Int.J.Curr.Microbiol.App.Sci (2022) *11*(06): 266-269

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 06 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1106.029

Algal Strain Improvement by Chemical Mutagenesis of Microalga AMS16 A Strain of Thraustochytrid Alga *Schizochytrium limacinum*

Sailendra Bhaskar[1, 2*], Krishnan Kathiravan[1] and Sachitra Kumar Ratha[2]

[1]*Department of Biotechnology, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India*
[2]*Department of Biotechnology, Aban Infrastructure Private Limited, Chennai - 600 008, Tamil Nadu, India*

**Corresponding author*

Keywords

Nutraceutical industries, biomolecules, non-mutated parent strains, mutate algae

Article Info

Received:
12 May 2022
Accepted:
31 May 2022
Available Online:
10 June 2022

A B S T R A C T

Random mutagenesis has for long been used as a tool to genetically modify organisms for various purposes, such as increasing biomass yield or yield of specific biomolecules or improved adaptation to cultivation conditions. Random mutagenesis is especially attractive for systems where it is not obvious which genes require modification and has been extensively used to beneficially modify crop plants. However, even with the renewed interest in microalgae for biofuel applications, there is relatively little current research available on the application of random mutagenesis in microalgae. In this research project we used the chemical mutagen NTG - N-methyl-N'-nitro-N-nitrosoguanidine in the medium that the thraustochytrid alga *Schizochytrium limacinum* was grown and algae that survived the mutagenesis were cultured and analysed for lipid production. One mutagenic strain of the alga was found to grow well and produced 30% more lipid than the control which was not exposed to NTG. This proves that random mutagenesis can be an important tool in the hands of algal scientists who are looking to improve productivity of biomolecules in specific algae.

Introduction

Random mutagenesis has been a tool used by plant scientists worldwide for several decades to create and isolate mutated strains of plants that yield higher or are adapted to specific environments better than the non-mutated parent strains. It is common knowledge that many varieties of pulses and grains grown across the world were developed from mutated strains however few if any attempts have been made to mutate algae to obtain improved strains of algae that may yield higher biomass or specific biomolecules of interest to pharmaceutical or nutraceutical industries. Chaturvedi *et al.*, used EMS (Ethyl MethaneSulfonate) to generate mutant microalgae. While their experiments and screening

were designed to increase the content of eicosapentaenoic acid within the algae, they noted that the generated mutants demonstrated better thermotolerance compared to the parent (Wild Type) strain. This study amply demonstrates the viability of using random mutagenesis to increase thermotolerance of phototrophic organisms.

Other alkylating mutagens such as Methyl Methane Sulfonate (MMS) have also been used by some researchers (Todd P *et al.,* 1979) who found it to be useful in producing beneficial mutagenesis in *Escherichia coli.*

The thraustochytrid alga *Schizochytrium limacinum* is of commercial importance because of its ability to produce significantly high amounts of Omega-3 fatty acids (DocosaHexaenoic Acid DHA and EicosaPentaenoic Acid EPA).

Omega fatty acids are of great importance because it is mandated for use in Infant Formulae and Follow-On-Formulae fed to new born children worlwide. Currently 75% of the Omega fatty acids are sourced from fish and this has resulted in the over-fishing of certain species of fish such as the Atlantic Menhayden. It is important to note here that fish do not produce these lipids in-vivo, they are bioaccumulators of lipids derived from the algae they consume as feed. Therefore there is a commercial case for culture of *Schizochytrium* sp for the extraction of Omega fatty acids. This would be ecologically sustainable because the fish stocks would not need to be harvested to produce Omega fatty acids.

Materials and Methods

AMS-16 *Schizochytrium limacinum* isolate, pure axenic culture

Medium with agar liquid broth

NTG (N-methyl-N'-nitro-N-nitrosoguanidine)

Petri plates and test tubes with 9 mL of media

Total lipids in the alga were extracted and estimated according to the method of Folch *et al.,* Protein was estimated using Lowry's method (Lowry *et al.,* 1951). Total carbohydrates were measured by the phenol-sulphuric acid method developed by Dubois *et al.,*

Procedure

1mL of the AMS16 culture was taken in three fresh centrifuge tubes and centrifuged at 1500rpm for 15minutes. The supernatant was discarded while the pellet was retained. To this pellet 1mL of NTG solution (conc.150mg/L) was added. This NTG added pellet was kept for incubation at three time intervals i.e. 20 min, 30 min and 40 min. After incubation the tubes were taken and centrifuged at 1500rpm for 15 minutes. The supernatant was discarded and the pellet was washed with 1mL of the nutrient medium three times. After thoroughly washing the pellet it was dissolved in 1mL of the nutrient media.

This 1mL of NTG-treated culture was serially diluted from 10^{-2} to 10^{-6} and from this the serial dilution of 10^{-3} was plated on agar plates with nutrient medium. The plates were kept for growing under dark conditions in an incubator at 25 ± 1^{0}C. Another plate with 10^{-3} serially diluted culture without NTG treatment was kept as control for each treatment. After three days the colonies which appeared on the mutant streaked plates were picked up and inoculated in liquid broth as was the control. After attaining a dense culture, biochemical composition estimation was done on the control and the mutant strains and was compared.

Results and Discussion

AMS16 *Schizochytrium limacinum*

The AMS16 strain was taken from the germplasm collection of the Biotechnology Division of M/s Aban Infrastructure Private Ltd. This is a heterotrophic marine microalga. Strain improvement studies were conducted using NTG mutagenesis and the mutant strain was assessed for its biomass productivity and biochemical composition.

Description of AMS16

The AMS16sp is spherical in shape and found in colonies. And the cells are colourless and non-motile. The diameter of the cells is approximately 9.6µm.The cells divide by binary fission to produce zoospores which are biflagellate.

Biomolecule Composition

The lipid percentage in the mutant and control were found to be highest for the day 1 treatment.

The biochemical composition of the wild strain of the microalga AMS16 under standard laboratory conditions was 16.8%, 10.7% and 56% of carbohydrate, protein and lipid, respectively. After NTG treatment the mutant strain's biomass productivity was almost similar while the biochemical composition differed considerably from the control – the biomolecule content in the mutant was 9.9%, 14% and 73% of carbohydrate, protein and lipid respectively. The protein content decreased 43% compared to control whereas lipid content increased from 56% to 73%, which was more than 30% when compared to control. (Fig.2). This study confirms that NTG mutagenesis is a potential tool to improve the lipid yield of algal strains. Lian *et al.*, (2010) earlier reported that the UV and NTG mutagenesis could improve the lipid yield of heterotrophic *Schizochytrium* mutant strain by 34% when compared to control.

They also reported that the activity of G6PDH Glucose- 6-phosphate dehydrogenase of the mutant was higher than the parent strain, which indicated that the HMP pathway of the mutant was strengthened, and more NADPH was thus produced. They also found that the activities of 3 key enzymes increased in the mutant strain i.e. 27.6% for Glucose- 6-phosphate dehydrogenase (G6PDH), 152.3% for Malic Enzyme (ME), and 200% for ATP–citrate lyase (ACL). Similar metabolic changes would have occurred in the mutant strain obtained in this study through NTG mutagenesis which could explain the increased lipid yield which is a commercially significant result. Benedetti *et al.*, 2018 have proposed the use of microalgae as biofactories for specific biomolecules and recommended strain improvement efforts while Ullrich *et al.*, 2012 have proposed methods of working out dosages of mutagenic agents for algae.

Fig.1 Photomicrograph of *Schizochytrium limacinum*AMS16 strain

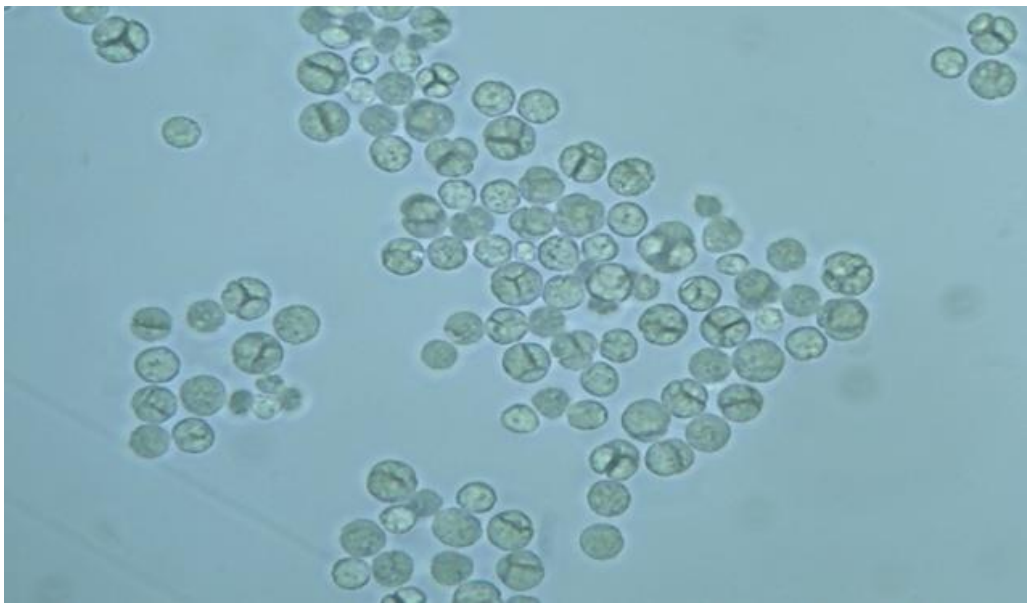
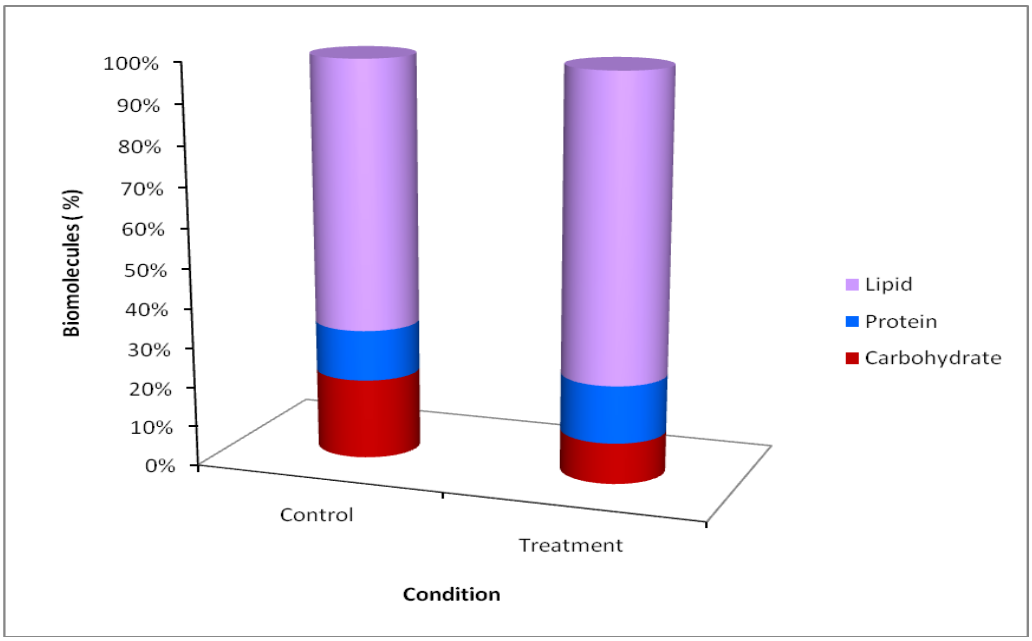


Fig.2 Comparison of biomolecules, carbohydrate, protein and lipids between control and NTG mutagen under heterotrophic conditions, of the alga *Schizochytrium limacinum*isolate



AMS16

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How to cite this article:

Sailendra Bhaskar, Krishnan Kathiravan and Sachitra Kumar Ratha. 2022. Algal Strain Improvement by Chemical Mutagenesis of Microalga AMS16 A Strain of Thraustochytrid Alga *Schizochytrium limacinum*. *Int.J.Curr.Microbiol.App.Sci.* 11(06): 266-269. doi: https://doi.org/10.20546/ijcmas.2022.1106.029