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Obtaining Mutant Strain of *Chlamydomonas reinhardtii* and Studying Its Phenotypic Characteristics

UV mutagenesis is a powerful tool for obtaining mutant strains of microalgae for using in biotesting of the polluted aquatic ecosystems. UV irradiation has a strong mutagenic agent, compared with chemical mutagenesis, UV mutagenesis offers many advantages such as less pollution, simple operation, and sterile cultivation condition. In this study the phenotypic characteristics of the wild and mutant strains of the green microalgae *Chlamydomonas reinhardtii* (*CC 1021*) were investigated. From the obtained results, selection of the mutant colony after irradiation time 1min. and named *CC1021Mut1* strain. The mutant strain showed differences in phenotypic features such as color and size of colonies in the solid medium and also different color in the liquid medium. The mutant cells under microscope appeared different in size and color than the wildtype cells.

Keywords: UV mutagenesis, mutant strain *Chlamydomonas reinhardtii CC 1021 Mut1*, Phenotype (light green color and small size colonies).

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Получение мутантных штаммов C*hlamydomonas reinchardtii* и исследование его фенотипических характеристик

УФ мутагенез является мощным инструментом для получения мутантных штаммов микроводорослей для использования в биотестировании загрязненных водных экосистем. УФ-облучение является сильным мутагенным веществом, по сравнению с химическим мутагенезом, УФ мутагенез имеет ряд преимуществ, такие как уменьшает загрязнения, простота в использовании, и стерильные условия выращивания. В данном исследовании были изучены фенотипические характеристики диких и мутантных штаммов зеленой микроводоросли *Chlamydomonas reinhardtii* (*CC 1021*). В результате были отобраны мутантные колонии штамма *CC1021Mut1*, полученные после 1 мин облучения. Мутантный штамм имеет отличные фенотипические признаки от дикого штамма, такие как размер и цвет колоний на твердой и жидкой средах. Микроскопирование показало, что мутантные клетки отличаются по размеру и цвету от клеток дикого типа.

Ключевые слова: УФ-мутагенез, мутантный штамм *Chlamydomonas reinhardtii CC 1021 Mut1,* фенотип (колонии светло зеленого цвета и малого размера).

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C*hlamydomonas reinchardtii*-дің мутантты штамдарын алу және оның фенотиптік қасиеттерін зерттеу

УК мутагенез микробалдырлардың мутантты дақылдарын ала отыра ластанған су экожүйелерін биотестілеу мүмкіншілігін беретін маңызды әдістердің бірі. УК- сәулесімен сәулелендіру химиялық мутагенезбен салыстырғанда маңыздылығы жоғары мутагендеу әдістерінің бірі, яғни УК мутагенез ластануды төмендету, қолдану қарапайымдылығы, өсірудің залалсыз жағдайын қамтамасыз ету секілді бірқатар артықшылықтарға ие. Зерттеме барысында жабайы және *Chlamydomonas reinhardtii* (*CC 1021*) жасыл мутантты микробалдыр дақылының фенотиптік сипаттамалары анықталды. Нәтижесінде 1 мин сәулелендіру арқылы мутантты *CC1021Mut1* дақылы таңдап алынды. Қатты және сұйық орталардағы мутантты дақыл жабайы дақылға қарағанда колонияларының көлемі, түсі секілді жоғарғы фенотиптік көрініске ие. Микроскоптау мутантты клеткалардың жабайы клеткалардан клетка көлемі мен түсі бойынша анық ерекшеленетінін көрсетті.

Түйін сөздер: УФ-мутагенез, *Chlamydomonas reinhardtii CC 1021 Mut1* мутантты штамы*,* фенотип (түсі ашық жасыл және мөлшері кіші колониялар).

The solar radiation is essential for life on earth. However, an increase in UV-radiation can inhibit many biological processes. The major cellular targets of UV are different biomolecules, which

directly absorb this radiation, or which are indirectly affected by various UV-induced photochemical reactions. The biological and, ultimately, ecological consequences are numerous [1].

It was known that UV induced some physiological effects such as declining photosynthetic rates which can be related not only to damaged biomolecules, but also to ultrastructural changes in organelles or membranes [2]. Typical alterations include swollen mitochondrial cristae, disrupted thylakoids or detached phycobilisomes in chloroplasts, bent-shaped dictyosomes, and damaged plasmalemma. An intact ultrastructure of the algal cell is a prerequisite for optimum functioning of all physiological processes [3].

DNA represent one of the most UV-sensitive biomolecules, and UV-induced damage occurs directly by the absorption of UV, especially UVB, quanta by the aromatic residues. The absorbed energy can be dissipated by different mechanisms involving single bases (e.g. single-strand breaks) or interactions between adjacent bases (e.g. dimerization) and between non-adjacent bases (i.e. inter- or intrastrand crosslinks). Such UV-induced DNA damage can significantly compromise the accuracy of nucleic acid transcription and replication, causing misreading or erroneous replication, leading to an increasing number of mutations. A higher mutation rate can result in reduced gene expression and, hence, debilitation or even increased mortality of algal cells [4]. The major product after UV radiation treatment is cyclobutane pyrimidine dimer (TT, TC, CC). Pyrimidine dimers are repaired by a direct reversal called photoreactivation or by excision of damage in a process of nucleotide excision repair [5].

In this study the phenotypic characteristics of the wild and mutant strains of the green microalga *Chlamydomonas reinhardtii* (*CC 1021*) were investigated. The genus *Chlamydomonas* is of worldwide distribution and is found in a diversity of habitats. *C. reinhardtii* has become the species of choice for genetic studies, because its life cycle was known and it would grow in the dark on an organic carbon source. Descriptive studies in the 19th century led to comprehension of the life cycle of *Chlamydomonas* and to its early recognition as an organism with possibilities for genetic analysis [6]. The discovery of non-Mendelian (uniparental) inheritance of certain antibiotic resistance mutations [7] opened the field of experimental organelle genetics, for which *Chlamydomonas* has remained one of the best model systems.

Materials and Methods

Microalgal strains and cultivation conditions. The microalgal strain of *Chlamydomonas reinhardtii* (*CC 1021(mt$^+$)*) obtained from Kazakh National university- Al-Farabi, Biotechnology Department culture collection. This wild type strain was grown in TAP medium in 250 ml Erlenmeyer flask at 28□C and was exposed to continuous illumination at a light intensity of 120μE $m^{-2}s^{-1}$.

UV irradiation and mutagenesis. According to Harris (1989) [8] , 5ml of the liquid culture with a density of 1x10^6/ml algal cells were placed in 9cm Petri dish forming a thin layer covering the bottom. The dish was placed on shaker with 20rpm and exposed to UVC lamp of 254nm and 40 erg/mm^2 at distance equal 15cm for 0, 1, 3, 5, 7 and 10 min respectively. After UV irradiation the cells were inoculated in solid TAP medium and incubated in dark for 24h to prevent photo-reactivation. Then after the 24h, some dishes incubated in light in photoautotrophic condition and some incubated in dark in heterotrophic condition for a period of 15days.

Results and Discussion

(1) Number of colonies after UV irradiation.

After 15 days, number of colonies was counted in the Photoautotrophic condition for *C. reinhardtii (CC1021)*. Fig 1 showed that the number of colonies decreased when the irradiation time increased and the curve was appeared as C-shape.

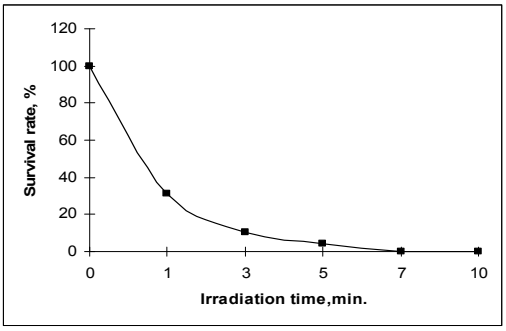

Figure 1 – Number of survival colonies of *C. reinhardtii(CC1021)* at different irradiation time under UV

(2) Description of the wild and mutant colonies of C. reinhardtii (CC1021) in both solid and liquid

media. The selected mutant colony was obtained after irradiation time of 1min. and was named *CC1021Mut1* and subcloned as shown in Fig 2a. After several successful times of subcloning, the mutant cells transferred into liquid TAP medium as shown in Fig.2b. after incubation in the phototrophic conditions for 5 days, the color of culture appeared with light green color than the color of the wildtype culture that was green. Then the wild and mutant cells were transferred to solid TAP medium to investigate the difference between wild and mutant colonies, the mutant colonies were had light green color and small size than the colonies of the wildtype as shown in Fig 3a and b.

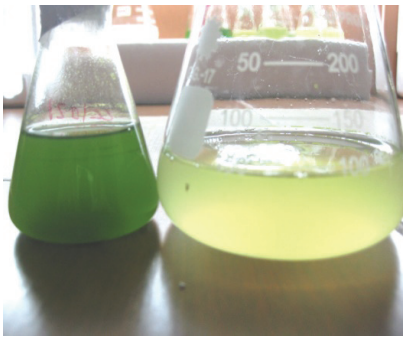

Figure 2a – Subcloning of colonies of wildtype *CC1021* and mutant *CC1021Mut1*

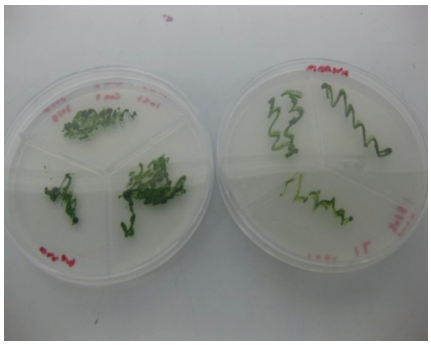

Figure 2b – Color of the liquid culture of *CC1021*and mutant *CC1021Mut1*

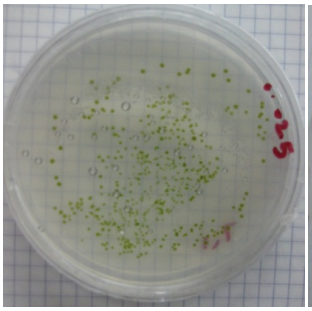

Figure 3a – Color of colonies of wildtype of *CC1021*

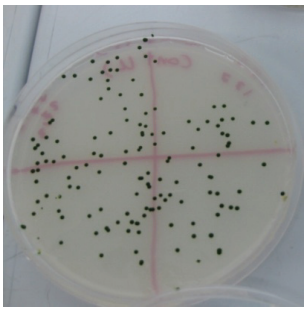

Figure 3b – Color of colonies of *CC1021Mut1*

(3) *Microscopic examination of the wildtype and mutant cells of C. reinhardtii.* The microscopic examination of the cells of both wild and mutant strains was done under the light microscope using the magnification power of 100x (oil immersion lens) as shown in Fig 4a and b. The examination of the fresh cells showed that, the mutant cells appeared larger in size than the wildtype cells, most of the mutant cells are slightly rounded while most of the wildtype cells were had oval shape. Also there was an obvious difference in the color of cells under the microscope in which the mutant cells had faint and pale green color while the wildtype cells had normal green color.

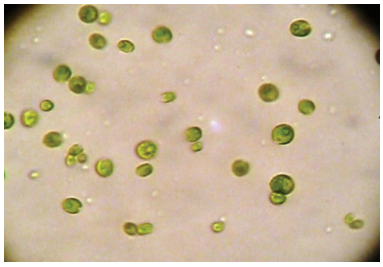

Figure 4a – Wildtype cells of *CC1021* strain

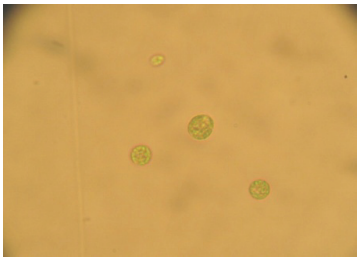

Figure 4b – Mutant cells of *CC1021Mut1* strain

Discussion

Ultraviolet (UV) irradiation has strong mutagenic biological effects on organisms, and UV mutagenesis is an effective breeding method [9]. Compared with chemical mutagenesis, UV mutagenesis offers many advantages such as less pollution, simple operation, and sterile cultivation condition [10]. Several successful cases on microalgae strains for UV mutagenesis have been documented. When photosynthetic organisms are exposed to ultraviolet radiation, significant, irreversible damage to important metabolic processes within the cell may occur such as lesions in DNA and inhibition of photosynthesis [11] that is why the *C. reinhardtii CC1021* unable to grow at irradiation time 7 and 10min, but the results in Fig.3 showed that the cells grown after irradiation time of 1, 3 and 5 min. with a percentages 35, 15 and 5% respectively. In the current study, the most stable colony that grown for several subclones was obtained after irradiation time of 1min, and was named by us as *CC1021Mut1* strain. On the other hand, the other colonies that obtained after irradiation time of 5 and 7 min didn't grow after 3 times of subclones. Generally under optimal light conditions, there is a certain balance between the pigment content in the algal cells which is a charasterstic feature of the species.Under exposure to mutagenic agent, the balance would exchange in either direction, UV irradiation can excite the electron shells, resulting in formation of photoelectrons cuasing a variety of chemical reactions leading to mutations. Upon irradiation, the cells begin to synthsise carotenoids . Quantity of carotenoids produced depends on the intensity of UV radiation. Concerning of UV effect on the photosynthetic pigments of plants and algae, some studies [12] revealed that the synthesis of pigments is blocked, retardation of cell growth as well as there is a strong trend towards increased levels of carotenoid in pigments of mutants. Also,[13] reported that in response to excess of light, a rapid increase in carotenoids propably reflecting the permanently increased needs for photoprotection. In spite of some literatures reported that response of carotenoids to UV is variIable: decreased carotenoids level were observed under UV but they were also stimulated by UV [14] . So, from the phenotepic features which appeared due to the UV irradiation, there was an obvious change in the color of colonies in the solid medium and color of the culture in the liquid medium (Fig 2 and 3) between the wildtype strain (*CC1021*) and the mutant strain (*CC1021Mut1*). As well as, the changes that investigated microscopically as shown in (Fig. 4), in which the mutant cells appearred slightly rounded and larger size than the wildtype cells with light and faint green color. The obtained mutant strain can be used in the biotesting assays of the polluted aquatic ecosystems and there are many prospects in usind such mutants.

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