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INVESTIGATION OF THE MALIC ACID CONCENTRATION ON EXTREMOPHILIC RED MICROALGA *GALDIERIA SULPHURARIA*

(MALİK ASİT KONSANTRASYONUNUN EKSTREMOFİLİK KIRMIZI MİKROALG GALDERİA SULPHURARİA DA ARAŞTIRILMASI)

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ABSTRACT

Malic acid is a dicarboxylic acid which is made by living organisms and the salts of malic acid are known as malates. Malates are used in pruvate/malate cycle that merges carbohydrate, fat and protein metabolism. Based on this cycle, the prediction was that malic acid could change carbohydrates ratio in *G.sulphuraria*. In this study, the effects of malic acid concentrations were investigated on growth (maximum specific growth rate and doubling time) of extremophilic, red alga *Galdieria sulphuraria* (SAG 108.79). By increasing malic acid concentration, specific growth rate and biomass concentration were also increased, however the highest concentration of protein (153.81±0.673 mg/L) was obtained in 5 mM. The total amount of carbohydrates was increased about 8.35%.

Keywords: Malic acid, Lipid, Protein, Carbohydrate, Galdieria sulphuraria, Microalga

ÖZ

Malik asit yaşayan organizmaların ürettiği ve malatlar gibi malik asit tuzları olduğu bilinen bir dikarboksilik asittir. Karbohidrat, yağ ve protein metabolizmasını birleştiren pürivat/malat döngüsünü içinde malatlar kullanılır. Bu döngü temelinde, malik asit Galderia sulphuraria nın karbonhidrat oranını değiştirebildiğini tahmin ediyoruz. Ayrıca bu konudaki benzer çalışmalara rastlanamamıştır. Bu çalışmada, malik asit konsantrasyonlarının ekstremofilik kırmızı alg G. Sulphuraria (SAG 108.79) büyümesi (maksimum spesifik büyüme oranı ve ikileneme süresi) üzerine etkisi incelenmiştir. Artan malik asit konsantrasyonunda spesifik büyüme oranı ve biykütle konsantrasyonu artarken, 5 mM konsantrasyonda proteinin en yüksek konsantrasyonu (153,81 ± 0,673 mg / L) belirlenmiştir. Sonuçlarımız karbonhidrat miktarında yaklaşık % 8,35 artış olduğunu göstermektedir.

Anahtar Kelimeler: *Malik asit, Yağ, Protein, Karbonhidrat, Galderia sulphuraria, Mikroalg*

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1. INTRODUCTION

Microalgae are microscopic algae, typically found in freshwater and marine systems living in both the water column and sediment. Extremophilic microalgae grow under acidic or alkaline pH, high temperature, light, CO_2 level and metal concentration [1]. Extremophile organisms have adapted to extreme environments, especially prokaryotes come to mind first. However, the unicellular red microalga *Galdieria sulphuraria* (Cyanidiales) is a eukaryote that naturally inhabits volcanic environments and hot sulfur springs with pH values of 0–4 and temperatures of up to 56 °C [2, 3].

Over 30,000 diverse microalgal species in the world have been described [4], but the most commercially important belong to the diatoms and the green algae genus *Chlorella*, *Dunaliella*, and *Haematococcus* [5]. Microalgal species are currently produced on a large scale in a sustainable economic process is limited [6]. Seasonal and diurnal fluctuations in light and temperature and contamination by other organisms affect growth and productivity in outdoor algal ponds [7]. Microalgal strains have reached a stage of a commercially traded product, *Dunaliella* and *Spirulina* are extremophiles. For example, *Dunaliella* which is a green unicellular microalga isolated from high salinity water bodies and *Spirulina* a filamentous cyanobacterium that blooms in alkaline lakes with high pH in the range of 9–10 [1]. *G. sulphuraria* is an extremophilic, spherical, spore-forming, eukaryotic red alga.

Endogenous organic acids are the source of both carbon skeleton and energy for cells, and are used in the respiratory cycle and other biochemical pathways. Malic acid is metabolized in plant mitochondria by reaction of malic enzyme [8]. Malate is an intermediate of the mitochondrion-based TCA cycle formed via reversible dehydration of oxaloacetate and hydration of fumarate [9].

In this study the effects of malic acid concentrations, changes in protein, lipid and glucose in *Galdieria sulphuraria* were analyzed.

2. MATERIALS AND METHODS

2.1. Organism

Galdieria sulphuraria strain was obtained from the culture collection of algae at the University of Göttingen, Germany (SAG 108.79) and grown aseptically in Cyanidium medium [10].

2.2. Culture Conditions

250 ml cultures were prepared with the same cell concentration (Figure 1). Malic acid concentrations were 0, 2, 5 and 10 mM and culture conditions were under 24^0C at the agitation rate of 100 rpm under continuous light intensity of 20 µmol photons $m^{-2}s^{-1}$ for 15 days. The culture growth was monitored by using spectrophotometer (Ultrospec 1100 pro, Amersham Biosciences) at 750 nm and cell growth (cells mL^{-1}) was estimated daily using a *Neubauer* counting chamber under an inverted microscope (Olympus CH40, Japan).

The specific growth rate (µ) of the cells was calculated from the exponential (straight line) phase, as $\mu = (\ln N_2 - \ln N_1)/d\ (t_2\text{-}t_1)$, where N_2 is the final cell concentration, N_1 is the initial cell concentration and dt is the time required for the increase in concentration from N_1 to N_2. Doubling time (DT) was also calculated as $DT = \ln 2/\mu$, according to Wood et al. 2005 [11].

The biomass was harvested after the culture reaches stationary phase by centrifugation (Pro-Research by Centrurion Scientific Ltd) at 5000 rpm for 5 min and washed twice with distilled water. The pellets were then lyophilized Christ (Alpha 1–2 LD plus, Germany) and stored at -20˚C until use.

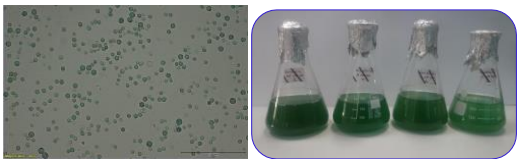

Figure 1. *Galdieria sulphuraria* cells and cultures

2.3. Protein Assay

Malic acid concentrations of *G. sulphuraria* total proteins were estimated by the method of Lowry Protein Assay [12]. Bovine serum albumin standards ranging in concentration from 35-175 μg/ml were run simultaneously with the test samples.

2.4. Lipid Content

Lipid was extracted from lyophilized microalgae biomass using the Blight & Dryer method, described by Demirel et al. 2015 [13].

2.5. Total Sugar Content

Total sugar content was estimated based on the phenol-sulphuric acid method of sugars [14]. D-glucose standards ranging in concentration between 0 and 100 μg/ml, were also run.

3. RESULTS AND DISCUSSION

The effect of autotrophic growth conditions in optimal medium and different malic acid concentrations were compared. The obtained growth curves are reported in Figure 2. Regarding maximum specific growth rates, control and concentrations of malic acid have shown the effects of cell growth on day 14 (Table 1).The highest growth rate of 0.0746 day^{-1} was found in 10mM malic acid concentration. The highest malic acid concentration was reported to be most effective in production of biomass. Also, doubling time was decreased by adding 10 mM malic acid when comparing with control.

G. sulphuraria grown in heterotrophic and mixotrophic batch cultures, the maximum specific growth rate on glucose, fructose or glycerol as carbon substrates was similar (μ_{max} = 1.2±0.1 day^{-1}). Cell growth ceased only when virtually all of the sugar and glycerol had been consumed. But, in phototrophic batch cultures grown at 42 $^{\circ}$C and 103 mol photonsm^{-2} s^{-1}, the specific growth rate was 0.10±0.01 day^{-1} [15].

Genomic analyses of carbon metabolism have suggested that *Cyanidioschyzon merolae*, as *G. sulphuraria*, harbors metabolic pathways for floridoside, trehalose, storage glucans, and matrix polysaccharides [16]. *C. merolae* is predicted to contain a minimal set of metabolic transporters but no aquaporin-type glycerol permease for uptake of glycerol from the environment, or plastidic dicarboxylate translocators, which are required for nitrogen assimilation and for photorespiration and which are conserved in green plants and algae [17].

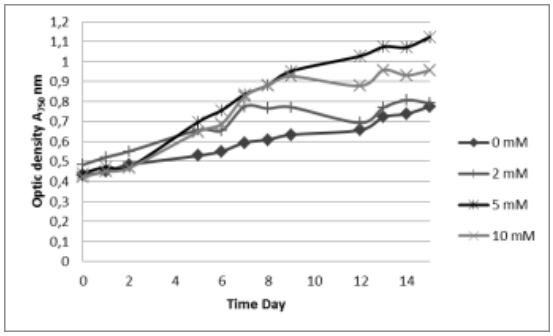

Figure 2. Growth curves for 2, 5, 10 mM malic acid concentrations in optimal medium

Table 1. Doubling time, specific growth rate and biomass of *G. sulphuraria* cultured at different malic acid concentrations

Malic Acid Concentration (mM)	Doubling Time (Day)	Growth Rate (μ_{max})	Biomass (mg)
Control	1.0817	0.0267	53.5
2	0.6505	0.0444	86.5
5	0.4569	0.0632	135.9
10	0.3871	0.0746	155.9

Biochemical studies in oleaginous fungi suggested that malic enzyme is a critical step in supplying the necessary amount of NADPH for fatty acid synthesis occurring under conditions of nitrogen starvation, which induces lipid accumulation [18, 19]. This research is necessary to acquire a complete picture of the interrelationships and dependence of lipid biosynthesis on the overall redox state of algae. Whereas, the highest concentration of protein (76.9047 ± 0.337 %) was obtained in 5 mM malic acid, the highest concentration of lipid (22.108 %) was found in 2 mM concentration.

G. sulphuraria, an algal extremophile, can grow at pH 0.5–4 and temperatures up to 56 °C, conditions that many competitors, predators, viruses, and pathogens will not tolerate [20]. Large-scale microalgal cultures are presently used in human, animal foods and production of fine chemicals, whereas the production of biodiesel from microalgae is in its starting [21].

Table 2. Percentage of protein, sugar and lipid in control, 2 mM, 5 mM and 10 mM malic acid concentrations

Malic Acid Concentration (mM)	Protein %	Sugar %	Lipid %
Control	75.8335 ± 9.933	11.2647 ± 0.790	28.522
2	63.6905 ± 6.902	16.5000 ± 0.624	22.108
5	76.9047 ± 0.337	16.3529 ± 1.539	14.919
10	70.0000 ± 7.071	19.6176 ± 0.125	19.614

4. CONCLUSIONS

In this study the red microalga *G.sulphuraria* was used to investigate the effect of malic acid in its extreme habitat. Considering the pruvate/malate cycle increasing amount of sugar was an expected result while increasing malic acid concentration. The decrease in the quantity of oil was predicted as a reason of increasing sugar concentration. The percentage of protein in the cells started to decrease in the presence of more than 5 mM malic acid concentration.

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ÖZGEÇMİŞ/CV

Neslihan DEMİRCİ; Master Student (Yüksek Lisans Öğrencisi)

Neslihan Demirci was graduated from Chemical Engineering in Ankara University (2014). She still studies as a master student at Biotechnology Department at Ege University. She is working as a scholarship student in a project named "Determination and Optimization of Appropriate Cryopreservation Methods of Microalgae and Cyanobacteria" supported by TUBITAK.

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Çiğdem DEMİRKAYA; PhD Student (Doktora Öğrencisi)

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Lisans derecesini 2012 yılında Ege Üniversitesi Biyomühendislik Bölümü'nden aldı. Yüksek Lisans derecesini 2014 yılında Ege Üniversitesi Biyomühendislik Bölümü'nden aldı. 2014 yılında Ege Üniversitesi Biyomühendislik Bölümü'nde doktora eğitimine başlamıştır. EU FP7 MAREX ve TÜBİTAK 113Z202 no'lu projelerde bursiyer olarak görev almıştır. 2013 yılında ERASMUS bursiyeri olarak Prometheus – Malzeme ve Metalurji Bölümü'nde (KU Leuven/Belçika) bulunmuştur. Temel ilgi alanları biyomühendislik, mikroalgal polimerler ve mikroalglerin doku mühendisliği uygulamalarıdır.

Zeliha DEMİREL; Post. Dr. (Doktora sonrası Araştırmacı)
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