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Variability of cultural and morphological traits of *Dunaliella salina* Teod. from different habitatsO.S.Pasiuga¹, S.P.Antonenko², V.P.Komaristaya¹, A.N.Rudas³¹V.N.Karazin Kharkiv National University (Kharkov, Ukraine)²Kharkov State University of Food Technology and Trade (Kharkov, Ukraine)³Betacar-XP LLC (Kharkov, Ukraine)

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Deficiency of nitrogen and phosphorus induced β -carotene accumulation in the cells of 35 clonal cultures of *D. salina* isolated from 6 habitats in Ukraine and 1 in Russia. Nitrate and phosphate re-supplied into the medium decreased cellular β -carotene content and cell size. Repeated passage into the medium without nutrients recovered inducible β -carotene level; the dimensional characteristics of the cells recovered partly. Isolates from different habitats (except one) broke up into 2 groups: low carotene – small celled – fast growing and high carotene – large celled – slow growing. Peculiarities of clonal cultures depended on habitats, where they were isolated from, and persisted for 3 passages, i.e. inherited. Each 5 isolates from the same habitat did not differ, i.e. *D. salina* populations were homogenous within a single habitat, at least at the time and location of sampling. The ability to β -carotene accumulation induced by the deficit of nutrients can be extrapolated to *D. salina* species on the whole, at least to the populations originated from Ukraine and lake Baskunchak in Russia.

Key words: *Dunaliella salina*, β -carotene, nitrogen, phosphorus, isolate, cultivation.

Варіабельність культуральних і морфологічних ознак *Dunaliella salina* Teod. из разных местообитаний

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У 35 клональних культур *D. salina*, виділених з 6 местообитаний в Україні і 1 – в Росії, дефіцит азота і фосфору індуцивав накоплення β -каротину в клітках. Внесення в середовище нітратів і фосфатів приводило до зниженню вмісту β -каротину на клітку і зменшенню розмірів кліток. Повторний посів культур на середовище без біогенів приводив до відновленню індукованого рівня вмісту β -каротину на клітку, розмірні характеристики кліток в повній мірі не відновлювалися. Ізоляти з різних водойм (крім однієї) розділилися на 2 групи: малокаротиноносні-мелкоклітинні-швидкозростаючі і багатокаротиноносні-крупноклітинні-повільнозростаючі. Особливості клональних культур зависели від местообитання, из которого они были выделены, и сохранялись в 3 пассажах, т.е. были обусловлены наследственно. Различий между 5 изолятами из одного местообитания выявлено не было, т.е. популяции *D. salina* были достаточно однородными в пределах одного местообитания, по крайней мере, в момент и в месте отбора проб. Способность к индукции накопления β -каротина дефицитом биогенов можно экстраполировать на вид *D. salina* в целом, по крайней мере, на те его популяции, которые происходят из Украины и оз. Баскунчак в России.

Ключевые слова: *Dunaliella salina*, β -каротин, азот, фосфор, изолят, культивирование.

Варіабельність культуральних і морфологічних ознак *Dunaliella salina* Teod. з різних місць зростання

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У 35 клональних культур *D. salina*, виділених з 6 місць зростання в Україні та 1 – в Росії, дефіцит азоту і фосфору індукував накоплення β -каротину в клітинах. Внесення в середовище нітратів і фосфатів призводило до зниження вмісту β -каротину на клітину і зменшення розмірів клітин. Повторний посів культур на середовище без біогенів приводив до відновлення індукованого рівня вмісту β -каротину на клітину, розмірні характеристики клітин повною мірою не відновлювалися. Ізоляти з різних водойм (крім однієї) розділилися на 2 групи: малокаротиноносні-дрібноклітинні-швидкозростаючі і багатокаротиноносні-крупноклітинні-повільнозростаючі. Особливості клональних культур залежали від місця зростання, з якого вони були виділені і зберігалися в 3 пассажах, тобто були обумовлені спадково. Відмінностей між 5 ізолятами з одного місця зростання виявлено не було, тобто популяції *D. salina* були досить однорідними в межах одного місця зростання, принаймні, в момент і в місці відбору проб. Здатність до індуції каротиногенезу дефіцитом біогенів можна екстраполювати на вид *D. salina* в цілому, принаймні, на ті його популяції, які походять з України та оз. Баскунчак в Росії.

Ключові слова: *Dunaliella salina*, β -каротин, азот, фосфор, ізолят, культивування.

Introduction

Unicellular green monad microalga *Dunaliella salina* Teod. massively develops in hyperhaline lakes and causes their red "bloom". The pigment β -carotene accumulates in lipid globules located in the chloroplast stroma at the periphery and determines orange-red color of the cells (Oren, 2005; Borowitzka, 2013). Different authors published contradictory data about the causes of β -carotene accumulation in *D. salina*. Lerche (1936/1937) observed change of cells color from green to orange-red when the cells underwent nitrogen and phosphorus deficiency. Later Ben-Amotz et al. (1982) found that neither deficit of nitrogen nor of phosphorus induced β -carotene accumulation. Many authors believe that high salinity and excess irradiation play the main role in that process (Borowitzka, 2013), together with sub-optimal temperature (Semenenko, Abdullaev, 1980). Loeblich (1982) considered the ability to accumulate at least 20 pg of β -carotene per cell, and change color from green to orange-red, under high salinity and irradiation to identify *D. salina* as a species. According to this criterion some laboratory strains unlikely belong to *D. salina* (Borowitzka, Siva, 2007).

Dunaliella cell has a very simple structure. Therefore, the main species diagnosis morphological traits in this genus appeared stigma distinguishability, the width and length of the cells, and their ratio giving to the cells the shape from spherical and pyriform to fusiform (Massyuk, 1973).

Cell size and β -carotene content may relate to cell division rate. Cultures of small celled algal species generally grow faster than large celled; and that is predetermined hereditarily (Fogg, Thake, 1987). In *D. salina* β -carotene accumulation usually accompanies inhibition of culture growth; and that depends on culture conditions – growth optimum disagrees with β -carotene accumulation optimum (Massyuk, 1973). Intraspecific variation of the complex of traits "cell size – division rate – β -carotene accumulation" may influence the productivity of the industrial culture of *D. salina*.

Given the contradictory literature data on the induction of β -carotene accumulation in *D. salina* by deficiency of nutrients, as well as the fact that most the data were obtained in the strains long maintained in laboratory, any observed effect should not be extrapolated to the species as a whole without additional studies of other strains and field isolates.

The objective of this work was to characterize 35 cultures of *D. salina* isolated from 7 different habitats in their ability to accumulate β -carotene under nutrient deficiency. In parallel, to clarify species affiliation, cell size was measured, and, to study the relation to cell β -carotene content and size, culture growth dynamics was registered.

Materials and methods

The starting material for the study was the brine, containing *D. salina*, sampled at different times from 6 different natural sources (table) and the strain IBSS-1 kindly provided by the A.O.Kovalevsky Institute of Biology of the Southern Seas. To avoid possible influence of laboratory cultivation conditions on genotype of the starting material, all batch cultures were maintained in the medium prepared from sea salt (density 1.15 g/cm³) without adding nutrients (Dogadina, Komaristaya, 2005) and were passed no more than once every six months.

Isolation of clonal cultures. To take into account genetic diversity in cultures from a single source, 5 clonal cultures were isolated from each of them. For that sea salt agar media in Petri dishes were inoculated, cell division and growth of colonies were monitored under microscope by placing the Petri dish onto the microscopic stage, 5 randomly selected colonies of algae derived from single cell were subcultured in liquid medium and maintained under the same conditions as the original cultures.

The experimental design envisaged 3 passages: the I and III passage under nutrient deficiency in modified Artari medium (Massyuk, 1973) without adding nitrates and phosphates. Cultivation of batch clonal cultures in sea salt medium without adding nutrients preceded the I passage; growth in full Artari medium with the both nutrients after the II passage preceded the III passage.

Culture conditions. Cultures were grown in Erlenmeyer flasks for 25 ml, 15 ml of culture in each, at irradiance 2 klx from two 32 Wt «Maxus» lamps with the color temperature of 2700 K, photoperiod: 16 hours – light, 8 hours – dark, and the temperature 24–28°C.

Growth dynamics registration. Cell number was counted in Goryev hemocytometer when passing cultures and every 7 days during their growth. Cell concentrations were expressed in thousands per 1 ml.

Cell size measurements. At the end of each cell growth cycle (after the I, II and III passages) sizes of 100 native cells were measured with ocular micrometer. Cells were not fixed to avoid distortion of their shape

and size (Massyuk, 1973). Watching each cell in motion, length from the base of the flagella to the opposite cell end and cell maximum width were recorded.

β -carotene quantification was carried out at the end of each culture growth cycle (after the I, II and III passages) with a rapid method. Aliquots of cell suspension were extracted with ethyl acetate. Extracts absorbances at 440 nm were measured. Cellular β -carotene content was calculated using the specific extinction coefficient $E_{1\%}^{1\text{cm}}=2500$ (IARC, 1998) and expressed in pg.

Statistical analysis. To account for possible variability within each clone the experiment was carried out in three replicates. Distributions of all data were significantly different from the normal according to Shapiro-Wilk test, so nonparametric Kruskal-Wallis test was used to compare variants. Discussed effects are significant at 95% level.

Table.

Origin and characteristics of the material for clonal cultures isolation

Isolate code	Origin	Cell color	β -carotene content, pg/cell	Sampled	Clonal cultures isolated
Heroyk	Zmievo lake, sedimentation pond of Heroyskoe salt works, Kherson region, Ukraine	red	91,1	July 2005	February 2008
Genichesk	Genicheskoe lake, sedimentation pond of Genichesk salt works, Kherson region, Ukraine	red	81,3	August 2005	November 2008
IBSS-1	IBSS collection of cultures, Siwash bay, AR Crimea, Ukraine, 2001	orange	24,8	June 2005	October 2007
Siwash	Siwash bay, behind the dam of salt works of Crimea soda plant, AR Crimea, Ukraine	red	100,0	August 2007	August 2008
Filatov	Filatovskaya salt flat bay, behind the dam of salt works of Crimea soda plant, AR Crimea, Ukraine	red	115,5	June 2005	October 2007
Sasyk	Sasyk lake, sedimentation pond of salt works of cooperative "Halite", AR Crimea, Ukraine	red	48,9	July 2004	November 2008
Baskunchak	Baskunchak lake, under a woody bridge at Baskunchak salt works, Astrachan region, Russia	red	not determined	May 2006	August 2008

Results

For each habitat 5 isolated clonal cultures did not differ by cellular β -carotene content, cell size and growth dynamics throughout 3 cultivation cycles.

Cellular β -carotene content. The cultures isolated from different habitats differed significantly in β -carotene content (fig. 1), that correlated neither with the time of sampling and isolation nor with cellular β -carotene content in the initial samples (table). After the I passage in the artificial medium cellular β -carotene content decreased significantly compared with the initial natural samples, except the strain IBSS-1, which had been already introduced into the laboratory culture. When subcultured in the medium with the both nutrients (the II passage) β -carotene content even more significantly decreased, and after the III passage in the medium depleted in nutrients increased again (fig. 1).

We refer to the minimum level of β -carotene in *D. salina* cells under optimal for culture growth conditions as baseline level and to the elevated levels under β -carotene accumulation conditions as inducible levels, as previously suggested (Bozhkov, Komaristaya, 2003). By cellular β -carotene content the cultures broke up into 3 groups. The first group comprised the cultures with high inducible β -carotene level: IBSS-1, Sasyk, Filatov. Within this group cultures differed in baseline β -carotene level (after the II passage): IBSS-1 had the highest baseline, Sasyk and Filatov, on the contrary, the lowest among all the variants

studied (fig. 1). Cultures of the second group – Heroysk and Siwash – possessed the average both baseline and induced β -carotene levels (fig. 1). Cultures of the third group – Genichesk and Baskunchak – possessed the lowest both baseline and induced β -carotene levels (fig. 1). The contents of β -carotene in the same cultures after the I and III passages differed insignificantly (fig. 1), i.e. single passage in the medium enriched with the nutrients did not affect the central tendency of this index for isolates from certain habitats.

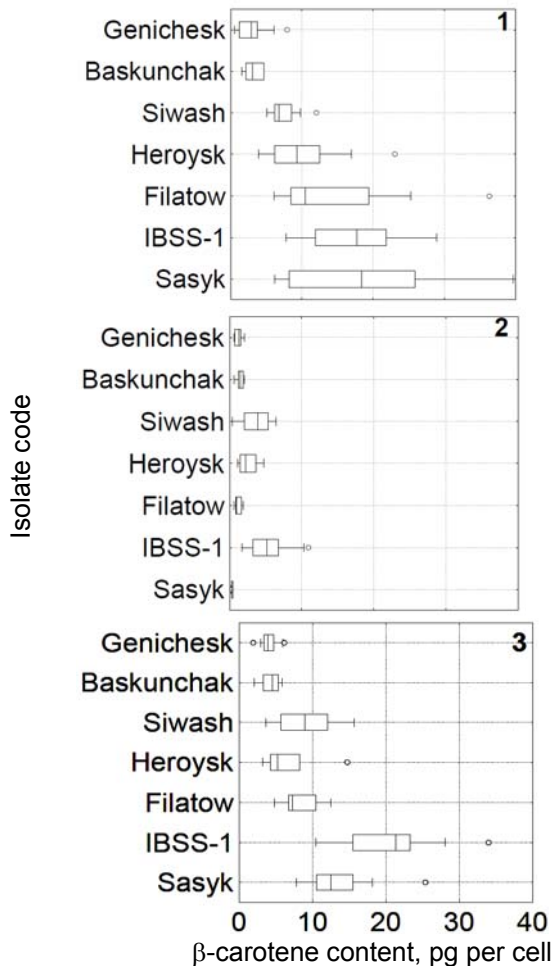


Fig. 1. Characteristics of *D. salina* isolates cultures from different habitat by cellular β -carotene content:

1 – after the I passage into the medium deficient in nitrogen and phosphorus;

2 – after the II passage into the full medium;

3 – after the III passage into the medium deficient in nitrogen and phosphorus

— – median,

□ – 25%–75%,

I – range,

○ – outliers

Cell sizes after the I passage somewhat correlated with the cellular β -carotene content in the most variants: the larger the cell size, the higher β -carotene content (fig. 1, 2). The group of the largest celled cultures, both by modal, mean and maximum values, comprised IBSS-1 and Filatov isolates (fig. 2), which contained more β -carotene per cell (fig. 1). The group of smallest celled cultures comprised Genichesk and Baskunchak (fig. 2), with low β -carotene content per cell (fig. 1). The other isolates possessed moderate cell size characteristics. At that, cells in Heroysk culture were large enough and had average β -carotene content, cells in Sasyk culture were quite small and had the largest β -carotene content among all the variants (fig. 2, 1).

After the II passage into the medium enriched with the nutrients, cell size decreased in all the cultures. The distributions of cell sizes became more compact. In the relatively large celled variants IBSS-1, Filatov and Heroysk the upper variation limits significantly decreased. Though these cultures preserved greatest cell dimensions among all studied, they approached the midsize cultures Siwash and Sasyk. The cells became even smaller in small celled cultures Genichesk and Baskunchak (fig. 2). It should be noted that after the II passage cells in Filatov culture revealed the distinct stigma, which during further cultivation became indistinguishable again.

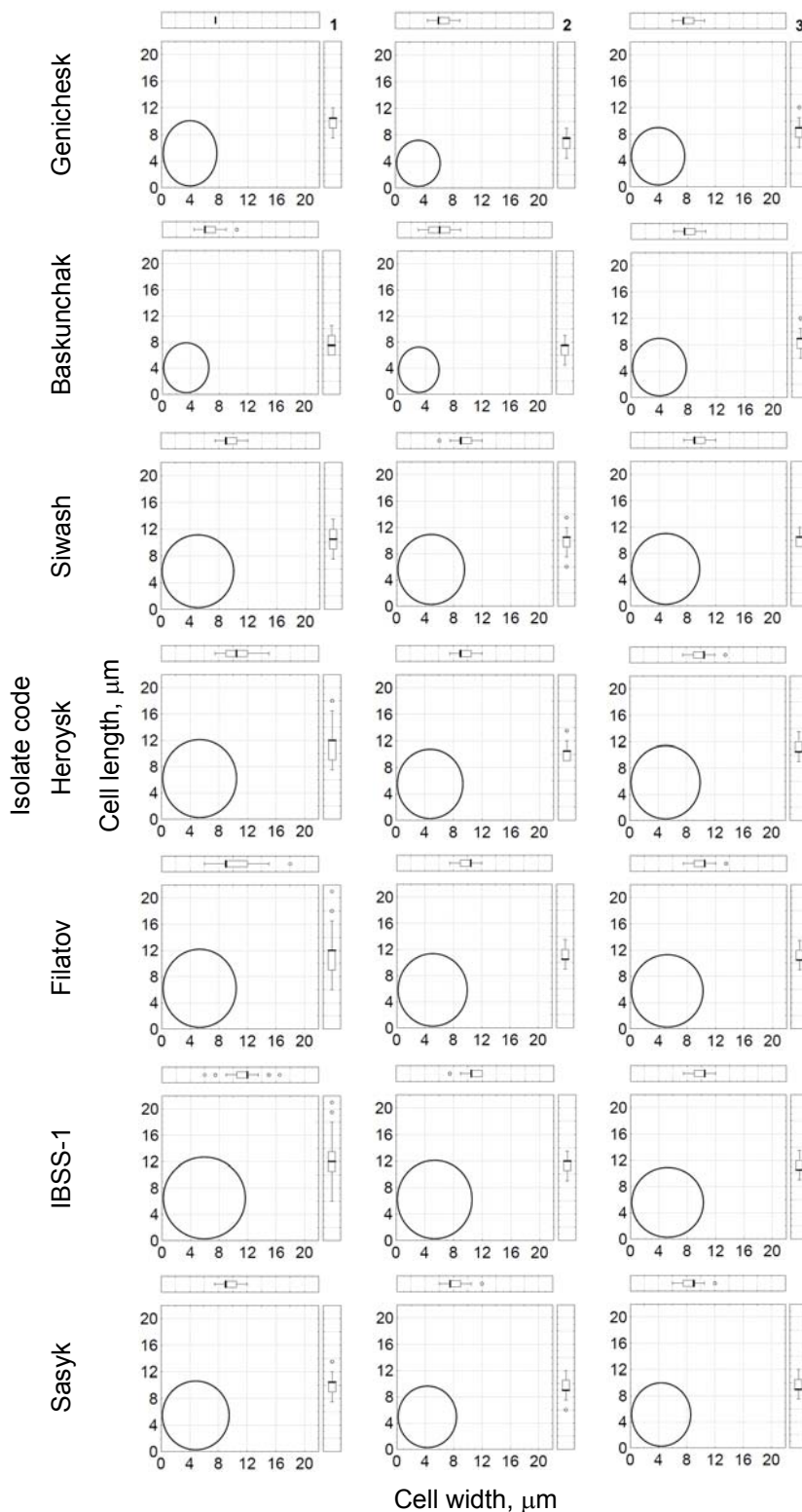


Fig. 2. Dimensional characteristics of cells in the cultures of *D. salina* isolated from different habitats:

1 – after the I passage into the medium deficient in nitrogen and phosphorus;

2 – after the II passage into the full medium;

3 – after the III passage into the medium deficient in nitrogen and phosphorus

○ – mean values,

— – median,

□ – 25%–75%,

I – range,

○ – outliers

After the following passage into the deficient in nutrients medium (the III passage), cell sizes recovered to the I passage values, but, unlike β -carotene content, only in small celled and low β -carotene cultures Genichesk and Baskunchak, as well as in Siwash, which closed the group of cultures with moderate

cell size and β -carotene values. In the other cultures the cell size after the III passage into medium without the nutrients remained the same as after the preceding II passage into the medium with the nutrients (fig. 2).

Culture growth dynamics. After the I passage into the medium without the nutrients the cultures rapidly reached stationary phase. In different isolates the maximum cell concentrations at stationary phase (medium carrying capacity) differed. The large celled high β -carotene isolate IBSS-1 reached the highest cell concentration within the shortest time. The other large celled isolates Heroysk and Filatov grew for the same time but reached almost 1.5 times lower concentrations of cells at stationary phase. The midsize Siwash and Sasyk isolates grew 1.5 times longer; moderate β -carotene Siwash reached a fairly high concentration of cells; and high β -carotene Sasyk reached the lowest cells concentration among all the isolates. The small celled and low β -carotene isolates Genichesk and Baskunchak grew most long and slowly under the nutrients deficiency (fig. 3).

Re-passing into the medium with the nutrients (the II passage) led to several tens of times increased medium capacity and 3–4 times increased duration of culture growth. Cell size slightly differently correlated with culture growth intensity than after the I passage. The small celled low β -carotene isolates Genichesk and Baskunchak reached the highest cell concentrations – 1.2–2 times higher than the rest of isolates. The small celled but high β -carotene isolate Sasyk almost approximated them. The large and medium sized high and moderate β -carotene isolates grew less intensively (fig. 3).

After the III passage into the medium without nutrients all the cultures reached the cell concentrations an order of magnitude lower than in the medium with the nutrients after the II passage, but 5–10 times higher than in the nutrients deficient medium after the I passage, apparently because of the nutrients stored in the cells during the preceding II passage. The growth of the majority of the cultures lasted almost twice longer than after the I passage. The growth did not correlate with the I passage, but did with the II passage, that could also be due to the surplus of the nutrients stored in the cells. Some correlation retained with the size of cells. Large celled isolates reached the lowest cell concentrations, small celled and midsize Siwash – the greatest. The Sasyk isolate reached the moderate cell concentration value comparing with all the isolates (fig. 3).

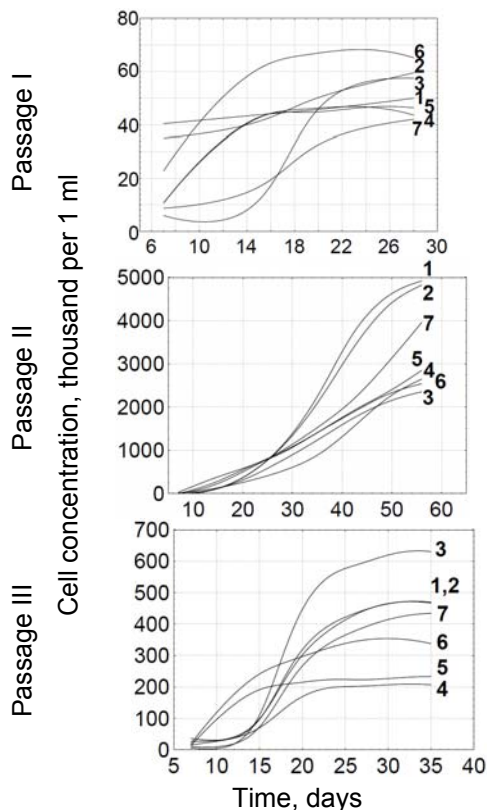


Fig. 3. Growth dynamics of the cultures of *D. salina* isolated from different habitats:

I – after the I passage into the medium deficient in nitrogen and phosphorus;

II – after the II passage into the full medium;

III – after the III passage into the medium deficient in nitrogen and phosphorus

- 1 – Genichesk
- 2 – Baskunchak
- 3 – Siwash
- 4 – Heroysk
- 5 – Filatow
- 6 – IBSS-1
- 7 – Sasyk

Discussion

In 35 clonal cultures of *D. salina* (isolated from 7 habitats, 5 cultures from each), we established that nitrogen and phosphorus deficiency under optimal salinity, irradiance and temperature induced cellular β -carotene accumulation. Addition of nitrates and phosphates into the medium decreased β -carotene content per cell and cell sizes. Re-passing the cultures from the medium with the nutrients into the medium without the nutrients recovered inducible β -carotene content level; though the dimensional characteristics of the cells did not recover fully. These effects expressed to varying extent in different isolates, but followed the same tendency, therefore the ability to induce β -carotene accumulation under nutrient deficiency can be extrapolated to *D. salina* species as a whole, at least to those of its populations that originate from Ukraine and lake Baskunchak in Russia.

Peculiarities of certain clonal cultures depended on habitat of isolation. Isolated from the same habitat 5 cultures did not differ, though that was likely as a result of genetic drift at isolation from a single cell. The populations of *D. salina* in different habitats differed among themselves, but were fairly homogenous within the same habitat, at least at the time and place of sampling.

In general *D. salina* isolates from different habitats broke up into 2 groups: low β -carotene – small celled – fast growing and high β -carotene – large celled – slow growing. This corresponds to the literature data on the inverse correlation of cell concentrations at stationary phase with cell size in *D. salina* (Cifuentes et al., 1992) and with cellular content of β -carotene (Gomez et al., 1999). In our study this pattern was not absolute: some isolates possessed moderate values of all parameters; Sasyk isolate, small celled – fast growing but high β -carotene, appeared an exception; the overall pattern of growth dynamics in the I passage was almost the opposite. Perhaps different isolates possessed different needs in the nutrients for culture growth and different abilities to store the surplus nutrients. This reflected in the different responses to nutrient deficiency and supply. The differences persisted in 3 cultivation cycles in identical for all isolates culture conditions, i.e. the differences were inherited. The differences between isolates of *D. salina* from different reservoirs likely were due to their origin from genetically isolated populations, the similarities – to the occupation of the same ecological niche.

Smaller cells in the medium with the nutrients compared with the medium deficient in the nutrients agreed with our previously obtained data on the dependence of size characteristics of IBSS-1 strain on nutrition conditions (Antonenko et al., 2010).

In general, the traits of all the isolates corresponded to the species diagnosis (Massyuk, 1973), but we observed some deviation: for a short time, when cultured in the medium containing the nutrients, the isolate Filatov possessed a clear stigma (instead of typical for the species unobtrusive diffuse one). N.P. Massyuk (1973) cited this as a characteristic trait for another species, *Dunaliella parva* Lerche, belonging to less β -carotene types, whereas Lerche herself (1936/1937) described that *D. parva*, as *D. salina*, differed from other species by diffuse stigma, but possessed smaller cell size compared with *D. salina*. In our opinion, the existence of the species *D. parva* described from the cultural material requires confirmation (Komaristaya et al., 2010), and the variability of traits in *D. salina* in nature and culture requires further study.

Many *D. salina* habitats all over the world demonstrated isolate-dependent variability of culture growth rate, cell size, response to β -carotene accumulation inducing factors and cellular β -carotene content: Chile (Cifuentes et al., 1992), Venezuela (Marin et al., 1998), Mexico (Garcia et al., 2007; Narvaez-Zapata et al., 2011), India (Jayappriyan et al., 2011), South Korea (Polle et al., 2008), Israel and Australia (Borowitzka, Siva, 2007). Our isolates from Ukraine and Russia lay in the same range of culture cell concentrations (Cifuentes et al., 1992; Marin et al., 1998; Garcia et al., 2007), cell size (Polle et al., 2008; Borowitzka, Siva, 2007; Jayappriyan et al., 2011) and β -carotene content (Cifuentes et al., 1992; Marin et al., 1998; Borowitzka, Siva, 2007; Garcia et al., 2007; Narvaez-Zapata et al., 2011) as the isolates from various locations except several unusually large celled (almost 5 times larger) and high β -carotene (up to 20 times higher) isolates from Australia (Borowitzka, Siva, 2007). That confirms once more that in algae habitat typology matters more than geography.

Significant deviations from the characteristic of *D. salina* traits in culture often challenges species affiliation of individual strains (Loeblich, 1982; Borowitzka, Siva, 2007). In our study three isolates after the I passage (Genichesk, Baskunchak and Siwash) and all but two (IBSS-1 and Sasyk) after the III passage did not meet Loeblich's biochemical criteria of *D. salina* – the ability to accumulate more than 20 pg per cell of β -carotene (Loeblich, 1982), whereas all the original samples met this criterion. The ability to accumulate β -carotene might depend on inducing conditions (Loeblich (1982) used high irradiance and salinity in non-chemostat culture) and on the condition preceding the passage.

All the studied traits distributed sharply skew in all the isolates throughout the 3 cultivation cycles. This might indicate that periodic variation of cultivation conditions (nutrients deficiency and nutrients supply) caused adaptive adjustments in the cultures recently isolated from natural habitats, which may occur at the level of selection of genotypes better adapted to the culture conditions in the laboratory.

On the basis of just 3 cultivation cycles it was impossible to judge how much the natural material of *D. salina* can differ from the culture maintained long enough in the laboratory, such as the strain IBASU-A D-11, which had been received from the collection of N.G.Kholodny Institute of Botany (Kiev) in 1995. This strain originated from the ponds of Heroyske salt works (former salt works Prognoi) – the same habitat as Heroysk isolate in the present study. The strain grew for more than 50 years (since 1962) in Artari medium enriched with the nutrients – the same composition as after the II passage in the present study (Catalogue..., 1991). Cells of IBASU-A D-11 significantly differed from the diagnosis by smaller size (length 10.5–(18.0)–18.0 μm , width 6.0–(6.0)–9.0 μm), fusiform shape, constant presence of stigma and inability to change color to red-orange. We cannot exclude that the other *Dunaliella* species from the same habitat with appropriate traits – *Dunaliella viridis* Teod. – contaminated and displaced *D. salina* in IBASU-A D-11 culture. It is also possible that this strain which is supported in culture medium rich in nutrients (Catalogue..., 1991) since 1962 had undergone genetic alterations that led to phenocopies morphologically similar to *D. viridis*. On that basis we excluded IBASU-A D-11 strain from the present study.

Another laboratory strain – IBSS-1 – was obtained from the collection of A.O.Kovalevsky Institute of Biology of the Southern Seas in 2005. It was isolated in 2001 from the Siwash bay (as the isolate Siwash in the present study), but the exact place of sampling, unfortunately, is not known. Before we get this strain it was maintained in the IBSS collection for 4 years on Ben-Amotz medium containing 0.505 g/l KNO_3 and 0.038 g/l KH_2PO_4 (Collection..., 2007) – 5 times lower than in modified Artari medium (2.500 g/l and 0.200 g/l correspondingly) (Massyuk, 1973). In IBSS-1 cell morphology corresponded to the species diagnosis (Massyuk, 1973), cell color and β -carotene content – to the biochemical criteria (Loeblich, 1982). The strain retained these species-specific traits not only throughout 3 passages in the present study, but during the preceding 4 years of cultivation in the laboratory, i.e. natural populations traits could retain in laboratory culture. Maintaining relatively low concentrations of the nutrients in the medium should be recommended as a precaution. In the present study, cellular β -carotene content reduced when the cells cultured in the medium with the nutrients after the II passage. Selection of the fittest variants for growth in culture would inevitably occur during prolonged cultivation (Tsoglin et al., 1970) that, in the presence of nutrients, could narrow the reaction norm of the trait “induced level of β -carotene content”.

We cannot judge on characteristics of natural populations of *D. salina* from certain habitats based on cultures isolated from just one sample. Despite the fact that clonal cultures derived from individual cells within the same sample did not differ, different local subpopulations likely exist in natural habitats (especially in such a large water body like Siwash bay) or likely dominate at different times. Some authors argue that some cryptic species of the genus *Dunaliella* can coexist in the same habitat being morphologically similar to *D. salina* but different in the ability for β -carotene accumulation and in some molecular genetic markers (Olmos et al., 2000; Olmos-Soto et al., 2002; Gomez, Gonzales, 2001; 2004; Borowitzka, Siva, 2007). To us it seems unlikely that in the same reservoir several genetically isolated species exist simultaneously, which have the same extreme (i.e. under high selection pressure) ecological niche and similar adaptive strategies (e.g. the ability to accumulate β -carotene). Further studies of genetic basis of *D. salina* variability by β -carotene accumulation, cell size and division rate, as well as of crossability of genetically distinct forms are necessary to judge the causes and dynamics of intraspecific diversity of *D. salina* and refute or confirm the existence of cryptic species.

Exploring diversity of *D. salina* by β -carotene accumulation and culture growth has the practical value as the basis for screening and selection of strains promising for commercial cultivation for the production of natural β -carotene – a valuable food coloring ingredient, provitamin and antioxidant.

The present study, although limited to a single sample from each reservoir, confirmed the induction of β -carotene accumulation by the deficit of the nutrients on the fairly extensive material (35 clonal cultures, 2 passages) and variable by origin (6 habitats, 1 laboratory strain); and showed hereditary differences among *D. salina* isolates in β -carotene accumulation ability, cell size and culture growth rate.

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