Effects of salinity increase on carotenoid accumulation in the green alga Dunaliella salina

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Abstract

The effect of sudden salinity increases on the kinetics of growth and carotenogenesis was studied in three geographically diverse isolates of Dunaliella salina. A sudden increase in salinity results in a lag phase in growth and the length of this lag phase is dependent on the final salinity and the magnitude of the salinity change (no lag at 10–15% w/v NaCl, 4-day lag at 30% NaCl). There is also a lag before an increase in the total carotenoid content can be measured following the salinity up-shock, and the length of the lag depends largely on the initial salinity and the magnitude of the salinity up-shock, whereas the rate of carotenogenesis and the final carotenoid content reached depend on the final salinity. The increase in total carotenoid content is mainly due to β-carotene. Following the salinity up-shock (especially from 10% to 20% NaCl) the proportion of lutein as a percentage of total carotenoids decreases, whereas zeaxanthin increases. This suggests that the pathway synthesising lutein is more sensitive to salt or osmotic stress and is inhibited at higher salinities, thus leading to β-carotene formation. The proportion of α-carotene does not change.

Introduction

The unicellular green alga Dunaliella salina Teodoresco (Chlorophyceae, Dunaliellales) is the most salt-tolerant eukaryote known (Borowitzka, 1981). In recent years D. salina has also achieved prominence as a commercial source of 'natural' β-carotene for the food and feed industry (Borowitzka & Borowitzka, 1988a). This is because this alga provides the richest natural source of β-carotene, with β-carotene contents of > 10% of dry weight (Mil'ko, 1963; Borowitzka et al., 1984).

A number of studies have shown that the highest β-carotene content in D. salina is achieved under conditions of high light, high temperature and high salinity (e.g. Mil'ko, 1963; Semenenko & Abdullayev, 1980; Loeblich, 1982; Ben-Amotz & Avron, 1983a; Borowitzka et al., 1984; Ben-Amotz, 1987). The content is also enhanced under conditions of nutrient limitation, especially nitrogen limitation (Semenenko & Abdullayev, 1980; Borowitzka & Borowitzka, 1988b). Little is known, however, of the mechanisms inducing this accumulation of β-carotene, nor of the kinetics of the accumulation process.
β-carotene accumulation is generally greatest when the growth rate is least, and this is the basic dilemma underlying any commercial process of β-carotene production using *D. salina*. One of the processes proposed by several workers (e.g. Mil'ko, 1963; Massyuk & Abdula, 1969; Borowitzka *et al.*, 1984) uses a low salinity (about 15% w/v NaCl) growth phase to achieve maximum biomass, followed by a β-carotene accumulation step at elevated salinity (about 20–25% w/v NaCl). This paper examines the effects of a rapid increase in salinity on the kinetics of changes in carotenoid content and distribution in three different isolates *D. salina*.

**Materials and methods**

Three isolates of *Dunaliella salina* from water samples of geographically diverse locations were used: Strain W5 isolated from salt works at Lake McLeod, Western Australia; Strain N41 from natural salt lakes near Wellington, South Australia; and Strain N43 from salt works at Bajool, Queensland. The strains were isolated by repeated plating on modified Johnson's medium at 20% w/v NaCl salinity (Borowitzka, 1988) and are now lodged in the Culture Collection of Commercial Algae at the School of Biological and Environmental Sciences, Murdoch University.

For the experiments presented here, the algae were grown in unshaken flasks illuminated by cool white fluorescent tubes at a photon flux density of approximately 180 μmol m$^{-2}$ s$^{-1}$, at a temperature of 26 °C in modified Johnson's medium containing 10% of the normal nitrate and phosphate levels. Salinities were adjusted by the addition of NaCl to exponentially growing cultures and are presented as% w/v NaCl.

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**Fig. 1.** Change in total carotenoid content in the three strains of *Dunaliella salina* when the salinity was increased from 5% NaCl to 10, 15 or 20% NaCl at day 0.