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Research Article

OPTIMIZATION STUDY OF BIOMASS AND ASTAXANTHIN PRODUCTION BY HYPERDUCING MUTANTS OF *HAEMATOCOCCUS PLUVIALIS* UNDER LABORATORY CONDITION

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ABSTRACT

Astaxanthin is a red ketocarotenoid, widely used as a natural red colourant in marine fish aquaculture and poultry and recently, as an antioxidant supplement for human and animals. The green microalga *Haematococcus pluvialis* is one of the richest natural sources of this pigment. In this study, strains were obtained from the physically and chemically mutated survivors of wild *Haematococcus pluvialis* by using UV irradiation and ethyl methane sulphonate (EMS). Mutated strains showed a maximum survival rate 31.9 % and 47.7 % increase in biomass compared with wild type. The maximum concentration of total carotenoids content was recorded in 5.780 µgmL^{-1} on 14th day at 0.24% ethyl methane sulphonate 44.21% as compare with wild culture, respectively. Similarly, 15 min UV irradiation favoured maximum concentration of total carotenoids 4. 990 µgmL^{-1} on 14th day which was more than 24.50 % that of control. We observe that the mutagenesis is an effective strategy for genetically improving strains of *Haematococcus pluvialis* and that improved carotenogenic capacity is maintained when the volume of culture is scaled up to a large size.

Keywords: Astaxanthin, *Haematococcus pluvialis,* Ethyl methane sulphonate, UV irradiation, Mutagenesis

1. INTRODUCTION

Green microalgae comprise more than 7000 species growing in a variety of habitats. *Haematococcus pluvialis (*Chlorophyceae, Volvocales) is unicellular freshwater microalga distributed in many habitats worldwide. It is considered as the best natural source of astaxanthin and the main producing organism of this commercial product Astaxanthin Production from *H. pluvialis* [1, 2]. Astaxanthin (3,3'-dihydroxy-b, b-carotene-4,4'-dione; $C_{40}H_{52}O_4$) is a high value keto-carotenoid, which is mainly found in aquatic animals. Astaxanthin has widespread applications, owing to its important biological functions, including protection against irradiation damage, promotion of oxidative stress resistibility,and enhanced reproduction [3, 4]. Beutner S *et al.* [5] indicated that carotenoids, as biological antioxidants (AO), can prevent other molecules from being destroyed with degradation of carotenoids, by assimilating excited energy of singlet oxygen into carotenoids. Astaxanthin as an AO is reported to have stronger antioxidant capacity than that of β-carotene and Vitamin E [6]. Besides, *H. pluvialis* mostly accumulates lutein and β-carotene in the green motile phase prior to stress exposure, and they are also valuable carotenoids, which benefit human health. Since the *Haematococcus* algae astaxanthin has the traits mentioned above, they have been involved in various industrial applications.

The market value of astaxanthin is expected to exceed $1.5 billion by 2020 [7], mainly incorporated in dietary supplements, nutraceuticals, cosmetics, as well as feed additives in the aquaculture and agriculture sectors [8, 9]. Currently over 95% of astaxanthin utilised for these applications is chemically synthesised, with <1% derived from *H. pluvialis* [10]. To a large extent this is due to the cost of production, as synthetic astaxanthin is around $1000/kg, compared to *H. pluvialis* derived astaxanthin at ~$3000–$3600/kg [11, 12]. However, concerns have been raised linked to the sustainability of synthetic astaxanthin production as it is derived from petrochemicals. Also, the stereochemistry differs between the synthetic and *H. pluvialis* derived forms with the (3S, 3'S) form predominant in *H. pluvialis* and a mixture of the three stereoisomers (3R, 3'R), (3R, 3'S) and (3S, 3'S) in ratios of 1:2:1 in synthetically synthesised material [13]. There are also concerns about efficacy and human health benefits as it has been reported that the isomer found in *H. pluvialis* has a higher bioactivity, compared to synthetic astaxanthin [14].

Additionally, this pigment is accepted as a natural product, has been approved as a colour additive for salmon feeds and as a nutraceutical for human use in the USA, Japan and several European countries [15].

Random mutagenesis has been successfully applied in the past to improve the productivity of various microalgal species with biotechnological application [16], including *H. pluvialis* [17, 18]. The advantage of this approach is its technical simplicity with no need for information on the genes involved or their regulation. This experimental strategy includes a first stage where random mutants are generated and second phase where mutants are generated and a second phase where mutants are selected under selection pressures imposed by chemical inhibitors of critical steps in the biosynthesis of the target metabolites.

2. MATERIAL AND METHODS

2.1. Procurement of strain

The green microalga *Haematococcus pluvialis* UTEX -2505 used in the present investigation was provided by Algal Biotechnology Lab., Department of P.G. Studies and Research in Biological Science, R. D. University, Jabalpur (M.P.).

2.2. Mutagenesis and Isolation of *Haematococcus* mutants

2.2.1. Mutation with UV irradiation

Algal cells taken from the mid-growth phase (1.8 x10^4 Cell mL^{-1}) were exposed to UV irradiation for different period of time (0, 15 to 60 minutes) in a sterile Petri dish using UV light 253.7nm at a distance of 15cm and cells were kept for one night in the dark [19].

2.2.2. Mutation with ethyl methane sulphonate (EMS)

Two ml of *H. pluvialis* (1.8 x 10^4 Cell mL^{-1}) was washed with 0.2 M sodium phosphate buffer and treated with EMS in the concentration range of 0.12% to 0.48%w/v level for 30 min. Treated cells centrifuged was re-suspended in Bold Basal Medium (BBM) medium and rewashed thrice and then kept in dark 12hrs. Then serial dilution of each culture were prepared and plated on solid Bold Basal Medium (BBM). Cultures were incubated in controlled air conditioned culture room maintained under 16:8 h (Light/Dark) period and maintained at temperature 25±2°C.When colonies were visible (after 25-30days), they were counted and transferred in liquid (BBM) Bold Basal Medium [21].

2.3. Mass production of *Haematococcus pluvialis* wild and mutant strain

The experiment was performed on flat plate type photobioreactors with dimensions 31.6×15.5×30.3cm the reaction volume was 7L in which the inoculums made up 10%. The reactors were connected to air bubble system, using an air compressor, ensuring, and the necessary tuberulance for the mass transfer. The light intensity 35µmol m^{-1} s^{-1}, the temperature was kept at 25±2°C and the pH ranged from 6.8-7. The reactors were inoculated inside a laminar air flow chamber and then transferred to another room with controlled and ambient light. The intensity of light was measured by a light meter and or a radiometer.

2.4. Evaluation of Chlorophyll and Carotenoid content

A known amount of the cells was extracted in methanol, chlorophyll (*a,b*) and Carotenoid content was estimated spectrophotometrically (SYSTRONICS VISIBLE SPECTRO 105 Mumbai India) by taking absorbance at 665nm, 652nm and 470 nm respectively using the method of Lichtenthlar *et al.*[20].

3. RESULTS

3.1. Effect of UV and EMS on *H. Pluvialis*

In the present attempt, the cells were exposed to UV irradiation. Results showed that the maximum survival rate of 31.9% when the cells were exposed to UV for 15 min. it was examined that the survival rate decrease with the increase in exposed time.

The algal cells were treated with EMS at different concentration (0.12% to 0.48% w/w) to obtain mutants. The cells treated at 0.24% (w/w) showed high survival rate of 47.7% when compared with control culture.

3.2. Mass production of mutants and wild culture

Higher survivor rate strains were selected for the mass production. Strain UV 15 min and EMS 0.24% (w/w) of them were compared on the growth and total carotenoid content with those of the wild type. The results obtained a maximum concentration of Chl *a* content 2.094 µgmL^{-1} and 2.300 µgmL^{-1} were recorded on 10^{th} day. The increment of mutants was more than 66.45 % and 82.82 % to that of wild type. Similarly maximum concentration of Chl *b* 7.753 µgmL^{-1} and 8.598 µgmL^{-1} recorded on 12^{th} days were more than 30.78% and 45.04% respectively, to that of wild culture. The mutant strains accumulated maximum amount of total carotenoids of

4.990 µgmL^{-1} and 5.780 µgmL^{-1} on 14th days, which were more than 24.50 %and 44. 21% when compared to wild type.

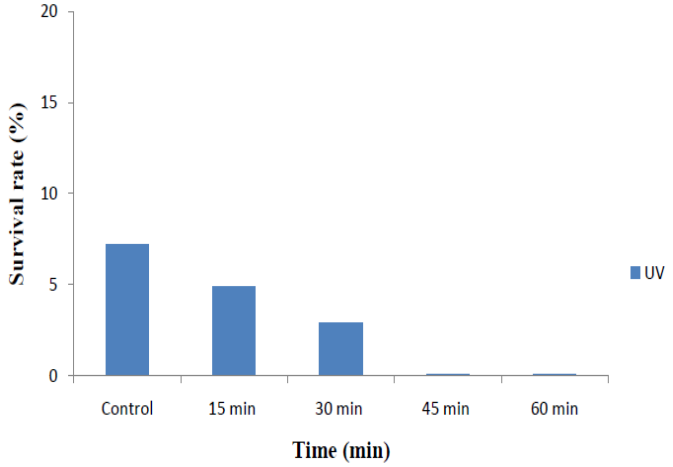

Fig. 1: Effect of UV irradition on survival of *H. Pluvialis*

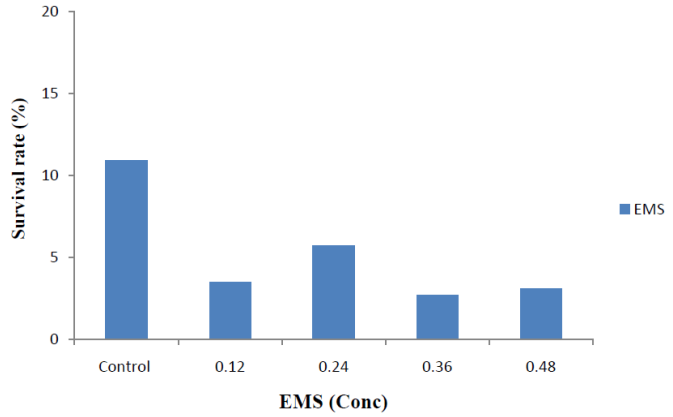

Fig. 2: Effect of ethyl methane sulphonate on survival of *H. Pluvialis*

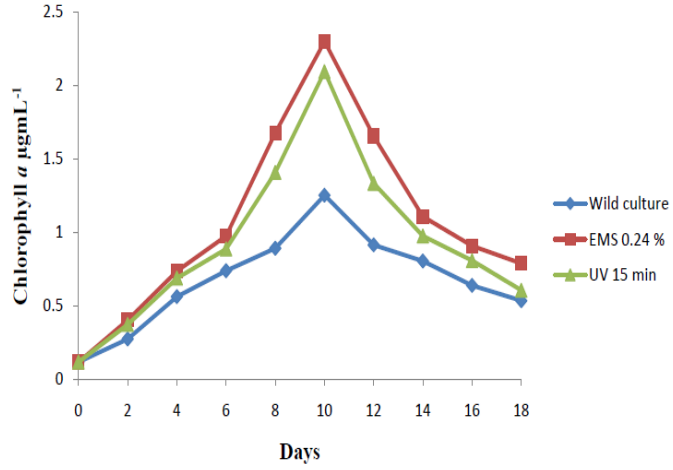

Fig. 3: Effect of each treatment on the Chl *a* of the *H. pluvialis* culture in photobiorectors

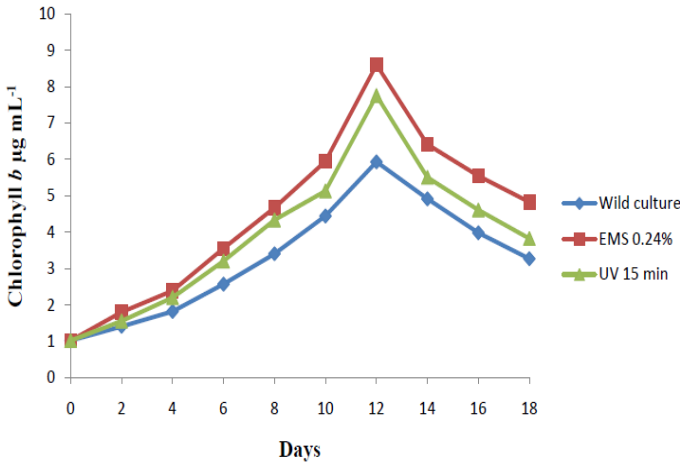

Fig. 4: Effect of each treatment on the Chl *b* of the *H. pluvialis* culture in photobiorectors

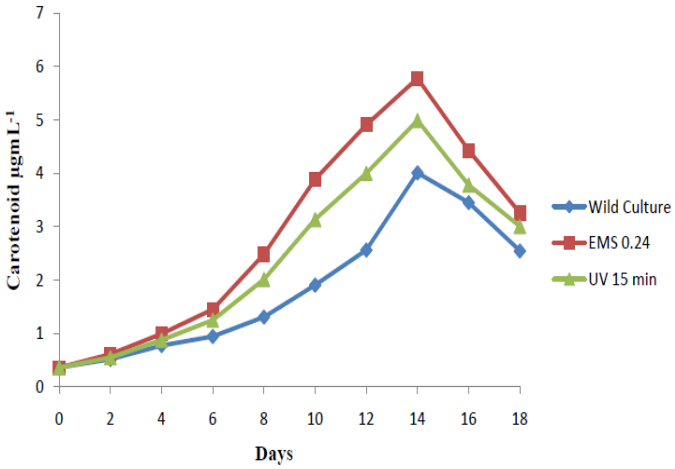

Fig. 5: Effect of each treatment on the total carotenoid of the *H. pluvialis* culture in photobiorectors

4. DISCUSSION

The global market for astaxanthin is worth more than US$ 200 million per year. About 130 tons of astaxanthin are consumed annually to feed the salmonids produced globally by aquaculture, of which > 90% is presently produced by chemical synthesis [21]. However, despite chemical synthesis providing a stable source of synthesis astaxanthin, there is concern about its biological functions and food safety. UV- irradiation has been known to affect the motility community, composition, pigmentation and several metabolic processes of algal system by changing the structure of the genes coding the key enzymes [22]. UV irradiation is known to affect the motility, community composition, pigmentation and several metabolic processes of algal systems [23]. EMS is a mutagenic agent which is capable of inducing point mutation by reactions with DNA and causing transitional

change in nucleotide sequences, such as A-T transition to G-C [24]. In recent year, numerous studies on culture conditions and the selection of suitable strains for mass culture of *Haematococcus pluvialis* have been conducted [25]. In our work, we grew wild and mutant strains in flat plate type reactors, a significantly higher culture volume, and obtained a maximum production of total carotenoid of mutants strains 24.50% and 44.21% as compared with wild type. UV irradiation and EMS compound mutagenesis was used to bread to high producing astaxanthin strain of *Haematococcus pluvialism.* These results were consistent with findings of [20, 4].

5. CONCLUSION

Improvement of *Haematococcus pluvialis.*by mutagenesis was showed to be a successful strategy to increase the amount of astaxanthin. Astaxanthin have great demand in food, feed, nutraceutical and pharmaceutical applications. Mutagenesis process will be effective strategy for genetically improving strain of *Haematococcus pluvialis.* Improved astaxanthin productivity of the mutant strain was maintained even when grown on large scale and holds promise as the basis for viable commercial production of this valuable biochemical by natural means.

6. ACKNOWLEDGEMENT

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