DR2005170

REPOSITORY DATA

Isolation and identification of pigments

Cells from 10 ml of cultures growing phototrophically with hydrogen or acetate as electron donor were harvested by centrifugation (10 min; 7,800 x *g*) after reaching stationary growth phase. For pigment extraction, 5 ml of a mixture of acetone and ethanol (1:1) were added and all following procedures were done under extremely dim light to protect the pigments from phototransformations. The suspension was sonicated for 2 min and incubated in the dark at 30°C for 1 h. The pigments were transferred to hexane by adding 3 ml of hexane and 0.5 ml of H_2O. The upper phase was collected and replaced several times until it stayed clear. The combined hexane fractions were concentrated ~10-fold under a stream of N_2 and stored at -20ºC before further analysis. The extracted pigments were separated using a normal-phase thin-layer chromatography (TLC) system with silica as adsorbent (Kieselgel 60, Merck, Darmstadt, Germany) and a mixture of petrolether and acetone (4:1) as mobile phase. The R_f values of the separated compounds were determined as the distance moved by the separated compound divided by the distance moved by the mobile front. The colored bands on the TLC plates were scraped off, the material was collected and extracted with dichloromethane. After filtration through glass wool in a Pasteur pipette to remove the silica particles, the solvent was removed under a stream of nitrogen gas and the isolated pigments were dissolved in hexane to record absorbance spectra in a quartz 96 well microtiterplate in a microtiterplate reader (Synergy HT, Bio-Tek, Winooski, VT). Hexane pigment extracts were analyzed with a high-pressure liquid

chromatography system (Waters, Milford, MA), equipped with a diodearraydetector and a Spherisorb ODS-2 column (5 μm particle size; 250 x 4 mm) with a mixture of acetonitrile/isopropanol (93:7) as solvent at 30°C and a flow rate of 1 ml/min.

Carotenoids were identified by comparison with reference compounds from strains with known carotenoid composition and comparison to reference absorption spectra and TLC data (Zuellig, 1985; Britton, G., 1995). Pigments from cells of strains SW2, F4, and KoFox growing with Fe^{2+} as electron donor were isolated as described above and the extracts showed colored bands on the TLC plates with the same R_f values as the hydrogen-/acetate-grown cells. Because of higher cell yields and thus higher contents of pigments, hydrogen- and acetate-grown cells were routinely used for all pigment analysis.

TABLE 1. MAIN CAROTENOIDS PRESENT IN THE FE(II)-OXIDIZING BACTERIA *RHODOBACTER FERROOXIDANS* STRAIN SW2, *THIODYCTION SP.* STRAIN F4, AND *CHLOROBIUM FERROOXIDANS* STRAIN KOFOX

Fe(II)-oxidizing bacteria	Compound (color)	Absorption maxima (nm)[a] [Ref. values][b]	HPLC t_R (min)	TLC R_f
Rhodobacter ferrooxidans strain SW2	spheroidene (yellow)	430, 455, 486 [429, 454, 486]	18.1	0.83-0.91
	spheroidenone (pinkish-red)	465, 480, 517 [460, 483, 515]	12.8	0.73-0.82
	OH-spheroidene (yellow)	430, 454, 486 [429, 455, 487]	10.0	0.39-0.54
Thiodictyon sp. strain F4	rhodopinal (purple-red)	362, 490-512(broad) [360, 496]	7.35	0.37
Chlorobium ferrooxidans strain KoFox	chlorobactene (yellow)	435, 462, 491 [435, 461, 491]	16.24	0.93

[a] Absorption maxima measured with a diode-array detector after separation of organic extracts by high-pressure liquid chromatography.

[b] Reference values in hexane or petrolether are from G. Britton, 1995 and Zuellig, 1985.

REFERENCES CITED IN REPOSITORY DATA

Britton, G., 1995, UV/Visible Spectroscopy, *in* Britton, G., Liaaen-Jensen, S., and Pfander, H., eds., Carotenoids: Vol 1B: Spectroscopy: Birkhäuser Verlag, Basel, Switzerland.

Zuellig, H., 1985, Pigmente phototropher Bakterien in Seesedimenten und ihre Bedeutung fuer die Seenforschung: Schweizerische Zeitschrift für Hydrologie, v. 47, p. 87-126.