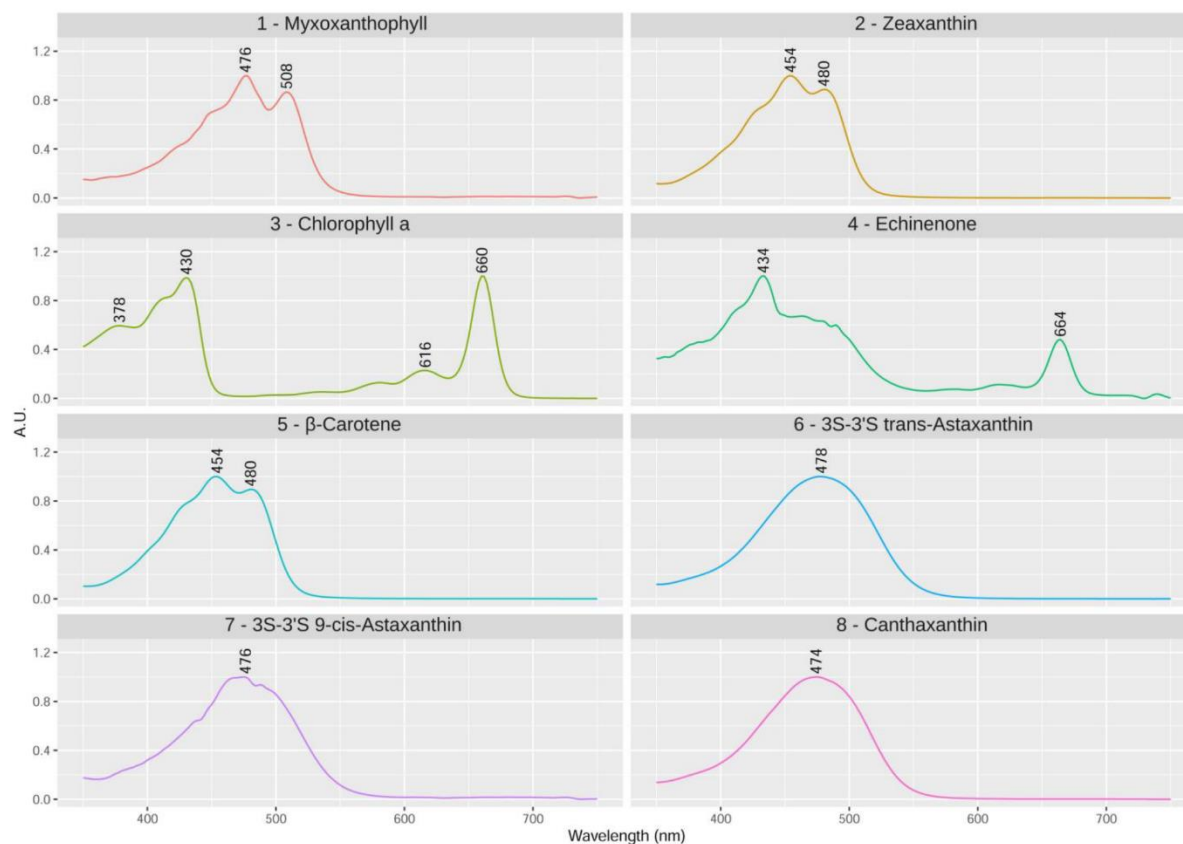
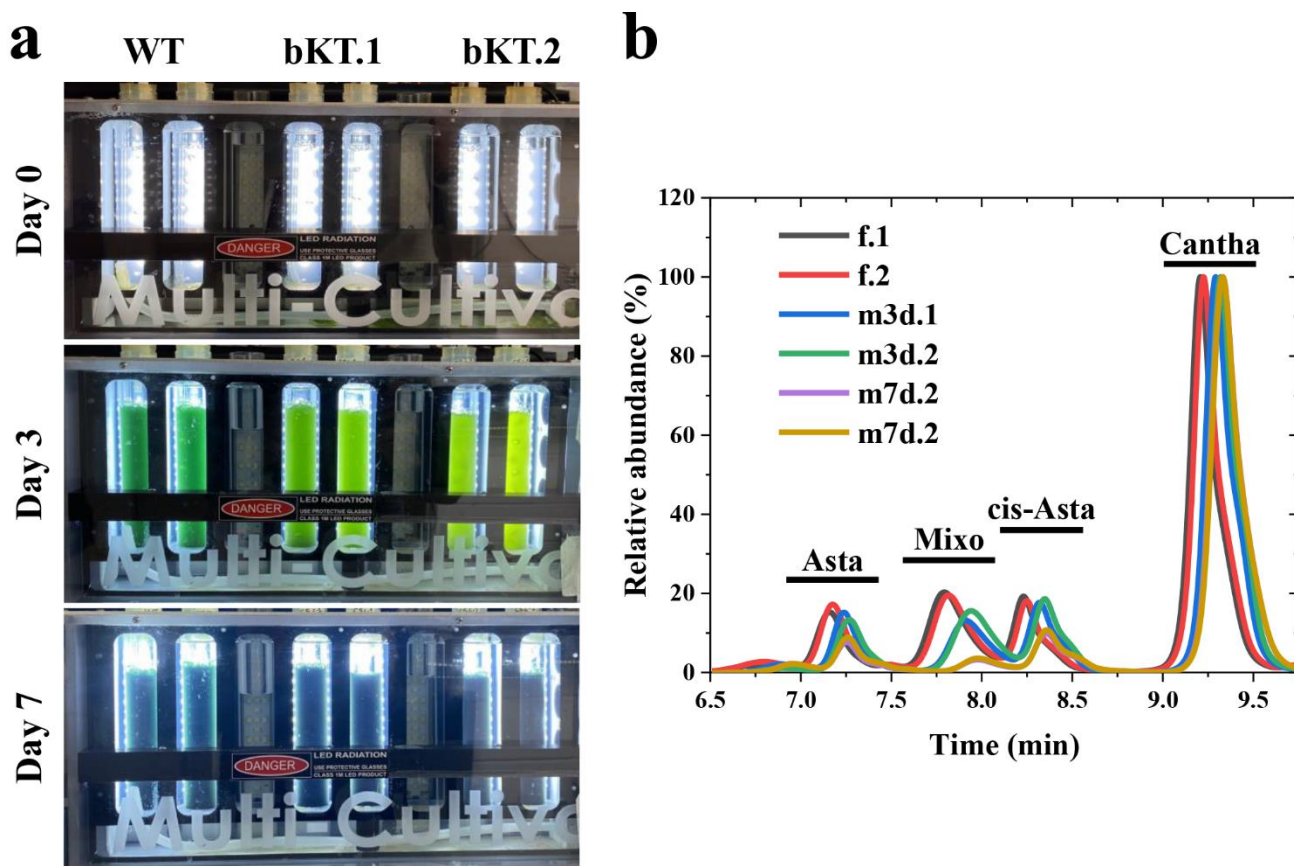


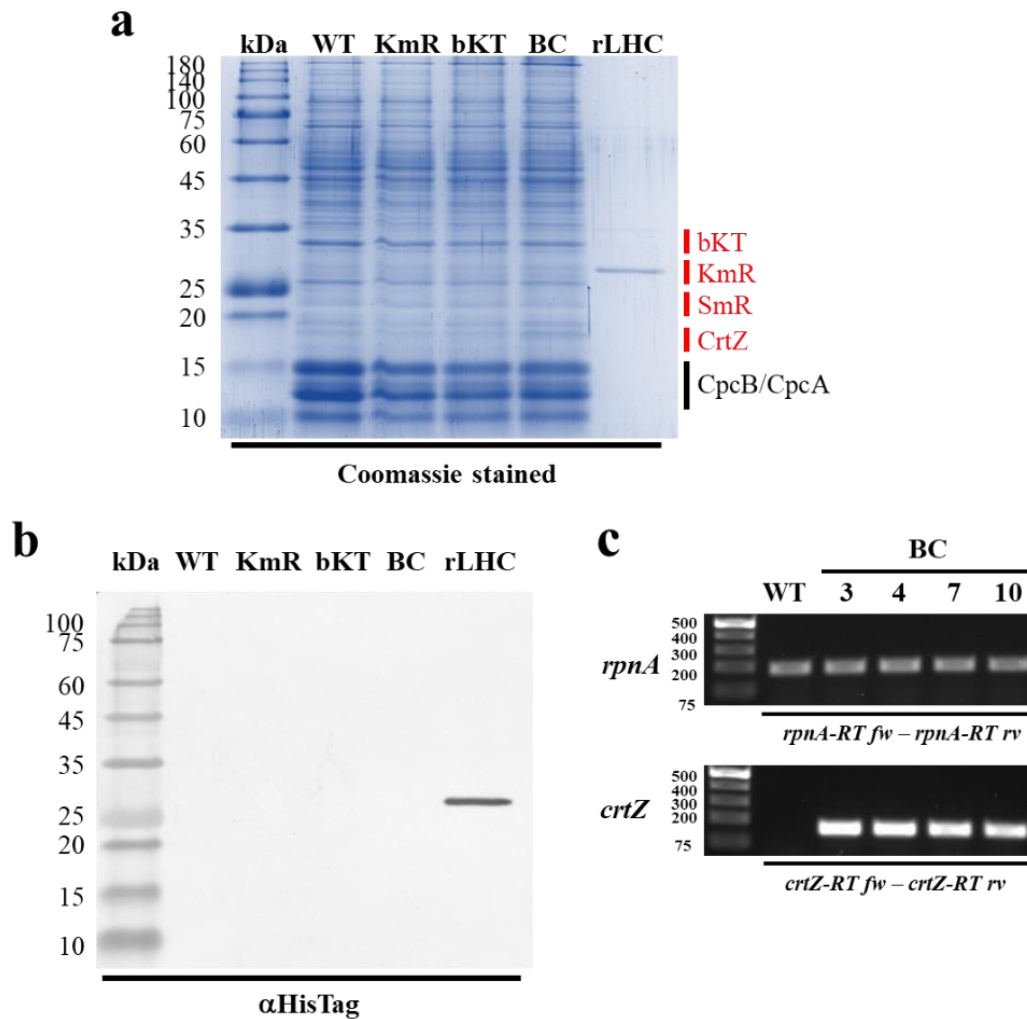
Supplementary Materials



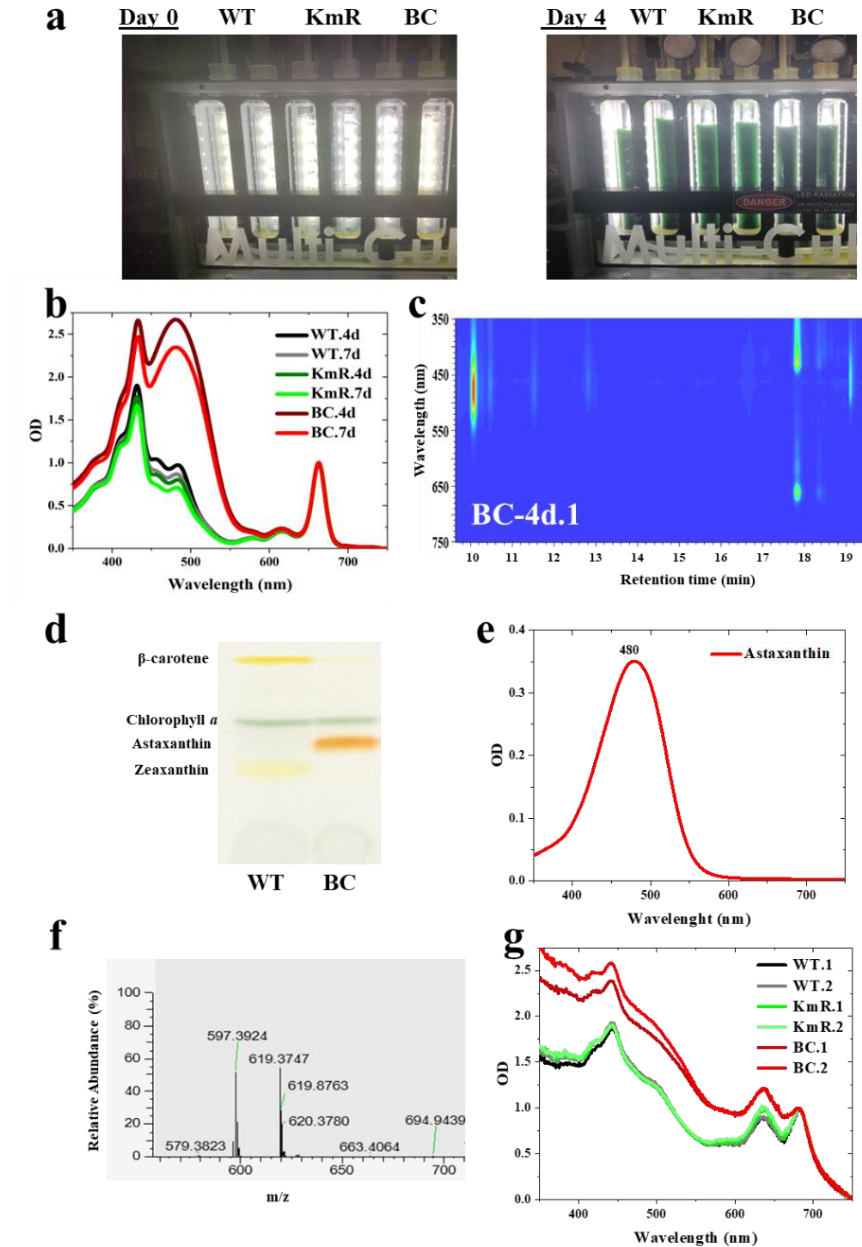
Supplementary Figure 1. Absorption spectra in the visible light range (350-750 nm) of eluted fractions from HPLC analysis of extracts from WT and bKT cultures, as shown in Figure 2f. 1, mixoxanthophyll; 2, zeaxanthin; 3, chlorophyll *a*; 4, echinenone (fraction contaminated with chlorophyll *a*); 5, β-carotene; 6, 3S,3'S trans-Astaxanthin; 7, 3S,3'S 9-cis-Astaxanthin; 8, canthaxanthin.



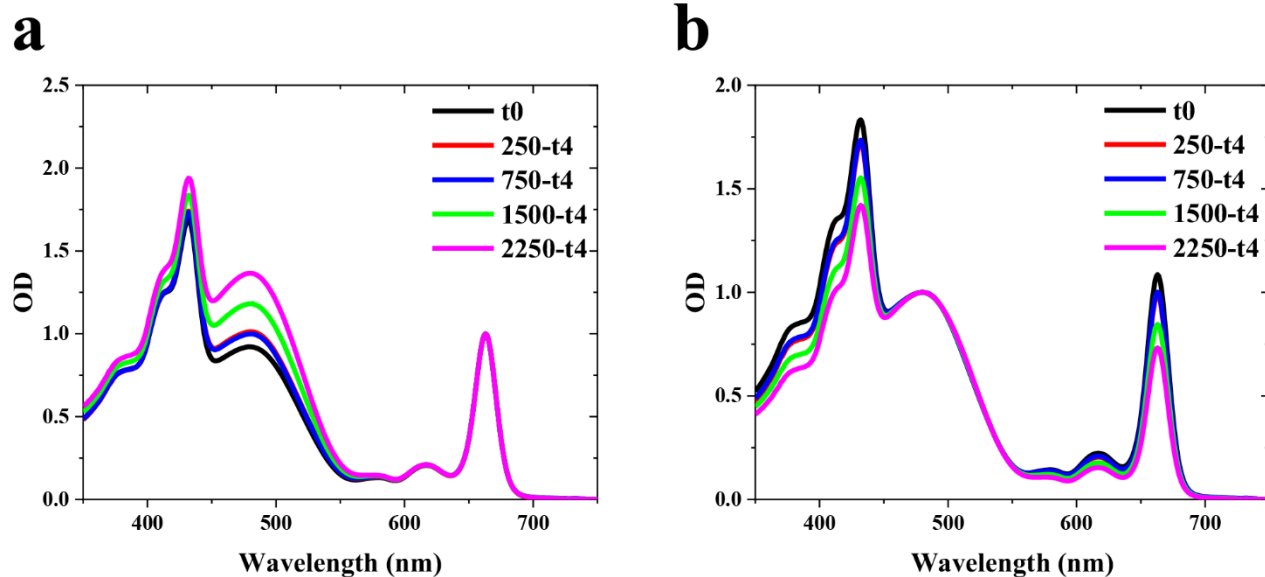
Supplementary Figure 2. Comparison of astaxanthin accumulation in bKT transformants grown in flasks or in 80 ml air-lift photobioreactors. **(a)** Growth of WT and bKT lines in air-lift PBRs in a Multicultivator system exposed to 1500 $\mu\text{mol}/\text{m}^2/\text{s}$ irradiance with 3% CO_2 -enriched air supply. Pictures were taken on days 0, 3, and 7. **(b)** Relative abundance of ketocarotenoids in bKT transformants grown in flasks, as shown in Figure 2d, and upon 7 days of growth in a Multicultivator system. HPLC chromatograms were normalized to the peak attributed to canthaxanthin. Asta, 3S,3'S trans-Astaxanthin; Mixo, mixoxanthophyll; cis-Asta, 3S,3'S 9-cis-Astaxanthin; Cantha, canthaxanthin.



Supplementary Figure 3. Protein expression and RT-PCR analysis of *Syn11901* wild type and transformants. **(a)** Total cellular protein extracts from WT, KmR, bKT, and BC cells were resolved by SDS-PAGE and visualized by Coomassie staining. Sample loading corresponds to 0.45 μ g of chlorophyll. CpcB and CpcA subunits of phycocyanin were recognized as the most abundant polypeptides and marked with a black bar, whereas putative migration regions of heterologous proteins were marked in red. A recombinant his-tagged protein (rLHC) was used as a control. **(b)** Total protein extracts of (a) were subjected to Western-blot analysis with loading of the lanes as above. Specific polyclonal antibodies against the his-tag domains were used to probe target proteins. **(c)** RT-PCR analysis for verifying the expression of transgenic *crtZ* gene and constitutive *rpnaA* gene transcripts in WT and BC cell cultures. The PCR products were separated on 1.5% agarose gel. The expected size of the PCR products was 152 and 231 bp for *crtZ* and *rpnaA* gene transcripts, respectively. Numbers represent the days of growth.



Supplemental Figure 4. HPLC, TLC and MS analysis of BC strain. **(a)** Cultivation of WT, KmR and BC cells in air-lift PBRs. Pictures were taken at day 0 and 4. **(b)** Representative absorption spectra (350-750 nm) of DMSO-acetone extracts from WT, KmR, and BC cells grown in air-lift PBRs. Cells were harvested after 4 (4d) and 7 days of growth (7d). **(c)** HPLC analysis of a representative sample of BC extracts obtained from cells grown for 4 days in an air-lift PBR. **(d)** Thin Layer Chromatography of isopropanol extracts from wild-type (WT) and BC cells. **(e)** Absorption spectrum of Asta fraction eluted from (d) in acetone 80%. Maximum of absorption was at 480 nm. **(f)** Mass Spectrometer analysis of eluted Asta fraction from (d). Identified ions derived from Asta were the protonated molecule $[M+H]^+$ at *m/z* 597 and the metal adduct ion $[M+Na]^+$ at *m/z* 619. **(g)** Whole-cell spectra in the visible range of light (350-750 nm) of cultures grown in air-lift PBRs.



Supplementary Figure 5. Absorption spectra in the visible range of light (350-750 nm) of pigments extracts from cultures grown 4 days in air-lift PBRs and described in Figure 6. The number comprised in each name of the different samples indicates the light intensity used for the cultivation ($\mu\text{mol}/\text{m}^2/\text{s}$), whereas “t” represents the day of cultivation. **(a)** Spectra normalized to the Chl *a* content. **(b)** Spectra normalized to the carotenoid content.