SUPLEMENTARY MATERIAL

Antiproliferative Activity of Carotenoid Pigments Produced by Extremophile Bacteria

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Experimental section

Bacterial Strains and Culture Conditions

Deinococcus sp. UDEC-P1 was isolated from an oligotrophic lake in Chilean Patagonia (44°22'S 72º25'W) (Fernandez-Bunster et al. 2012). *Arthrobacter* sp. UDEC-A13 was isolated from a seawater sample collected from Antarctica (63°19'S 57°53'W). In order to identify this strain, its 16S rRNA gene was amplified by PCR using universal primers for bacteria (Weisburg et al. 1991). PCR product was sequenced by Macrogen Inc. (Seoul, Korea) and the sequence was analyzed using Blastn of NCBI databank (http://www.ncbi.nlm.nih.gov/BLAST/).

Production and Purification of Bacterial Pigments

Both bacterial strains were cultured in 10 L of R2A broth (shaking 120 rpm). *Deinococcus* sp. UDEC-P1 was incubated 48 h at 30 °C and *Arthrobacter* sp. UDEC-A13 96 h at 10 °C. Bacterial cultures of *c.a.* 1.0 x 10^9 CFU ml^{-1} were centrifuged at 5000 *g* for 10 min. Cellular pellets were washed three times using distilled sterilized water. Then, pellets were centrifuged (5000 *g* for 10 min) and suspended in 30 mL methanol four times (Choi et al. 2014). Solvent from supernatants (120 mL) were evaporated at reduced pressure in a rotary evaporator at 40 °C (Heildolph Unimax 2010). Dried extracts were weighted in order to obtain the yield, which were *c.a.* 60 mg per liter of cultures.

Afterward, 10 mg of extracts were dissolved in 2 mL of acetone (to obtain a final concentration of 5 mg mL^{-1}) and analyzed by HPLC using a Young Lin apparatus (Gyeonggi, Korea) equipped with a binary pump (YL 9111S), PDA detector (YL 9160) and a Kromasil C18 250 x 4.6 mm column. An acetone (70–100%)/water gradient was used for elution (flow rate of 1.1 mL min^{-1}).

From crude extracts, different fractions were purified by preparative HPLC and then dissolved in DMSO to be used in further antiproliferative assays. Preparative HPLC were done using a Kromasil C18 250 x 10.0 mm column and an acetone (70–100%)/water gradient for elution (flow rate 5 mL min^{-1}). A β-carotene standard (EMD Chemicals Inc., USA) was used as control. From *Deinococcus* sp. UDEC-P1 the main signal (signal 1, deinoxanthin) was purified and from *Arthrobacter* sp UDEC-A13 three fractions were obtained according to their retention times: F1 (signals 1, 2 and 3 collected at times 13.0 - 13.6 min), F2 (signal 6 collected at 17.5 min) and F3 (signals 7 and 8 collected at times 17.9 - 18.3 min).

Tumour Cell Lines and Culture Conditions

Cancer cells used were Neuro-2a (ATCC CCL-131, mouse neuroblastoma), Saos-2 (ATCC HTB-8, osteosarcoma) and MCF-7 (ATCC HTB-22, human breast cancer) obtained from the American Type Culture Collection (ATCC) (Manassas, USA). Neuro-2a and Saos-2 cells were cultured in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS), 50 U mL^{-1} penicillin and 50 μg mL^{-1} streptomycin. MCF-7 cell line was cultured in DMEM medium supplemented with 10% FBS plus the same antibiotic mixture as above. The cell lines were cultured routinely at 37 ˚C in a 5% CO_2 atmosphere.

Evaluation of Antiproliferative Activity of Bacterial Pigments

One hundred microliter of tumour cells were seeded in 96-well plates to obtain 5.0×10^4 cells mL^{-1} and incubated 24 h under above described conditions. Then, the culture medium was replaced by 100 μl of fresh media containing 40 μg mL^{-1} of each pigment (in DMSO at 0.1 % v/v) and incubated 24 h (Pasquet et al. 2011). Next, the culture medium was replaced by basal medium containing 0.83 mg mL^{-1} of 3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium (MTT) solution (Torres et al. 2016). The viability of tumour cells was evaluated by MTT reduction into formazan. After solubilization of formazan crystals with DMSO, coloured solution was measured at 570 nm using a Synergy H1 microplate reader (Biotek, USA). Controls included the cell lines plus fresh medium and fresh medium plus 0.1% DMSO.

Statistical Analysis

Statistical analysis was performed using SPSS v.18.0 (IBM Corp., Armonk, NY, USA). The data were treated with one-way analysis of variance (ANOVA). Differences were considered statistically significant when $p<0.05$.

References

Choi Y, Hur J, Lim S, Jo M, Kim D, Choi J. 2014. Induction of apoptosis by Deinoxanthin in human cancer cells. *Anticancer. Res*., vol. 34, pp 1829-1836.

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Table S1. Pigments detected in *Deinococcus* sp. UDEC-P1 and *Arthrobacter* sp. UDEC-A13 extracts.

Bacterial Strain	Signal HPLC analysis	Retention time (min)	λ_{Max} (nm)
Deinococcus sp. UDEC-P1	1	9,9	458, 482, 510
	2	10,3	456, 580, 507
	3	10,9	554, 578, 504
	4	11,47	556, 579, 504
	5	12,5	461, 483, 511
Arthrobacter sp. UDEC-A13	1	13,0	417, 442, 471
	2	13,6	417, 442, 471
	3	13,9	417, 442, 471
	4	15,5	414, 438, 468
	5	15,9	412, 436, 464
	6	17,5	417, 441, 471
	7	17,9	417, 441, 471
	8	18,3	418, 442, 472
	9	18,8	414, 337, 467
	10	19,0	414, 437, 467
	11	19,3	413, 436, 466
	12	19,5	413, 436, 466

Figures

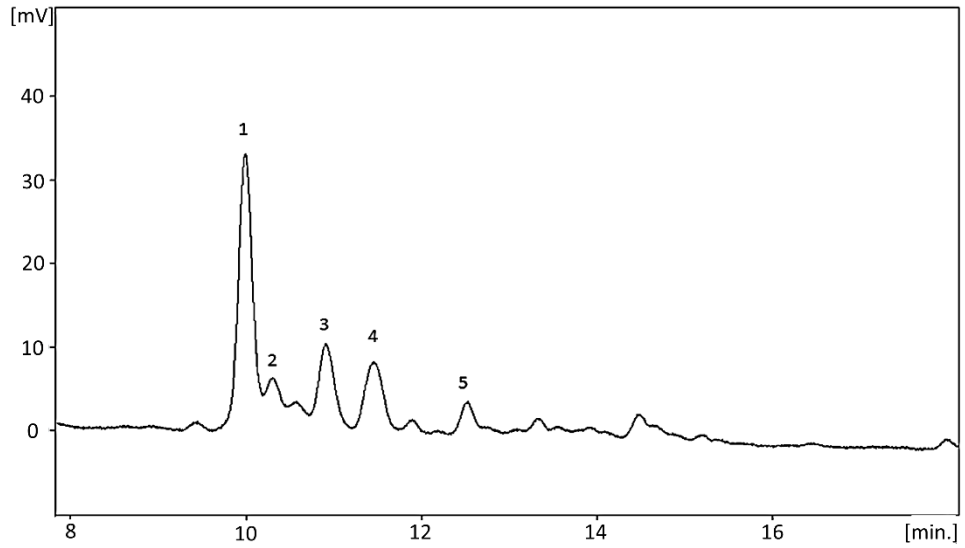

Figure S1. HPLC chromatographic profile at 440 nm of the carotenoid extract from *Deinococcus* sp. UDEC-P1. Main signal (signal 1, retention time 9.9 min) corresponds to the carotenoid deinoxanthin. This compound was purified and used for further antiproliferative assays.

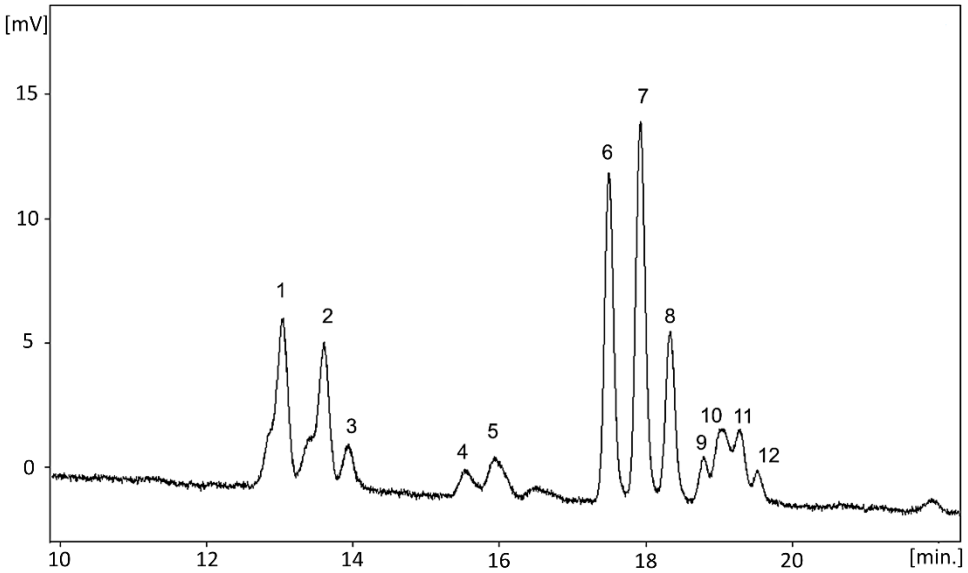

Figure S2. HPLC chromatographic profile at 440 nm of the carotenoid extract from *Arthrobacter* sp. UDEC-A13. Main signal (signal 7, retention time 17.9 min) corresponds

to the carotenoid decaprenoxanthin. Three fractions were purified and used for antiproliferative assays: F1 (signals 1, 2 and 3), F2 (signal 6) and F3 (signals 7 and 8).

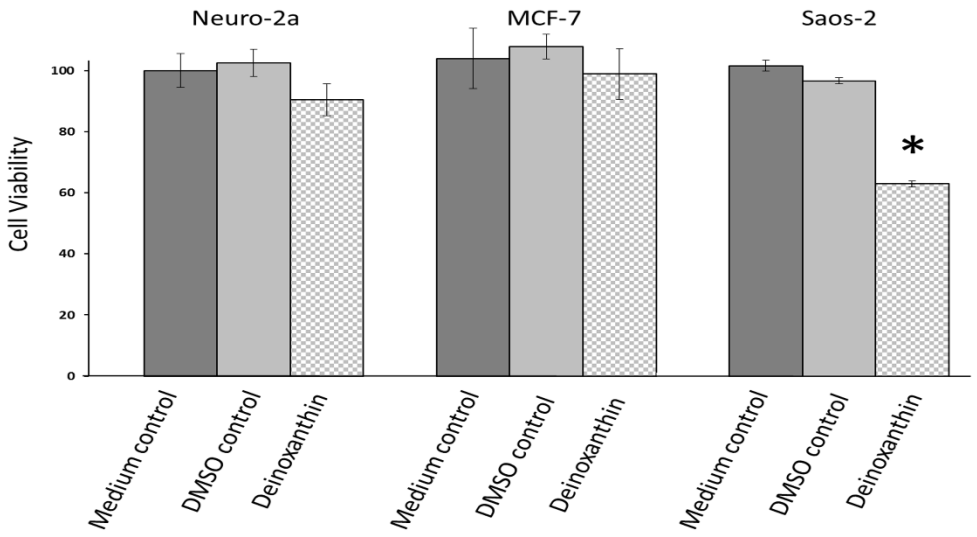

Figure S3. The effect of *Deinococcus* sp. UDEC-P1 deinoxanthin (40 μg mL^{-1}) on viability of Neuro-2a, MCF-7 and Saos-2 cell lines after 24 h of incubation. A control corresponds to cells cultured in fresh medium and another control corresponds to cells cultured in fresh medium plus 0.1 % DMSO. (*) indicates statistically significant differences ($P<0.05$).

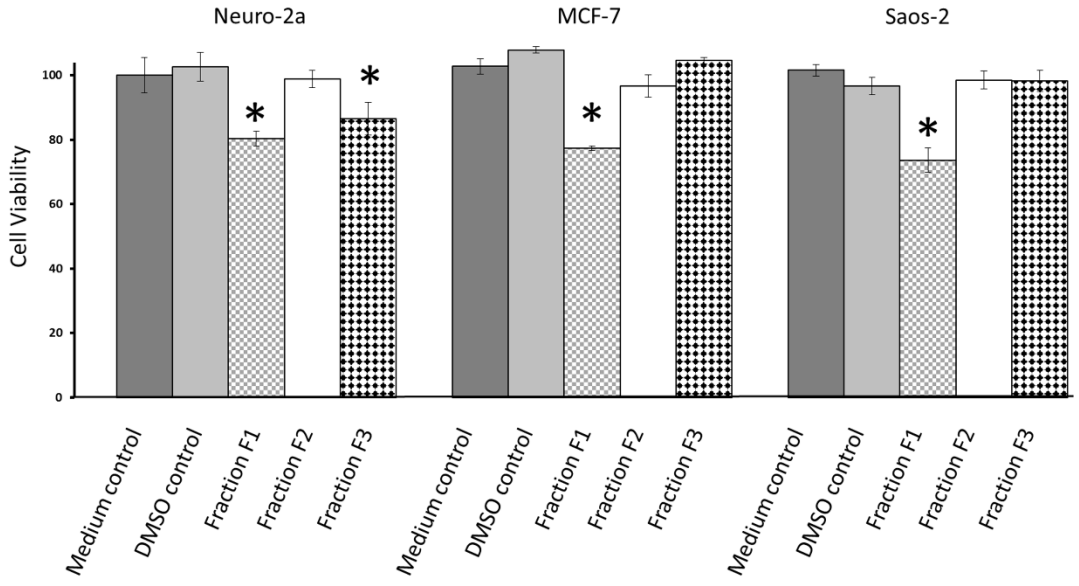

Figure S4. The effect of *Arthrobacter* sp. UDEC-A13 carotenoids fractions (40 μg mL^{-1}) on viability of Neuro-2a, MCF-7 and Saos-2 cell lines after 24 h of incubation. A control corresponds to cells cultured in fresh medium and another control corresponds to cells

cultured in fresh medium plus 0.1 % DMSO. (*) indicates statistically significant differences ($P<0.05$).