MICROALGAE FROM NORTHERN CHILE. III. GROWTH AND BETA-CAROTENE CONTENT OF THREE ISOLATES OF *Dunaliella salina* FROM THE ATACAMA DESERT.

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PATRICIA ARANEDA[1], CYNTHIA JIMENEZ[1] and BENITO GOMEZ-SILVA[1].

Patricia Araneda[1], Cynthia Jiménez[1] and Benito Gómez-Silva[1]: Microalgae from northern Chile. II. Growth and beta- carotene content of three isolates of *Dunaliella salina* from the Atacama Desert.

Three carotenogenic isolates of *Dunaliella salina* Teodoresco 1905, from athalassohaline and thalassohaline habitats of the Atacama Desert in Chile were grown in two qualitatively different growth media at two light intensities and nitrate concentrations. Specific growth rates, cell densities and beta-carotene contents were determined on the three isolates. Isolate CONC-007 accumulate 100 pg beta- carotene cell^{-1} during outdoor growth in a low-cost medium prepared with locally produced technical-grade chemicals. This isolate represents a natural renewable resource of desertic northern Chile and alternative source of natural beta-carotene.

Key Words: Beta-carotene; *Dunaliella*; Growth media; Microalgal biotechnology.

(1) Instituto del Desierto, Universidad de Antofagasta. P.O. Box 170- Antofagasta, Chile.

RESUMEN. Microalgas del norte de Chile. III. Cultivo y contenido de beta-caroteno de tres cepas de *Dunaliella salina* del Desierto de Atacama.

Tres cepas carotenogénicas de *Dunaliella salina* Teodoresco, 1905, aisladas de habitats talasosalinos y atalososalinos del Desierto de Atacama en Chile, se cultivaron a dos intensidades de luz y concentraciones de nitrato en dos medios de cultivos cualitativamente diferentes. Tiempos de duplicación, densidades celulares y contenido de beta-caroteno se determinaron en los tres organismos. Uno de ellos, CONC-007, alcanzó concentraciones de 100 pg de beta-caroteno por célula, al ser cultivado a la intemperie en un medio de bajo costo preparado con sales de grado técnico producidas localmente. Este organismo representa un recurso natural renovable del Desierto chileno y una fuente alternativa natural de beta-caroteno.

INTRODUCTION

Carotenogenic species of the halotolerant green alga *Dunaliella* are able to accumulate large amounts of beta-carotene, more than 10% of the algal dry weight, under appropiate growth conditions (Loeblich, 1982; Ben-Amotz & Avron, 1983). High salt concentrations, extreme temperatures, nitrogen limitations and high light intensities trigger beta-carotene biosynthesis in *Dunaliella salina* and *Dunaliella bardawil* (Ben-Amotz & Avron, 1983; Borowitzka *et al.*, 1990). The pigment is accumulated in globules in the interthylakoidal spaces of the algal chloroplast (Ben-Amotz *et al.*, 1982). Thus, *Dunaliella* has been

recognized as a commercial source of natural beta-carotene for the food and feed industry (Borowitzka & Borowitzka, 1988). Estimates of aproximately US$ 10 kg^{-1} beta-carotene have been reported as costs of production for the pigment, excluding extraction and processing costs (Borowitzka & Borowitzka, 1988). Even though these costs of production are understimations, natural beta- carotene from *Dunaliella* is sold in US$ 1,000- 2,000 kg^{-1} beta- carotene, depending upon the commercial product formulation (Vonshak, 1990).

Availability and introduction of new or improved strains/species of carotenogenic *Dunaliella* should impact positively on commercial beta-carotene production. The present work examines the effect of light intensity and nitrate concentration on the beta-carotene content of three *Dunaliella salina* isolates from northern Chile cultured in a low-cost growth medium. One isolate accumulates beta-carotene to levels 2-4 fold higher than *Dunaliella* strains used nowadays in outdoor microalgal biomass production systems. A brief report of this work was presented at the XII Jornadas de Ciencias del Mar (1992) Santiago, Chile.

MATERIALS AND METHODS

ALGAE. D. salina isolate CONC-001 was obtained from La Rinconada pond, a thalassohaline water body located 30 km north of Antofagasta city (Goméz-Silva *et al.*, 1990). CONC-003 and CONC-007 were isolated from the Atacama Salar, a 3,000 km^2 salt-flat at 2,340 m above sea level (Goméz-Silva, 1991). Unialgal cultures were obtained from the microalgal collection at the Botany Department, Universidad de Concepción, Chile.

GROWTH CONDITIONS. Duplicates of algal cultures were grown in 50-100 ml of a growth medium defined by Pick *et al.* (1986) and in a low-cost KSP growth medium formulated by Araneda (1992a,b). Table 1 shows the chemical composition of the growth media and source of the chemicals used. Cell cultures were maintained at 26-28°C in a growth room with a light regime of 14 h light: 10 h darkness, at an irradiance of 45 Wm^{-2} at the flask level, given by a bank of eight cool white fluorescent lamps. For higher light intensities, cultures were grown outdoors (18-25°C), where maximum solar irradiances reached 800 Wm^{-2}. Cultures in Pick's medium were shaken only during the light day hours, and since KSP medium did not contain bicarbonate, these cultures were aerated during the light hours.

GROWTH PARAMETERS AND PIGMENTS. Cell number was determined in an improved Neubauer hemacytometer, 0.1 mm deep. Beta-carotene was extracted from the algal pellets with 80% (v/v) acetone and measured according to Ben-Amotz & Avron (1983). Specific growth rates, cell densities and beta-carotene contents are given as mean values obtained from experiments carried out in duplicate.

RESULTS AND DISCUSSION

In order to evaluate the carotenogenic capabilities of native strains of *Dunaliella*,

three *D. salina* isolates from the Atacama Desert were grown in two growth media made of qualitatively different chemicals (Table 1). The cells were grown until the cultures reached exponential growth (6-8 days) at low or high irradiance. Besides light intensity, nitrate concentration was included as another enviromental factor known to affect beta-carotene biosynthesis in *Dunaliella*. Table 2 shows the pigment content of the three isolates under the various growth conditions. Isolate CONC-007 showed the highest beta-carotene content under all experimental conditions with maximun pigment content (90-100 pg cell^{-1}) 2-4 times higher than those reported by Ben-Amotz *et al.* (1982, 1987); instead, isolates CONC-001 and CONC-003 showed beta-carotene contents similar to *D. bardawil* (Ben-Amotz *et al.*, 1982, 1987). Growth in Pick's medium at low nitrate concentration and high light intensity resulted in a 5-fold increase in beta-carotene accumulation in all three isolates. At high nitrate concentration, high light intensity allowed a 7-fold increase in the pigment content of CONC-007, while the other isolates showed a 4-5 fold increase. These results indicate that light intensity is the major factor stimulating beta-carotene accumulation in the three isolates from northern Chile, while the effect of sub-optimal nitrate concentration on pigment biosynthesis is only significant when growth in low nitrate is accompanied by growth at high irradiances. This synergic effect is evident in isolates CONC-001 and CONC-003. Isolate CONC-007 reacts mainly to light intensity, while the nitrate effect is only present at low irradiances. Table 2 also shows the beta-carotene content of isolates grown in a low-cost growth medium. Under all conditions, isolates CONC-007 showed the highest pigment content. Isolates CONC-001 and CONC- 003 showed similar beta-carotene content, irrespective of nitrate concentration in the medium. Isolate CONC-007 however, accumulates maximal amounts of the pigment at high nitrate concentration. As in Pick's medium, light is the major environmental factor to trigger beta-carotene accumulation in cell grown in KSP medium. Recent studies on eight natives strains of *Dunaliella* from northern Chile (Cifuentes *et al.*, 1992) showed that total carotenoids content was higher in those strains isolated from the Atacama Salar.

Since beta-carotene accumulation was maximal in isolate CONC-007 grown under high light intensity and 5 mM nitrate in KSP medium, it was of interest to compare the specific growth rates and cell densities of *Dunaliella salina* isolates grown outdoors. These results are shown in Table 3. Isolate CONC-007 presented similar or higher doubling times than CONC-001. After stationary growth was reached, cell density in CONC-007 cultures was nearly one half the density of the other isolates. However, if beta-carotene content is considered under these growth conditions, CONC-001 and CONC-003 only accumulate 14-17 pg cell^{-1} (Table 2). Final cell numbers (4 x 10^5 cell ml^{-1}) and doubling times (0.40 day^{-1}) decreased when isolate CONC-007 was grown in Pick's medium at high irradiances and 5 mM nitrate, even though beta-carotene accumulated at the same level in the cells grown in either media (Table 2). Finally, higher specific growth rate (0.73 day^{-1}) and same

cell density (5.4×10^5 cell ml^{-1}) were obtained if CONC-007 cells were grown in KSP medium supplemented with 0.5 mM nitrate under high irradiance although, under this set of conditions beta-carotene cell's content was 46 pg $cell^{-1}$ (Table 2).

Isolate CONC-007 is therefore, a carotenogenic microorganism that can be cultured outdoors in a low-cost growth medium accumulating massive amounts of beta-carotene. It also represents a natural renewable resource, native of a athalassohaline aquatic habitat from the Atacama Desert in Northern Chile whith the biotechnological potential of becoming an alternative source of natural beta-carotene.

Table 1. Growth media composition. Pick's medium contained Sigma-quality chemicals; low-cost KSP medium was prepared with technical-grade salts produced locally: nitrate and superphosphate from Soquimich, Antofagasta, and sodium chloride from Punta de Lobo Salt Mine, Iquique, Chile.

COMPONENT	PICK'S MEDIUM	KSP MEDIUM
KNO_3	5.0 mM	5.0 mM
KH_2PO_4	0.2 mM	-
$CaH_4(PO_4)_2$	-	0.2 mM
$CaCl_2 \times 2H_2O$	0.3 mM	-
$MgSO_4 \times 7H_2O$	5.0 mM	-
$NaHCO_3$	50.0 mM	-
EDTA	6.0 mM	-
$FeCl_3$	1.5 uM	-
H_3BO_3	185.0 uM	-
$MnCL_2$	7.0 uM	-
$CoCl_2$	20.0 nM	-
$CuCl_2$	0.2 nM	-
NaCl	2.0 M	2.0 M
pH	7.5	7.8
Air (ml min^{-1})	-	230.0
H_2O	Distilled water	seawater

Table 2. Beta-carotene content of three *Dunaliella salina* isolates from hypersaline habitats at the Atacama Desert, Chile.

IRRADIANCE (Wm^{-2})	ISOLATE	BETA-CAROTENE CONTENT (pg $cell^{-1}$) 0.5 mM nitrate Pick's Medium	0.5 mM nitrate KSP Medium	5.0 mM nitrate Pick's Medium	5.0 mM nitrate KSP Medium
45	001	9.32	6.70	8.65	6.49
	003	4.01	9.80	3.70	9.50
	007	20.12	15.45	12.59	26.50
800	001	46.70	16.80	31.00	17.20
	003	20.23	16.90	16.50	14.20
	007	94.82	45.94	92.26	100.80

Table 3. Specific growth rates and cell densities of *Dunaliella salina* isolates from northern Chile. Cells were grown out-doors in the low-cost KSP growth medium at 5 mM nitrate. Cell numbers shown were obtained from cultures at early stationary phase of growth.

ISOLATE	SPECIFIC GROWTH (doubling 24 h^{-1})	CELL DENSITY (cells x 10^{-4} ml^{-1})
CONC-001	0.50	88.8
CONC-003	0.46	92.8
CONC-007	0.60	51.1

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LITERATURE CITED

Araneda, P. 1992a. Cultivo intensivo de *Dunaliella salina* (Teodo-resco, 1905). Evaluación de cepas nativas, medios de bajo costo y de un sistema prototipo de producción masiva. Tésis para optar al título de Ingeniero en Acuicultura, Universidad de Antofagasta, 162 pp.

Araneda, P., Tapia, I., Gómez-Silva, B. & L. Tapia. 1992b. Micro- algas del Norte de Chile. II. Cultivo en medios de bajo costo de dos cepas de *Dunaliella salina* (Teodoresco, 1905) nativas del Desierto de Atacama, Chile. Estudios Oceanológicos, **11**: 5-10.

Ben-Amotz, A. & M. Avron. 1983. On the factors which determine massive beta-carotene accumulation in the halotolerant alga *Dunaliella bardawil*. Plant Physiology, **72**: 593-597.

Ben-Amotz, A., Kantz, A. & M. Avron. 1982. Accumulation of beta-carotene in halotolerant algae: purification and characterization of beta-caroten-rich globules from *Dunaliella bardawil* (Chlorophyceae). Journal of Phycology, **18**: 529- 537.

Ben-Amotz, A., Gressel, J. & M. Avron. 1987. Massive accumulation of phytoene induced by norflurazon in *Dunaliella bardawil* (Chlorophyceae) prevents recovery from photoinhibition. Journal of Phycology, **22**: 176-181.

Borowitzka, M. A. & L. J. Borowitzka. 1988. *Dunaliella*. In : Borowitzka, M.A. & L.J. (eds), Microalgal Biotechnology, pp. 27-58. Cambridge University Press, Cambridge.

Borowitzka, M.A. Borowitzka, L.J. & D. Kessly. 1990. Effects salinity increase on carotenoid accumulation in the green alga *Dunaliella salina*. Journal of Applied Phycology, **2**: 111-119.

Cifuentes, A., González, M., Conejeros, M., Dellarrosa, V. & O. Parra. 1992. Growth and carotenogenesis in eight strains of *Dunaliella salina* Teodoresco from Chile. Journal of Applied Phycology. **4**: 111-118.

Gómez-Silva, B., Olivares, H. & L. Rodriguez. 1990. Microalgae from northern Chile. I. La Rinconada, a hypersaline aquatic habitat in the Atacama Desert. Estudios Oceanológicos, **9**: 73-76.

Gómez-Silva, B. 1991. Salina and hypersaline habitats from northern Chile. Salinet, 5: 44.

Loeblich, L.A. 1982. Photosynthesis and pigments influenced by light intensity and salinity in the halophile *Dunaliella salina* (Chlorophyta). Journal of the Marine Biological Association, UK, 62: 493-508.

Pick, U., Karni, L. & M. Avron. 1986. Determination of ion content and ion fluxes in the halotolerant alga *Dunaliella salina*. Plant Physiology, 81: 92-98.

Vonshak, A. 1990. Recent advances in microalgal biotechnology. Biotechnological Advances, 8: 709-727.

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