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REVIEW

Insights into random mutagenesis techniques to enhance biomolecule production in microalgae: Implications for economically viable bioprocesses

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Abstract Microalgae, as a diverse photosynthetic group of microorganisms with substantial ecological significance, have enormous potential for use in the food, medicine, and energy industries. However, current limitations in cost-effective production restrict their ubiquitous use. The purpose of this comprehensive mini-review is to investigate the impact of random mutagenesis techniques on microalgae and their biomolecule content, with the intention of improving the productivity and sustainability of cell factories for economically viable bioprocesses. Moreover, due to their simplicity and ease of cultivation in laboratory settings, microalgae serve as valuable model organisms for numerous scientific studies. This study investigates the effects of chemical and physical mutagens on microalgae in order to generate a heterogeneous microalgal population with advantageous traits such as increased lipids, proteins, and carotenoids, accelerated development, and enhanced nutrient absorption. This study findings will provide valuable insights into the manipulation of microalgae to increase biomolecule production and release their potential for a variety of applications.

Keywords Microalgae . Random mutagenesis . Chemical mutagens . Physical mutagens . Strain development . Microalgal biotechnology

Introduction

Microalgae are small aquatic microorganisms belonging to the Protista kingdom (Varshney et al. 2015). They can undergo photosynthesis and can be found in a range of habitats, including freshwater, marine, and terrestrial ones (Metting 1996; Singh and Saxena 2015). Additionally, they are used in numerous commercial and research applications, as well as in the global carbon and nitrogen cycles, and as food for other creatures (Chu 2012; Richmond 2008). These microorganisms can create a large variety of products that are useful in industry, including carbohydrates, lipids, pigments, and vitamins (Chen et al. 2016; Fathy et

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al. 2023). Furthermore, they can build a variety of lipids, such as trans-fatty acids, fatty acid methyl esters, polyunsaturated fatty acids, and omega-3 fatty acids (Maltsev and Maltseva 2021). As a result, microalgae are considered crucial in developing alternative feedstocks to deal with the global food and feed shortage (Caporgno and Mathys 2018). Despite the remarkable potential of microalgae for many biotechnological uses, the industrialization of microalgae-based goods, such as the biomass itself or additives like pigments and polyunsaturated fatty acids, is still confined to high-value specific markets.

The current high manufacturing costs and unsuccessful marketing and sales efforts have hampered the growth of the microalgal market and business (Kumar et al. 2020). Challenges in microalgal cultivation include poor biomass conversion efficiencies, low target bio-compound productivities, significant environmental changes in outdoor settings, culture contamination, and expensive inputs such as culture media and energy consumption (Trovão et al. 2019; Trovão et al. 2022). Additionally, exposure to abiotic stress factors like temperature and salt negatively impacts overall productivity unless a robust and stress-tolerant strain is utilized (Sun et al. 2018; Tredici 2004). To overcome these limitations in industrial-scale microalgal biomass cultivation, a multistage optimization strategy is necessary across the entire production and processing pipeline (Xue et al. 2021). Adopting a biorefinery and circular economy strategy can lead to the redesign of the pipeline, resulting in more commercially viable industrial-scale processes (Lai et al. 2019). While only a few naturally occurring microalgal strains possess the desired qualities for successful industrial production in various biotechnological applications (Song and Pei 2018), these strains need further improvement to achieve large-scale production and profitability.

Random mutagenesis and targeting strategies, such as genetically modified organism creation, can be employed to develop more resilient and productive strains compared to their wild-type counterparts (Spicer and Molnar 2018). However, natural processes like random mutagenesis and adaptive laboratory evolution are slow and unfocused, necessitating the use of strategies to accelerate them. These approaches enable the creation and selection of mutant organisms with industry-suitable characteristics (Hu et al. 2017). The concept of random mutagenesis involves subjecting microalgae cells to chemical or physical mutagens, leading to the generation of a diverse population of mutants with distinct genetic and phenotypic characteristics. These mutants undergo a thorough examination to identify desired cellular traits and improved metabolic capabilities (Bleisch et al. 2022; Zhang et al. 2018b). Mutation is a natural or synthetic process that occurs in all living organisms, including microalgae. It entails changes to a gene's DNA sequence that can result from a variety of things, including mistakes in DNA replication, exposure to toxins or radiation, or viral infection (Hlavova et al. 2015). Mutations can bring about notable changes in microalgae, including alterations in cell size, shape, pigment synthesis, and metabolic activity (Damsky and Bosenberg 2017; Sohrabi et al. 2016). Some mutations may confer advantages to the microorganism, such as enhanced adaptation to specific environments or the ability to produce desired chemicals (Betancourt 2007; Elena and Lenski 2003). However, the impact of mutations can vary, with some being detrimental or neutral depending on their effect on normal microorganism functioning and their specific consequences (Pamilo et al. 1999; Soskine and Tawfik 2010).

Microalgae can be mutated using a variety of techniques as depicted in (Fig. 1), such as genome editing, adaptive laboratory evaluation, chemical mutagenesis, and physical mutagenesis (Sproles et al. 2021; Trovão et al. 2022). Chemical mutagenesis is the process of damaging the DNA of microalgae by exposing them to mutagenic compounds, such as alkylating agents or DNA intercalating agents (Cid et al. 2012). While microalgal cells are exposed to physical agents, such as high-energy particles or ionizing radiation, that can also induce DNA damage (Bleisch et al. 2022). Further genetic engineering techniques such as CRISPR/Cas9-mediated genome editing alter the microalgal genome in a particular manner (Fathy et al. 2021; Patel et al. 2019). The optimal strategy for a particular application will depend on the precise objectives and requirements of the research, as each of these techniques has its benefits and drawbacks. Chemical mutagenesis, for instance, is relatively simple and inexpensive, but it has the potential to produce toxic or hazardous mutations (Khan et al. 2009). Physical mutagenesis is also quite simple, but it can be expensive and requires specialized equipment (Bleisch et al. 2022). Although genetic engineering techniques are becoming increasingly specialized and precise, their application can be difficult and time-consuming (Trovão et al. 2022).

Mutation serves as a valuable tool for enhancing microalgae strains to improve their growth rate or lipid production, thereby benefiting various applications (Senthamilselvi and Kalaiselvi 2022). Furthermore,

inducing mutations can lead to the development of microalgae strains with increased tolerance to harsh environmental conditions such as high salinity or temperature, which can be advantageous for environmental applications including bioremediation (Ong et al. 2010). Mutations can be deliberately induced to enhance the production of valuable substances like pigments or proteins, providing opportunities for diverse industrial purposes (Cecchin et al. 2022; Schüler et al. 2020). However, it is crucial to carefully evaluate the risks and benefits associated with each approach for inducing mutations, and thorough screening and testing of the resulting mutants must be conducted. Critical considerations include assessing the stability of mutants and ensuring the absence of any toxic or hazardous substances released (Gepts 2002; Sauer 2001). Additionally, when introducing genetically engineered microalgae into natural ecosystems, it is essential to take into account potential adverse ecological and environmental impacts (Bajpai 2022; Henley et al. 2013).

Consequently, the study of microalgal mutation is a significant and dynamic field that holds immense importance in various domains, including business, academia, and environmental protection. To develop safe and efficient techniques for generating mutant strains of microalgae and utilizing them across a wide range of applications, a comprehensive understanding of the causes and effects of microalgal mutation is

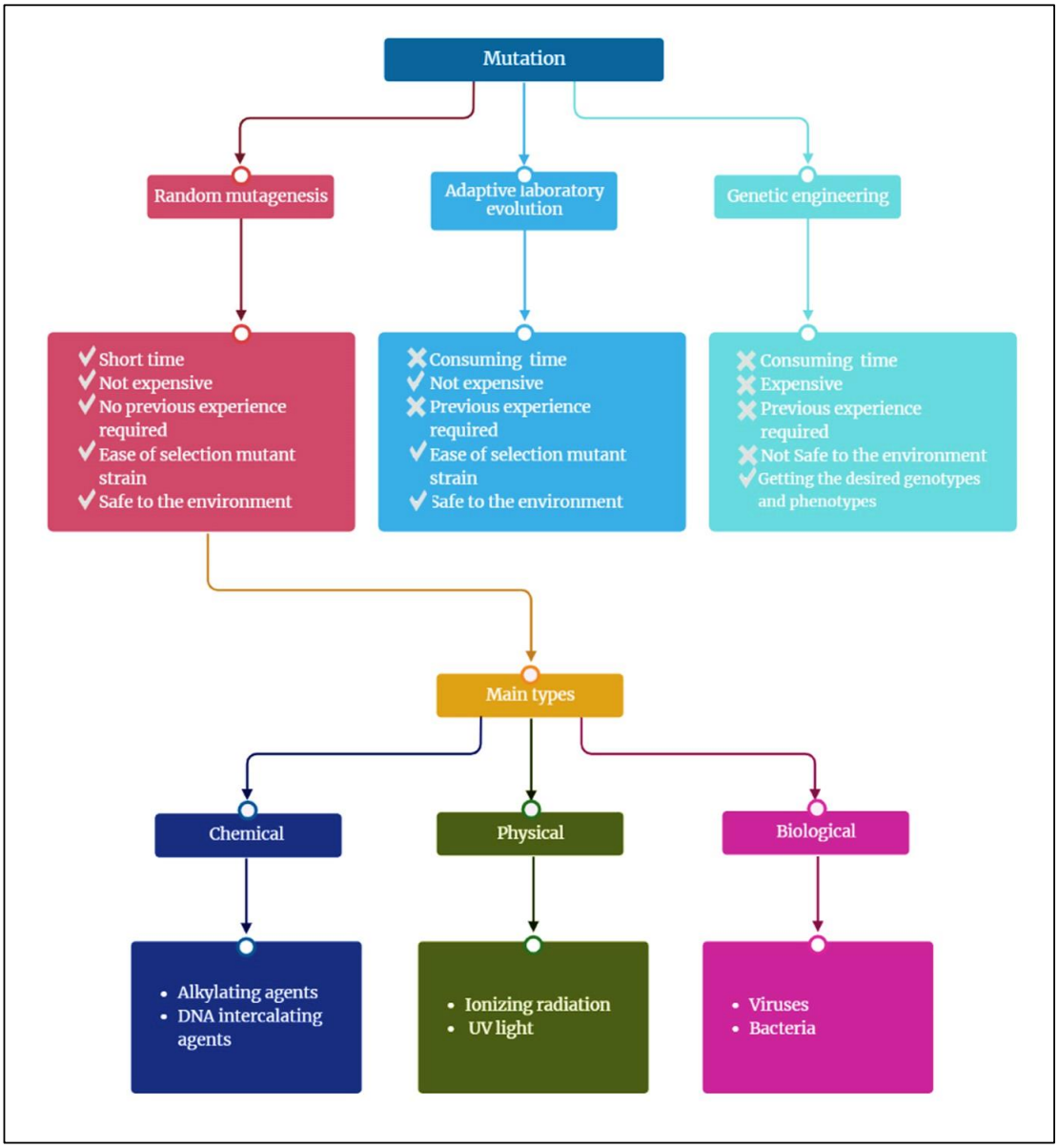

Fig. 1 Illustrating the different techniques utilized in microalgae mutagenesis, emphasizing the advantages and disadvantages associated with each method, along with notable examples of their application

essential. Mutagenesis serves as a vital approach for altering microalgae to achieve diverse objectives, such as biofuel production, pharmaceutical applications, cosmetic formulations, and enhancing nutritional value. Therefore, this study provides a comprehensive overview of techniques employed for inducing random mutations in microalgae, with a specific focus on chemical and physical methods. To present novel perspectives on random mutagenesis and emphasize their approach and outcomes, published studies spanning from 1968 to 2022 in this field were thoroughly examined and critically discussed in the subsequent sections.

Random mutagenesis; Techniques and effects

Random mutagenesis usage

Random mutagenesis is an accelerated process that induces mutations in microorganisms by exposing them to potent chemical or physical mutagens. The mutants exhibiting desired traits are selected from the resulting population (Hlavova et al. 2015). This well-established and accessible technique has been employed for over a century, primarily for phenotype-driven mutant generation rather than specific gene alterations (Cheng et al. 2019). Ethyl Methane Sulfonate (EMS) has emerged as a frequently used mutagenic agent (Flibotte et al. 2010). Random mutagenesis has gained prominence in the field of microalgae due to its effectiveness in developing strains with improved biomass productivity, target compound production, and environmental adaptability, requiring less expertise in microalgal genetics (Dinesh Kumar et al. 2018; Tillich et al. 2012). However, the isolation of stable mutant strains is often hindered by the occurrence of cell death or growth inhibition, with the possibility of reverting to the wildtype state (Garrido-Cardenas et al. 2018).

Factors influencing random mutagenesis

Several factors throughout the pre-, during, and post-mutagenesis stages can influence the outcomes of random mutagenesis in microalgae. Carbon and nitrogen availability, as well as the quality and quantity of light, are crucial considerations when handling photosynthetic microalgae (Kim et al. 2014; Morschett et al. 2017). The type and concentration of the mutagen, duration of exposure, ambient conditions, and other variables impact the results of mutagenesis (Bleisch et al. 2022). Parameters such as cell survival rate and mutation rate serve as indicators for assessing the effectiveness of mutagenesis, with a desired survival rate of around twenty percent to achieve a favorable mutation rate within the surviving cell population (Carino and Vital 2022). To minimize the photoactivation of cellular repair pathways, many studies recommend keeping the cells in darkness for at least the following night or up to 24 hours after mutagen exposure (Yamamoto et al. 2017). Chemical, physical, or insertional mutagens can be used to induce random mutation in microalgae, causing alterations in the organism's DNA. Mutants with desired metabolic characteristics can then be selected from the mutant population.

Types of mutations and their effects

Mutations can occur in various ways, resulting in different impacts on the microorganism. Examples of different categories of mutations as shown in (Fig. 2) include (a) Point mutation: A single base pair in the DNA sequence is altered, leading to base pair deletions, insertions, or substitutions (Cooper and Krawczak 1990). (b) Gene mutation: The structure or function of a gene is modified, influencing its functionality through variations such as deletions, duplications, and other alterations in the gene sequence (Levine 1993). (c) Insertional mutations: These mutations involve the insertion of additional DNA into the genome, potentially affecting the normal function of nearby genes (Van Lijsebettens et al. 1991). (d) Knockout mutation: This type of mutation results in the loss or inactivation of a specific gene, often used in research to study the function of a particular gene (Tamae et al. 2008). (e) Missense mutation: A single base pair change leads to the incorporation of a different amino acid in the protein encoded by the gene, potentially affecting its functionality (Benzer and Champe 1962). (f) Nonsense mutation: This mutation involves the insertion of a stop codon or a frame shift, resulting in the synthesis of a truncated or non-functional protein (Aoufouchi et al. 1996). (g) Silent mutation: A change in the DNA sequence that has no impact on the functionality of the protein (Chamary and Hurst 2009).

Summary of publications utilizing random mutations in microalgae

Overview of published studies

A comprehensive analysis of the literature revealed a total of 109 publications employing random mutagenesis techniques to enhance microalgae. These studies spanned from 1968 to the end of 2022, indicating the sustained interest in this field (Fig. 3). Among these publications, 51 employed chemical approaches, 54 papers utilized physical methods, and 4 utilized a combination of both methods.

Chemical methods

Chemical mutagenesis accounted for 46.78% of the studies utilizing random mutagenesis. Of the chemical methods employed, EMS emerged as the most frequently used mutagenic agent, featuring in 34.9% of all studies and 75% of the chemical publications. Other chemical agents were also employed, although less frequently, highlighting the diversity of approaches within this category.

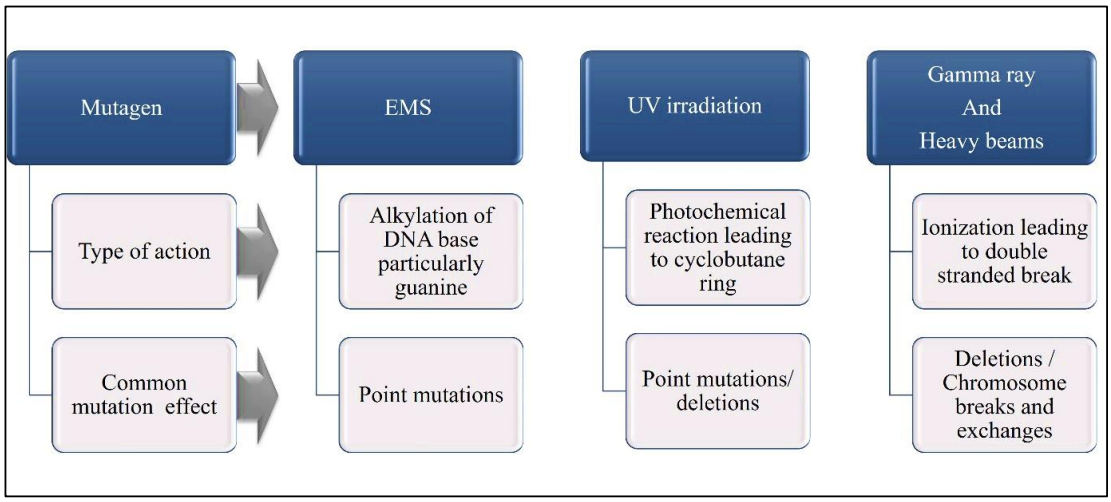

Fig. 2 Effects of commonly used mutagenic agents on microalgal cells and their DNA mechanisms

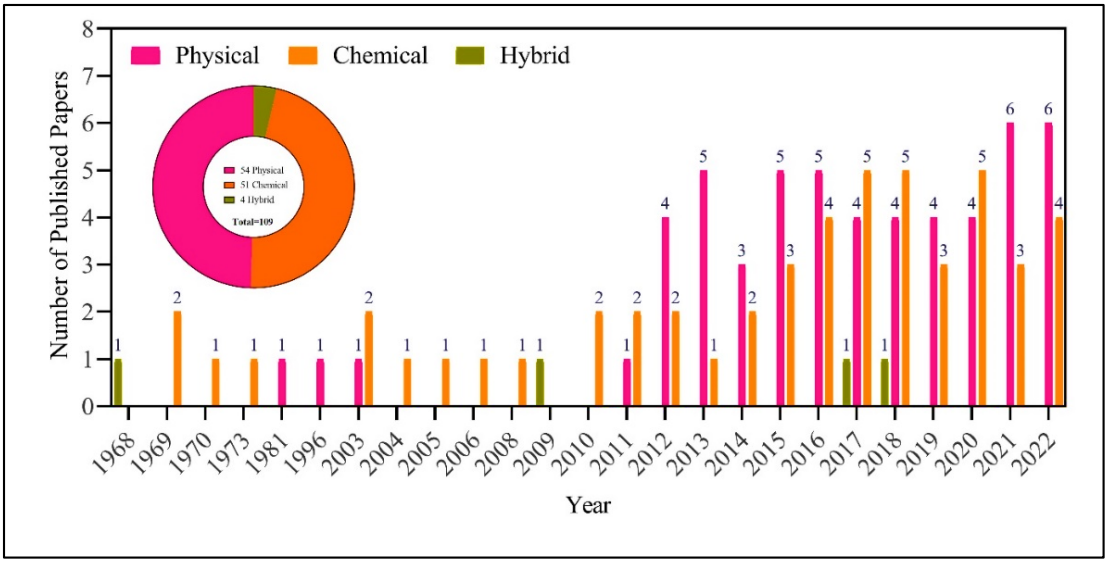

Fig. 3 Categorical distribution of published literature on the topic of random mutagenesis using physical, chemical methods, or a hybrid of both.

Physical methods

Physical mutagenesis constituted 49.54% of the research, with UV treatment being the predominant technique in 55.5% of these investigations. UV treatment proved to be an effective method for inducing mutations in microalgae. Other physical mutagenic agents, such as ion beams and radiation, were employed in a smaller proportion of the studies.

Targeted genera/species

The publications encompassed a range of microalgal genera/species. The six most targeted genera/species were *Chlorella*, *Nannochloropsis*, *Scenedesmus*, *Haematococcus*, *Chlamydomonas*, and *Tetraselmis* (Fig. 4). *Chlorella* accounted for the largest proportion, with 40.4% of all articles focusing on this genus. *Chlorella vulgaris* alone represented 19.3% of the articles. *Nannochloropsis*, *Scenedesmus*, *Haematococcus*, and *Chlamydomonas* also received significant attention in the research, while *Tetraselmis* and *Phaeodactylum* were the subjects of a smaller number of studies. *Desmodesmus* had the least representation among the targeted genera/species.

Objectives of the studies

The majority of studies (57 papers) aimed to increase lipid content, indicating the significance of enhancing

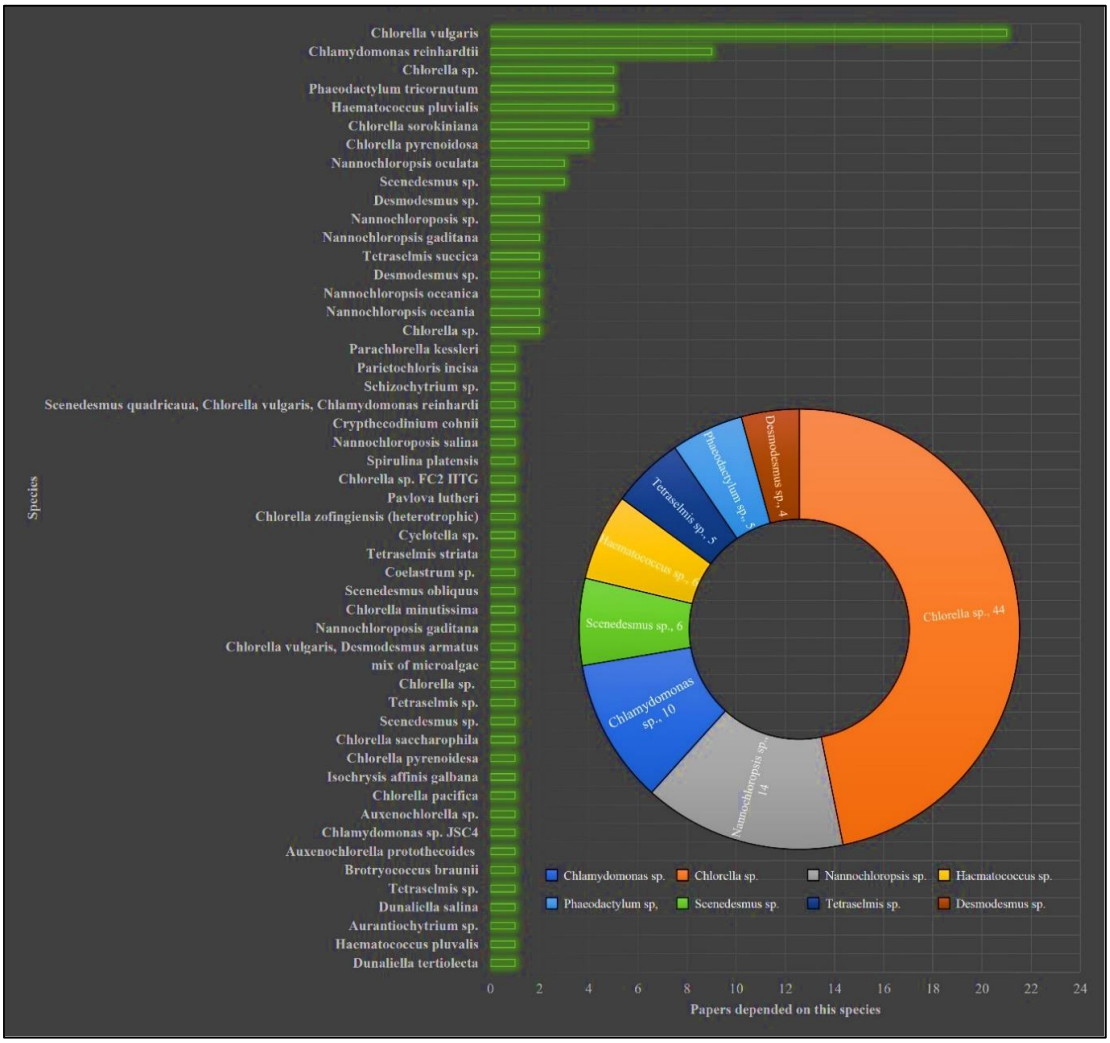

Fig. 4 A graphical depiction of the various microalgal strains employed in the field of random mutagenesis research

lipid metabolism in microalgae. Thirteen papers focused on improving carotenoid productivity, while nine articles aimed to enhance biomass production. Additionally, researchers focused on developing microalgal strains capable of bioremediation, aiming to remove harmful substances such as heavy metals or gases from the environment. Overall, the published studies reflect a diverse range of objectives, highlighting the broad applications and potential benefits of random mutagenesis in microalgae research.

Chemical mutagenesis in microalgae: A versatile approach for inducing mutations

Advantages and simplicity of chemical mutagenesis

Chemical mutagenesis is a popular technique for inducing mutations in microalgae due to its relative ease and affordability compared to other methods (Hlavova et al. 2015). Compared to physical mutagenesis or genetic engineering approaches, chemical mutagenesis offers the advantage of being a more straightforward process (Bleisch et al. 2022). It allows for the creation of various alterations in the DNA sequence, including base pair substitutions, deletions, and other forms of DNA damage, leading to a diverse range of mutants for evaluation and application (Cheng et al. 1992).

Alkylating agents and mutation induction

The selection of the specific chemical mutagen and the desired level of mutation induction determines the appropriate treatment concentration and duration. After the treatment, the microalgae are typically cultivated in selective media to promote the growth and persistence of mutants while wild-type cells degrade (Lopez-Rodas et al. 2001). Alkylating agents, such as ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), diethyl sulphate (DES), and N-methyl-N-nitro-N-nitrosoguanidine (NTG), are commonly used to increase the lipid output of oleaginous microalgae (Elisabeth et al. 2021). Among all chemical and physical agents, alkylating agents are frequently employed (Fig. 5) (Patel et al. 2016). These agents interact with DNA, causing base pairing failures, purine deamination, transitions, and frameshift mutations (Elisabeth et al. 2021). DNA replication can be affected when the DNA polymerase misreads nucleotides on the chemically modified template strand, resulting in nucleotide substitutions, insertions, and deletions (Yamamoto et al. 2017). Different alkylating compounds, such as N-methyl-nitrosourea (NMU) and N-meth-

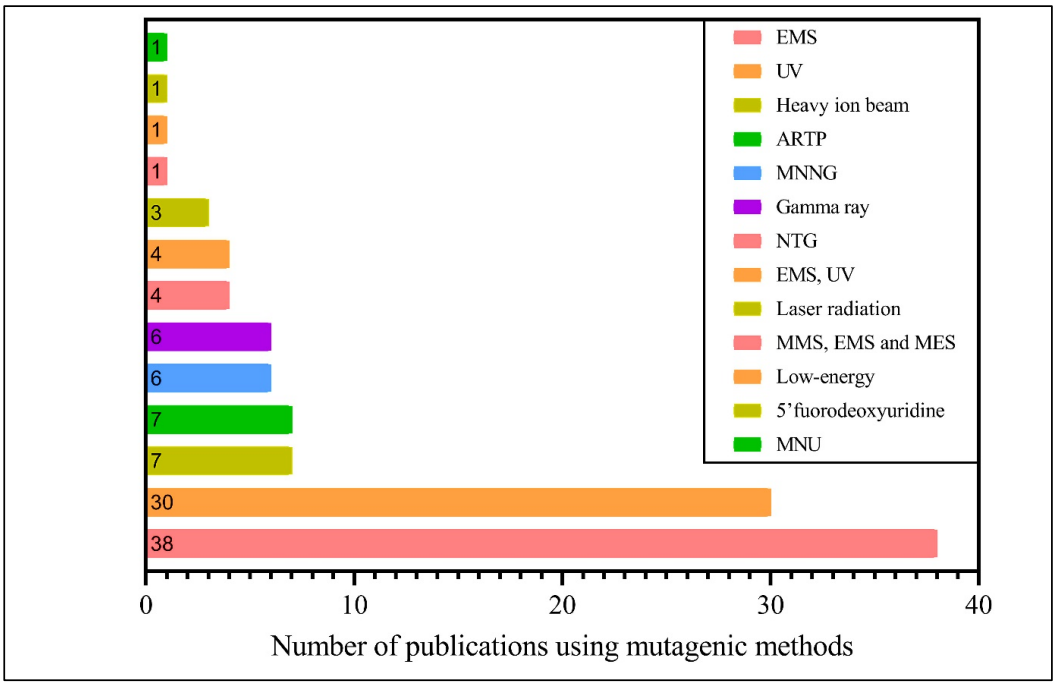

Fig. 5 Prevalence of different mutagenesis agents employed in microalgal studies, as reported by researchers

yl-N'-nitro-N-nitrosoguanidine (MNNG), can induce a wide range of mutations by efficiently methylating O- and N-atoms (Chaturvedi et al. 2004; Krasovec et al. 2018)

While chemical mutagenesis offers several advantages, it is essential to carefully consider the potential risks and benefits. Toxic or unstable mutants may arise from this process, requiring thorough evaluation. Further research is necessary to comprehensively understand the mechanisms and outcomes of chemical mutagenesis in microalgae and develop safe and efficient procedures for inducing and utilizing mutant strains for various applications. Long-term applications, such as biofuel production, should also consider the stability and reliability of the generated mutants.

Physical mutagenesis

In contrast to the previously discussed approaches, physical mutagenesis involves subjecting microalgae to physical elements, such as UV light or ionizing radiation, to induce DNA damage and mutations (Trovão et al. 2022). This technique is straightforward, affordable, and offers unique advantages in the generation of genetic variation.

UV radiation-mediated mutagenesis

UV radiation is a commonly used physical mutagen due to its ease of implementation, simplicity, and cost-effectiveness (Liu et al. 2015c). Microalgae are exposed to UV lamps, typically found in flow chambers, to induce point mutations, deletions, and substitutions (Altenburg 1934). UV radiation is absorbed by DNA molecules, leading to the formation of pyrimidine dimers that disrupt the DNA double-helix structure and inhibit normal base pairing (Rastogi et al. 2010). The wavelength of UV radiation determines the type and extent of mutations induced. UV radiation is divided into three categories as shown in (Fig. 6) UV-A, UV-B, and UV-C the commonly used area is UV-C. Although UV exposure causes a variety of DNA modifications, phototrophic cells may be resistant to some physical mutagens because of their photon-capturing and quenching capabilities. Furthermore, the creation of pyrimidine dimers within the DNA is linked to 80% of mutation events brought on by UV radiation, particularly UV-C radiation. As DNA absorption reaches its peak at this spectral range, radiation at 260 nm promotes the most effective production of cyclobutene pyrimidine dimers. As a result, UV-C irradiation has been suggested for methods involving random mutation, such as those using microalgae (Yi et al. 2015).

Ionizing radiation-mediated mutagenesis

Ionizing radiation, such as gamma rays, X-rays, or ion beams, can also be used as physical mutagens (Sikder et al. 2013). Compared to UV radiation, ionizing radiation has a higher energy density, resulting in

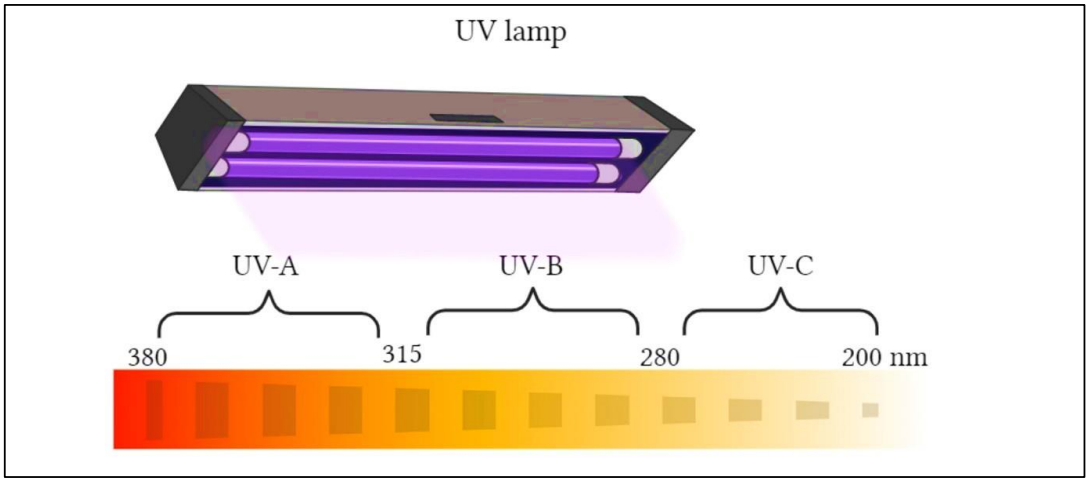

Fig. 6 Spectrum of ultraviolet radiation used for physical mutagenesis

severe genetic damage (Min et al. 2003). It causes ionization of molecules, alteration of DNA bases, phosphodiester bond breakage, and chromosomal aberrations, including deletions, translocations, and fragmentation (Klug and Cummings 2003). Research on the effects of gamma radiation on microalgae has revealed modifications in energy transfer and photosynthetic activity due to the formation of reactive oxygen species (Gomes et al. 2017).

Laser mutagenesis

Visible and near-infrared laser light has been used as a physical mutagen in microalgae (Ouf et al. 2012). Microalgae show higher resistance to radiation in the visible light spectrum due to natural heat dissipation and fluorescence quenching. Laser mutagenesis, using lasers such as semiconductor lasers (632.8 nm), (He-Ne) lasers (808 nm), or Nd: YAG lasers (1064 nm), allows for brief exposure to microalgae, resulting in mutagenesis effects, including increased lipid production (Xing et al. 2021).

Atmospheric and Room-Temperature Plasma (ARTP) mutagenesis

ARTP mutagenesis employs room-temperature plasma to produce mutations and mutagenic chemical species, making it a potential physicochemical technique (Fridman et al. 2007). This method shows promise due to its low gas temperatures, quick performance, high diversity of mutants, and environmentally friendly operation (Gaunt et al. 2006). However, comprehensive datasets on cell survival rates and mutation rates are currently limited (Zhang et al. 2014).

Physical mutagenesis techniques offer unique advantages in generating genetic variation in microalgae. However, understanding the mechanisms and effects of physical mutagenesis on microalgae, as well as evaluating the stability and applicability of the generated mutants, requires further research. Additionally, exploring advanced techniques like ARTP mutagenesis and optimizing laser mutagenesis methods hold the potential for enhancing the efficiency and outcomes of physical mutagenesis in microalgae.

Effect of random mutagenesis on biomolecule content

Chemical mutagenesis and lipid content in microalgae

The lipid content of microalgae has been a primary focus for researchers due to its significant importance in various industrial applications, making it a pivotal area of study within the field of biotechnology. One prominent application of microalgae lipids lies in the production of biofuels, particularly biodiesel, as microalgae have the capability to generate substantial amounts of lipids that can be converted into renewable fuel sources (Khoo et al. 2023). Microalgae offer several advantages over conventional sources such as corn and soybeans for biofuel production. They can be cultivated on non-arable land with minimal resource inputs, thereby presenting a sustainable and promising biofuel source (Gouveia and Oliveira 2009). Additionally, microalgae lipids find extensive use in the production of cosmetics and personal care products. The incorporation of lipids derived from microalgae into various cosmetic formulations, including moisturizers, sunscreens, and anti-aging creams, has gained significant traction. These lipids contribute to the functionality and performance of such products (Wijffels et al. 2010). Moreover, microalgae lipids play a crucial role in the production of animal feed. They serve as a valuable source of energy and essential fatty acids for fish and other aquaculture species. The inclusion of microalgae lipids in animal feed formulations enhances nutritional value and supports optimal growth and development (Patil et al. 2005).

Chemical mutagenesis represents the initial approach employed to enhance the lipid content of microalgae. In this study, a comprehensive literature review was conducted, and data were gathered to determine the most effective method for increasing lipid content using chemical mutagenesis across diverse microalgae species. The findings are summarized in (Table 1). The analysis revealed that the highest lipid content achieved through chemical mutagenesis was reported by Beacham et al. (2015) in *Nannochloropsis salina*. The researchers utilized EMS and achieved a 1.95 fold increase compared to the wild strain. Similarly, Michela et al. (2020) employed chemical mutagenesis in *Nannochloropsis gaditana* and achieved a 1.8 fold increase relative to the control strain. Notably, Mehtani et al. (2017) and Lee et al. (2014) also found that

chemical mutagenesis increased the amount of lipids in *Chlorella minutissima* and *Chlamydomonas reinhardtii* by 1.56 and 1.5 fold, respectively, compared to the control. Furthermore, Doan and Obbard (2012) achieved a 1.49 fold enhancement in lipid content in *Nannochloropsis* sp. In conclusion, chemical mutagenesis utilizing EMS emerges as the most effective method for augmenting lipid content in microalgae, particularly within the *Nannochloropsis* genus. However, further investigations are warranted to refine this approach and explore the potential commercial applications of microalgae exhibiting high lipid content.

Physical mutagenesis and lipid content in microalgae

Physical mutagenesis, in addition to chemical mutagenesis, represents another viable method for enhancing the lipid content of microalgae. In this analysis, the existing literature was examined to identify the highest lipid content achieved through physical mutagenesis. A comprehensive review of the literature was conducted, and relevant data on the highest lipid content achieved using physical mutagenesis in various microalgae species were collected in (Table 2). Whereas Zhang et al. (2017) reported that *Chlorella pacifica* had the highest lipid content using physical mutagenesis, according to the analysis of the data. The researchers employed laser radiation and achieved a remarkable 2.66 fold increase compared to the wild

Table 1 Effects of chemical mutagenesis on microalgal strains concerning the improvement of lipid content

Mutagenic agent	Species	Method	Improvement by fold compared to control strain	Reference
EMS	*Auxenochlorella* sp.	0.25M for 13 min	1.12	(Polat and Altınbaş 2022)
EMS	*Chlamydomonas reinhardtii*	300 mM for 80 min	1.12	(Xie et al. 2014)
EMS	*Chlamydomonas reinhardtii*	20 - 40 µL/mL for 120 min	1.5	(Lee et al. 2014)
EMS	*Chlorella minutissima*	0.45, 0.8, 1.4, 1.7, 2.0 and 2.4 M for 30 min	1.56	(Mehtani et al. 2017)
EMS	*Chlorella* sp.	50, 100, and 200 mM for 30 or 60 min	1.24	(Noochanong et al. 2018)
5'Fuorodeoxyuridine	*Chlorella vulgaris*	0.25 mM for 1 week	1.23	(Anthony et al. 2022)
EMS	*Chlorella vulgaris*	100 mM for 30 min	1.44	(Nayak et al. 2022)
EMS	*Chlorella* sp.	100 mM for 60 min	1.26	(Ong et al. 2010)
EMS	*Desmodesmus* sp.	600-800 mM for 30-60 min	1.29	(Zhang et al. 2016)
EMS	*Nannochloropsis gaditana*	0.75%, 1.5%, 2% and 2.5% for 120 min	1.8	(Michela et al. 2020)
EMS	*Nannochloropsis oceanica*	1 mol/L for 60 min	1.29	(Wang et al. 2016b)
MNU	*Nannochloropsis oculate*	5 mM for 60-90 min	1.22	(Chaturvedi et al. 2004)
EMS	*Nannochloropsis oculate*	100 mM for 60 min	1.22	(Chaturvedi and Fujita 2006)
EMS	*Nannochloropsis salina*	0.24 mol/L for 30 min	1.95	(Beacham et al. 2015)
EMS	*Nannochloropsis* sp.	0.1 M and 0.5 M	1.36	(Kawaroe et al. 2015)
EMS	*Nannochloropsis* sp.	0.1, 0.5, 1, and 1.2 M for 60 min	1.49	(Doan and Obbard 2012)
MNNG	*Parietochloris incisa*	100 µg/ml for 60 min	1.05	(Iskandarov et al. 2011)
NTG	*Schizochytrium* sp.	1, 1.5, and 2 mg/ml for 20, 30, and 40 min	1.34	(Lian et al. 2010)
EMS	*Tetraselmis* sp.	25, 50, 75, and 100µmol mL^{-1} for 30, 60, 90, and 120 min	1.48	(Dhanalakshmi et al. 2018)
EMS	*Tetraselmis* sp.	25, 50, 75, and 100µmol mL^{-1} for 30, 60, 90, and 120 min	1.25	(Dinesh Kumar et al. 2018)

Table 2 Effects of physical mutagenesis on microalgal strains concerning the improvement of lipid content

Mutagenic agent	Species	Method	Improvement by fold compared to control strain	Reference
Heavy ion beam	*Aurantiochytrium* sp.	137Se- γ-ray irradiation	1.13	(Cheng et al. 2016)
Gamma-ray	*Auxenochlorella protothecoides*	$^{12}C^{6+}$ ion beam (80 MeV/u), irradiated to 100 Gy with a rate of 40 Gy/min	1.15	(Shao et al. 2022)
UV	*Brotryococcus braunii*	UV-Clamp (254 nm, 15W GE lightning). UV-C exposed for 0-30 min	1.62	(Thurakit et al. 2018)
Heavy ion beam	*Chlamydomonas* sp. JSC4	Irradiated with 50 or 100 Gy of the carbon ion beams $^{12}C^{5+}$, 220 MeV	1.89	(Kato et al. 2017)
Laser radiation	*Chlorella pacifica*	Nd: YAG laser 1064 nm, 40 mW, for 2 min	2.66	(Zhang et al. 2017)
Laser radiation	*Chlorella pyrenoidesa*	He–Ne laser 808 nm, 6 W, for 4 min	2.2	(Xing et al. 2021)
Low energy	*Chlorella pyrenoidosa*	Three ion source gases (N_2, Ar, and C_2H_2), energy 10 KeV, current of 20 mA. pressure 10^{-2} Pa, dose implantation 0.3×10^{15} to 3.3×10^{15} ions cm^{-2} s^{-1}	1.3	(Tu et al. 2016)
ARTP	*Chlorella pyrenoidosa*	(P = 120 W, G = 10 SLM, D = 2 mm)	1.12	(Cao et al. 2017)
UV	*Chlorella* sp.	354 nm	1.96	(Rachmayati et al. 2020)
Gamma-ray	*Chlorella* sp.	Co^{60} γ rays, 800 Gy for 50 min	1.36	(Senthamilselvi and Kalaiselvi 2022)
UV	*Chlorella vulgaris*	18W UV light, distance 15 cm for 13 min	2.4	(Xiaodong et al. 2011)
UV	*Chlorella vulgaris*	254 nm for 0.5-10 min	1.64	(Sarayloo et al. 2018)
laser radiation	*Chlorella vulgaris*	Nd:YAG laser 1064 nm, 40 mW for 8 min	1.66	(Xing et al. 2021)
UV	*Chlorella vulgaris*	UV-2 / UV-A light for 60 s, distance 15 cm	1.26	(Anthony et al. 2022)
UV	*Chlorella vulgaris*	UV-C, for 1–10 min, distance 40 cm	1.3	(Carino and Vital 2022)
UV	*Chlorella vulgaris* *Desmodesmus armatus*	354 nm	1.33	(Smalley et al. 2020)
UV	*Chlorella* sp. FC2 IITG	254 nm UV-C, distance 20 cm, time 60 min	1.2	(Muthuraj et al. 2019)
Heavy ion beam	*Desmodesmus* sp.	$^{12}C^{6+}$ ion beam, 90 Gy	1.2	(Hu et al. 2013)
ARTP	*Desmodesmus* sp.	(G = 5 SLM, D = 2 mm, U = 120 V, I = 1 A)	1.64	(Li et al. 2021)
ARTP	*Desmodesmus* sp.	(G = 5 SLM, D = 2 mm, U = 120 V, I = 1 A)	2.32	(Sun et al. 2020)
UV	*Isochrysis affinis galbana*	254 nm, for 3–32 min	1.56	(Bougaran et al. 2012)
UV	*mix of microalgae*	254 nm, 15 W, for 30 -180 s, distance 5 cm	1.96	(Ardelean et al. 2018)
Heavy ion beam	*Nannochloropsis oceania*	$^{12}C^{6+}$ ion beam 31 keVµm^{-1}, 160 Gy	1.39	(Ma et al. 2013)
UV	*Nannochloropsis oceania*	354 nm for 120 min	1.16	(Moha-León et al. 2019)
UV	*Nannochloropsis oculata*	UV.A	1.16	(Srinivas and Ochs 2012)
ARTP	*Parachlorella kessleri*	Power 100 W, the distance between slide and plasma emitter jet 2 mm, helium gas flow rate of 10 L/min, and exposure time from 10 to 60 s	1.75	(Elshobary et al. 2022)
UV	*Pavlova lutheri*	254 nm, for 40 min	1.14	(Meireles et al. 2003)
UV	*Phaeodactylum tricornutum*	250 nm	1.34	(Alonso et al. 1996)
UV	*Scenedesmus obliquus*	254 nm	2.39	(de Jaeger et al. 2014)
Gamma-ray	*Scenedesmus* sp.	10 doses of irradiation 50–7000 kGy, Co^{60} gamma ray irradiator at room temperature	1.5	(Liu et al. 2015a)
UV	*Scenedesmus* sp.	253.7 nm, distance 15 cm, time from 0 to 40 min.	1.5	(Sivaramakrishnan and Incharoensakdi 2017)
UV	*Scenedesmus* sp.	20 W UV, λ = 254 nm, distance 35 cm, time intervals 30, 60, 100, 120, 180, 240, and 270 s	1.21	(Zhang et al. 2018a)
Heavy ion beam	*Scenedesmus* sp.	Pressure 10^{-2} Pa, energy 10 keV, beam current 20 mA, The dose of implantation ranged from $0.2x10^{15}$ to $3.4x10^{15}$ ions cm^{-2} s^{-1}	1.18	(Qu et al. 2020)
UV	*Tetraselmis suecica*	UV-C	1.23	(Lim et al. 2015)
UV	*Tetraselmis suecica*	254 nm, 8 W, time durations of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 min	1.4	(Lo et al. 2022)

strain. Similarly, Xiaodong et al. (2011) utilized physical mutagenesis in *Chlorella vulgaris*, employing UV radiation to achieve a substantial 2.4 fold increase in lipid content. Furthermore, other studies reported successful improvements in lipid content using physical mutagenesis. For instance, de Jaeger et al. (2014) employed UV radiation in *Scenedesmus obliquus*, resulting in a 2.39 fold increase compared to the control strain. Similarly, Sun et al. (2020) utilized ARTP treatment in *Desmodesmus*, achieving a significant 2.32 fold increase. Moreover, Xing et al. (2021) employed laser radiation to enhance lipid content in *Chlorella pyrenoidosa*, resulting in a 2.2 fold increase. Collectively, these findings indicate that physical mutagenesis using laser radiation and UV radiation is highly effective in improving lipid content in microalgae, particularly within the *Chlorella* genus. Additionally, ARTP has demonstrated superior results compared to other methods, suggesting its potential as a valuable approach. With further experimentation and refinement, ARTP could potentially yield even more significant improvements.

Enhancing carotenoid production in microalgae through mutation

Carotenoids, alongside lipids, hold substantial market value in the food and nutrition industry (Priyadarshani and Rath 2012). With diverse industrial applications, carotenoids have emerged as a significant research area within biotechnology (Dhanda and Shankar 2022). Additionally, carotenoids serve as a source of essential vitamin A, which is crucial for human health (Rao and Rao 2007). Certain microalgae species, such as *Dunaliella salina* and *Haematococcus pluvialis*, are renowned for their high carotenoid production, particularly beta-carotene, a precursor to vitamin A (Rammuni et al. 2019). These microalgae are widely utilized as a source of beta-carotene for food supplements and as natural food colorants (Gupta et al. 2007). Moreover, carotenoids derived from microalgae exhibit antioxidant properties, which can aid in safeguarding against cellular damage inflicted by free radicals (Maadane et al. 2015).

Thirteen articles centred around carotenoids as their primary focus. This discussion will highlight the top five articles reporting significant improvements in carotenoid content (Table 3). Cordero et al. (2011) employed MNNG treatment to enhance carotenoid production in *Chlorella sorokiniana*, resulting in a two-fold increase compared to the wild strain. Wang et al. (2016a) employed UV radiation to improve carotenoid content in *Haematococcus pluvialis*, yielding a 1.7 fold increase compared to the control strain. Similarly, Jin et al. (2003) utilized EMS treatment in *Dunaliella salina* and achieved a 1.25 fold increase compared to the control strain. Fan et al. (2021) employed a heavy ion beam, while Yi et al. (2018) employed EMS treatment, resulting in 1.25 fold and 1.2 fold increases, respectively, in carotenoid content in *Phaeodactylum tricornutum*. These studies collectively demonstrate the efficacy of chemical mutagenesis in enhancing carotenoid production in microalgae. The most effective methods led to significant improvements in carotenoid content compared to the control. MNNG treatment exhibited the highest fold increase, followed by UV radiation and EMS treatment. It is important to note that different microalgae species may respond differently to chemical mutagenesis.

Improving microalgae biomass through different mutagenesis techniques

Microalgae biomass, encompassing cells, cell debris, and extracellular substances, holds significant industrial interest and serves as a crucial subject of study in biotechnology (Fathy et al. 2021). The production of biofuels stands out as a prominent application of microalgae biomass, with large-scale cultivation capa-

Table 3 Enhancement of carotenoid content in microalgae using different chemical and physical mutagenesis techniques

Mutagenic agent	Method	Species	Method	The improvement compared to the control strain	Reference
EMS	Chemical	*Chlamydomonas reinhardtii*	NG*	Faster growth and pigment change	(Loppes 1969)
MNNG	Chemical	*Chlorella sorokiniana*	0.1 mg/mL, for 60 min	2 fold	(Cordero et al. 2011)
MNNG	Chemical	*Chlorella sorokiniana*	0.1 mg/mL, for 60 min	Lutein productivity and content	(Chen et al. 2017)
EMS	Chemical	*Chlorella vulgaris*	2.2% (w/v), for 120 min	Carotenoid content and oxidative stress tolerance	(Guardini et al. 2021)
MNNG	Chemical	*Chlorella zofingiensis (heterotrophic)*	0.5-10 mg/mL, for 60 min	Zeaxanthin, β-carotene, and lutein accumulation	(Huang et al. 2018)
EMS	Chemical	*Dunaliella salina*	200 mM, for 120 min	1.25 fold	(Jin et al. 2003)
EMS	Chemical	*Dunaliella tertiolecta*	200 mM, for 120 min	1.015 fold	(Kim et al. 2017)
EMS	Chemical	*Haematococcus pluvialis*	100 mM	1.025 fold	(Chen et al. 2003)
NTG	Chemical	*Haematococcus pluvialis*	0.1 mM, for 60 min	1.038 fold	(Sandesh Kamath et al. 2008)
UV	Physical	*Haematococcus pluvialis*	1.5% to 2.0% (V/V), for 60 min	1.7 fold	(Wang et al. 2016a)
Gamma-ray	Physical	*Nannochloropsis oceanica*	100 to 1000 Gy using ^{60}Co irradiator	Violaxanthin productivity	(Park et al. 2021)
EMS	Chemical	*Phaeodactylum tricornutum*	0.1- 0.2 M	1.2 fold	(Yi et al. 2018)
Heavy ion beam	Physical	*Phaeodactylum tricornutum*	Carbon ions, 200 Gy ion beam	1.25 fold	(Fan et al. 2021)

* NG = Not Given

ble of yielding biohydrogen and bioethanol (John et al. 2011). Moreover, microalgae biomass is a valuable source of protein, vitamins, and minerals, finding utility in animal feed manufacturing (Saadaoui et al. 2021). Additionally, it finds application in a range of cosmetic and personal care products, including moisturizers, sunscreens, and anti-aging lotions (Ariede et al. 2017). Furthermore, microalgae biomass serves as a precursor for various chemicals and polymers, such as bio-based plastics, lubricants, and surfactants (Lambert and Wagner 2017). Utilizing microalgae biomass in the production of industrial chemicals and polymers offers the potential for reduced dependence on fossil fuels and decreased greenhouse gas emissions.

This section presents a review of the utilization of mutagenesis techniques to enhance microalgae biomass, as depicted in (Table 4). Several studies have reported successful increases in biomass, particularly in *Chlorella*, through the application of chemical and physical mutagenesis. For instance, Kuo et al. (2017) employed NTG treatment, resulting in a remarkable 5.83 fold increase in biomass compared to the control strain of *Chlorella* sp. Similarly, Cheng et al. (2013) utilized gamma rays to achieve a 2.88 fold increase in biomass for *Chlorella vulgaris* and a 1.93 fold increase for *Chlorella pyrenoidosa*. Furthermore, Sachdeva et al. (2016) employed EMS treatment, leading to a 1.97 fold increase in biomass for *Chlorella pyrenoidosa*. Finally, Shin et al. (2016) utilized EMS treatment to achieve a 1.44 fold increase in biomass for *Chlorella vulgaris*. The reviewed literature suggests that chemical mutagens, particularly NTG, exhibit greater efficacy in enhancing biomass in *Chlorella* compared to physical mutagens. Therefore, when targeting biomass enhancement as the primary objective, the utilization of *Chlorella* sp. in conjunction with chemical mutagenesis holds promise for yielding favorable results.

Hybrid approaches in random mutagenesis

Combined mutagenesis approaches have garnered significant interest due to their potential to achieve higher success rates compared to individual methods (Wang et al. 2016a). A recent review of the literature, presented in (Table 5), demonstrates the application of combined mutagenesis approaches and their impact on various aspects of microalgae. Nečas (1968) employed the combination of EMS and UV methods on different microalgae strains, observing notable improvements in cell growth. However, Huesemann et al. (2009) reported a negative effect on chlorophyll content when applying the same combined method to Cyclotella sp. On the other hand, Sarayloo et al. (2017) evaluated the effect of a hybrid mutagenesis approach on the lipid content of Chlorella vulgaris, resulting in a significant 1.67 fold improvement. Furthermore, Sarayloo et al. (2018) also reported a 1.67 fold increase in lipid content in Chlorella vulgaris using the same hybrid method. These findings indicate that the effectiveness of the hybrid mutagenesis approach is yet to be fully realized. Further investigations involving diverse species and a range of doses will shed light on whether this method exhibits antagonistic or synergistic effects.

Conclusions and future perspectives

Microalgal strains hold significant potential as sources of renewable energy, food, and valuable compounds.

Table 4 Improvement of microalgal biomass through the utilization of various chemical and physical mutagenesis agents

Mutagenic agent	Method	Species	Method	Improvement by fold compared to control strain	Reference
Gamma-ray	Physical	*Chlorella pyrenoidosa*	100, 300, 500, 700, and 900 Gy of ^{60}Co γ rays, the dose rate of 15 Gy min^{-1}	1.93	(Cheng et al. 2013)
EMS	Chemical	*Chlorella pyrenoidosa*	1.75 and 2%	1.97	(Sachdeva et al. 2016)
UV	Physical	*Chlorella sorokiniana*	254 nm, for 60 -120 min	1.27	(Cazzaniga et al. 2014)
EMS	Chemical	*Chlorella sorokiniana*	0.5% (w/v), for 4 h	1.11	(Wu et al. 2019)
Gamma-ray	Physical	*Chlorella vulgaris*	100, 300, 500, 700, and 900 Gy of ^{60}Co γ rays, the dose rate of 15 Gy min^{-1}	2.28	(Cheng et al. 2013)
EMS	Chemical	*Chlorella vulgaris*	0.1, 0.19, 0.24, and 0.28 M, for 120 min	1.44	(Shin et al. 2016)
NTG	Chemical	*Chlorella* sp.	5 μg/mL, for 60 min	5.83	(Kuo et al. 2017)
ARTP	Physical	*Crypthecodinium cohnii*	He RF power 150 W, for 100 s	1.18	(Liu et al. 2015b)
EMS	Chemical	*Nannochloropsis gaditana*	70 mM, for 60 min	1.27	(Perin et al. 2015)

Table 5 Effects of utilizing hybrid mutagenic agents on microalgae cells: EMS and UV radiation

Target	Species	Method	Improvement by fold compared to control strain	Reference
Lipid content	*Chlorella vulgaris*	UV 254 nm, for 0.5-10 min / EMS 25 mM for 60 min	1.43	(Sarayloo et al. 2017)
Lipid content	*Chlorella vulgaris*	UV 254 nm, for 0.5-10 min / EMS 25 mM for 60 min	1.67	(Sarayloo et al. 2018)
Chlorophyll	*Cyclotella sp.*	0.2 M for 120 min / 15 W UV for 15 - 20 s	Reduced chlorophyll	(Huesemann et al. 2009)
Growth	*Scenedesmus quadricaua, Chlorella vulgaris, Chlamydomonas reinhardi*	NG*	Better growth on minimal media as compared to wild type	(Nečas 1968)

* NG = Not Given

However, the challenges faced in industrial microalgae production hinder their widespread utilization. Strain improvement is crucial to overcome these challenges and develop more productive and resilient strains. The choice of strain improvement approach should be based on the specific improvement targets and intended applications. Random mutagenesis is a well-established technique for generating genetic diversity in microorganisms. It involves subjecting microorganisms to physical or chemical agents that introduce random mutations into the genome. This can lead to the emergence of novel traits, such as increased productivity or environmental stress resistance. Although there is a wide range of physical and chemical mutagens available for random mutagenesis, not all of them have been extensively studied in microalgae.
In this review, we discussed the use of random mutagenesis as a cost-effective and time-efficient strategy to develop more robust strains for the microalgae industry. We found that physical mutagens have demonstrated the ability to enhance the cellular lipid content of microalgae, while chemical mutagens like EMS and MNNG have been effective in increasing pigment production and biomass. Furthermore, we observed that the recent trend of utilizing combined mutagenesis approaches to increase the mutation rate of cells has shown limited effectiveness. Further research is necessary to explore the advantages and disadvantages of different mutagenesis strategies to optimize their outcomes. Whereas the study of microalgal genomics and metabolomics plays a crucial role in understanding the regulation of pathways involved in the biosynthesis and breakdown of target compounds. This knowledge can aid in the identification, selection, and isolation of key factors, such as gene products and environmental conditions, for the improvement of specific microalgal strains with desired phenotypes. Genomic and metabolomic data can also be utilized to predict the effects of mutations on phenotypes, assisting in the selection of the most promising mutants. Despite the power of random mutagenesis as a strain improvement tool, several challenges still need to be addressed. Industries like food and medicine are currently limiting the use of genetically modified organisms and molecular toolboxes for microalgae. Thus, approaches that facilitate the natural optimization of microalgal cell factories are being explored. Additionally, the development of high-throughput screening technologies and scalable phototrophic cultivation systems is needed.

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References

Alonso DL, Segura del Castillo CI, Grima EM, Cohen Z (1996) First insights into improvement of eicosapentaenoic acid content in *Phaeodactylum tricornutum* (bacillariophyceae) by induced mutagenesis 1. J Phycol 32(2):339-345

Altenburg E (1934) The artificial production of mutations by ultra-violet light. Am Nat 68(719):491-507

Anthony J, Rangamaran VR, Sivasankarasubbiah KT, Nagaraj N, Gopal D, Ramalingam K (2022) An insight into the influence of random mutagenesis on growth and lipid accumulation in *Chlorella vulgaris* - a transcriptome study. Biomass Convers Biorefin https://doi.org/10.1007/s13399-022-03249-8

Aoufouchi S, Yélamos J, Milstein C (1996) Nonsense mutations inhibit RNA splicing in a cell-free system: recognition of mutant codon is independent of protein synthesis. Cell 85(3):415-422

Ardelean AV, Ardelean II, Sicuia-Boiu OA, Cornea P (2018) Random mutagenesis in photosynthetic microorganisms further selected with respect to increased lipid content. Agric Life Conf Proc 1:501-507

Ariede MB, Candido TM, Jacome ALM, Velasco MVR, de Carvalho JCM, Baby AR (2017) Cosmetic attributes of algae-A review. Algal Res 25:483-487

Bajpai P (2022) Environmental and health risks. Fourth Generation Biofuels, Springer, pp 41-47

Beacham T, Macia VM, Rooks P, White D, Ali S (2015) Altered lipid accumulation in *Nannochloropsis salina* CCAP849/3 following EMS and UV induced mutagenesis. Biotechnol Rep 7:87-94

Benzer S, Champe SP (1962) A change from nonsense to sense in the genetic code. Proc Natl Acad Sci 48(7):1114-1121

Betancourt A (2007) When the going gets tough, beneficial mutations get going. Heredity 99(4):359-360

Bleisch R, Freitag L, Ihadjadene Y, Sprenger U, Steingröwer J, Walther T, Krujatz F (2022) Strain development in microalgal biotechnology- random mutagenesis techniques. Life 12(7):961

Bougaran G, Rouxel C, Dubois N, Kaas R, Grouas S, Lukomska E, Le Coz JR, Cadoret JP (2012) Enhancement of neutral lipid productivity in the microalga *Isochrysis affinis Galbana* (T-Iso) by a mutation-selection procedure. Biotechnol Bioeng 109(11):2737-2745

Cao S, Zhou X, Jin W, Wang F, Tu R, Han S, Chen H, Chen C, Xie G-J, Ma F (2017) Improving of lipid productivity of the oleaginous microalgae *Chlorella pyrenoidosa* via atmospheric and room temperature plasma (ARTP). Bioresour Technol 244:1400-1406

Caporgno MP, Mathys A (2018) Trends in microalgae incorporation into innovative food products with potential health benefits. Front Nut 5:58

Carino JDG, Vital PG (2022) Characterization of isolated UV-C-irradiated mutants of microalga *Chlorella vulgaris* for future biofuel application. Environ Dev Sustainability. https://doi.org/10.1007/s10668-021-02091-8

Cazzaniga S, Dall'Osto L, Szaub J, Scibilia L, Ballottari M, Purton S, Bassi R (2014) Domestication of the green alga *Chlorella sorokiniana*: reduction of antenna size improves light-use efficiency in a photobioreactor. Biotechnol Biofuels 7(1):157. https://doi.org/10.1186/s13068-014-0157-z

Cecchin M, Cazzaniga S, Martini F, Paltrinieri S, Bossi S, Maffei ME, Ballottari M (2022) Astaxanthin and eicosapentaenoic acid production by S4, a new mutant strain of *Nannochloropsis gaditana*. Microb Cell Fact 21(1):117. https://doi.org/10.1186/s12934-022-01847-9

Chamary J, Hurst LD (2009) The price of silent mutations. Sci Am 300(6):46-53

Chaturvedi R, Fujita Y (2006) Isolation of enhanced eicosapentaenoic acid producing mutants of *Nannochloropsis oculata* ST-6 using ethyl methane sulfonate induced mutagenesis techniques and their characterization at mRNA transcript level. Phycol Res 54(3):208-219

Chaturvedi R, Uppalapati SR, Alamsjah MA, Fujita Y (2004) Isolation of quizalofop-resistant mutants of *Nannochloropsis oculata* (Eustigmatophyceae) with high eicosapentaenoic acid following N-methyl-N-nitrosourea-induced random mutagenesis. J Appl Phycol 16(2):135-144

Chen C-Y, Hsieh C, Lee D-J, Chang C-H, Chang J-S (2016) Production, extraction and stabilization of lutein from microalga *Chlorella sorokiniana* MB-1. Bioresour Technol 200:500-505

Chen J-H, Chen C-Y, Chang J-S (2017) Lutein production with wild-type and mutant strains of *Chlorella sorokiniana* MB-1 under mixotrophic growth. J Taiwan Inst Chem Eng 79:66-73. https://doi.org/https://doi.org/10.1016/j.jtice.2017.04.022

Chen Y, Li D, Lu W, Xing J, Hui B, Han Y (2003) Screening and characterization of astaxanthin-hyperproducing mutants of *Haematococcus pluvialis*. Biotechnol Lett 25(7):527-529. https://doi.org/10.1023/A:1022877703008

Cheng J, Huang Y, Feng J, Sun J, Zhou J, Cen K (2013) Mutate *Chlorella* sp. by nuclear irradiation to fix high concentrations of CO_2. Bioresour Technol 136:496-501. https://doi.org/https://doi.org/10.1016/j.biortech.2013.03.072

Cheng J, Zhu Y, Zhang Z, Yang W (2019) Modification and improvement of microalgae strains for strengthening CO_2 fixation from coal-fired flue gas in power plants. Bioresour Technol 291:121850. https://doi.org/https://doi.org/10.1016/j.biortech.2019.121850

Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes GT and AC substitutions. J Biol Chem 267(1):166-172

Cheng Y-r, Sun Z-j, Cui G-z, Song X, Cui Q (2016) A new strategy for strain improvement of *Aurantiochytrium* sp. based on heavy-ions mutagenesis and synergistic effects of cold stress and inhibitors of enoyl-ACP reductase. Enzyme Microb Technol 93-94:182-190. https://doi.org/10.1016/j.enzmictec.2016.08.019

Chu W-L (2012) Biotechnological applications of microalgae. IeJSME 6(1):S24-S37

Cid Á, Prado R, Rioboo C, Suarez-Bregua P, Herrero C (2012) Use of microalgae as biological indicators of pollution: looking for new relevant cytotoxicity endpoints. In: Johnsen, M. N. (ed.) microalgae: Biotechnology, microbiology and energy. Nova science publishers, New York pp:311-323

Cooper DN, Krawczak M (1990) The mutational spectrum of single base-pair substitutions causing human genetic disease: patterns and predictions. Hum Genet 85(1):55-74

Cordero BF, Obraztsova I, Couso I, Leon R, Vargas MA, Rodriguez H (2011) Enhancement of lutein production in *Chlorella sorokiniana* (Chorophyta) by improvement of culture conditions and random mutagenesis. Mar Drugs 9(9):1607-1624

Damsky W, Bosenberg M (2017) Melanocytic nevi and melanoma: unraveling a complex relationship. Oncogene 36(42):5771-5792

de Jaeger L, Verbeek RE, Draaisma RB, Martens DE, Springer J, Eggink G, Wijffels RH (2014) Superior triacylglycerol (TAG) accumulation in starchless mutants of *Scenedesmus obliquus*:(I) mutant generation and characterization. Biotechnol Biofuel 7(1):1-11

Dhanalakshmi B, Dinesh Kumar S, Kim M, Latha S, Park M, Santhanam P, Sojin K (2018) Triggering of fatty acids on *Tetraselmis* sp. by ethyl methanesulfonate mutagenic treatment. HERO (2)21-28. DOI: 10.1016/j.biteb.2018.04.001

Dhanda PS, Shankar A (2022) Microalgae for carotenoids production: An update. Pharma Innov J 11(3):2036-2045

Dinesh Kumar S, Sojin K, Santhanam P, Dhanalakshmi B, Latha S, Park MS, Kim M-K (2018) Triggering of fatty acids on *Tetraselmis* sp. by ethyl methanesulfonate mutagenic treatment. Biores Technol Rep 2:21-28. https://doi.org/10.1016/j.biteb.2018.04.001

Doan TTY, Obbard JP (2012) Enhanced intracellular lipid in *Nannochloropsis* sp. via random mutagenesis and flow cytometric cell sorting. Algal Res 1(1):17-21

Elena SF, Lenski RE (2003) Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. Nat Rev Genet 4(6):457-469

Elisabeth B, Rayen F, Behnam T (2021) Microalgae culture quality indicators: a review. Crit Rev Biotechnol 41(4):457-473

Elshobary ME, Zabed HM, Qi X, El-Shenody RA (2022) Enhancing biomass and lipid productivity of a green microalga *Parachlorella kessleri* for biodiesel production using rapid mutation of atmospheric and room temperature plasma. Biotechnol Biofuel Bioprod 15(1):122. https://doi.org/10.1186/s13068-022-02220-z

Fan Y, Ding X-T, Wang L-J, Jiang E-Y, Van PN, Li F-L (2021) Rapid sorting of fucoxanthin-producing *Phaeodactylum tricornutum* mutants by flow cytometry. Mar Drugs 19(4):228

Fathy W, Essawy E, Tawfik E, Khedr M, Abdelhameed MS, Hammouda O, Elsayed K (2021) Recombinant overexpression of the *Escherichia* coli acetyl-CoA carboxylase gene in *Synechocystis* sp. boosts lipid production. J Basic Microbiol 61(4):330-338

Fathy WA, AbdElgawad H, Essawy EA, Tawfik E, Abdelhameed MS, Hammouda O, Korany SM, Elsayed KNM (2023) Glycine differentially improved the growth and biochemical composition of *Synechocystis* sp. PAK13 and *Chlorella variabilis* DT025. Fronti Bioeng Biotechnol 11. https://doi.org/10.3389/fbioe.2023.1161911

Flibotte S, Edgley ML, Chaudhry I, Taylor J, Neil SE, Rogula A, Zapf R, Hirst M, Butterfield Y, Jones SJ, Marra MA, Barstead RJ, Moerman DG (2010) Whole genome profiling of mutagenesis in caenorhabditis elegans. Genetics 185(2):431-441. https://doi.org/10.1534/genetics.110.116616

Fridman G, Brooks AD, Balasubramanian M, Fridman A, Gutsol A, Vasilets VN, Ayan H, Friedman G (2007) Comparison of direct and indirect effects of non-thermal atmospheric-pressure plasma on bacteria. Plasma Processes Polym 4(4):370-375

Garrido-Cardenas JA, Manzano-Agugliaro F, Acien-Fernandez FG, Molina-Grima E (2018) Microalgae research worldwide. Algal

Res 35:50-60. https://doi.org/10.1016/j.algal.2018.08.005
Gaunt LF, Beggs CB, Georghiou GE (2006) Bactericidal action of the reactive species produced by gas-discharge nonthermal plasma at atmospheric pressure: a review. IEEE Trans Plasma Sci 34(4):1257-1269
Gepts P (2002) A comparison between crop domestication, classical plant breeding, and genetic engineering. Crop Sci 42(6):1780-1790
Gomes T, Xie L, Brede D, Lind O-C, Solhaug KA, Salbu B, Tollefsen KE (2017) Sensitivity of the green algae *Chlamydomonas reinhardtii* to gamma radiation: photosynthetic performance and ROS formation. Aquat Toxicol 183:1-10
Gouveia L, Oliveira AC (2009) Microalgae as a raw material for biofuels production. J Ind Microbiol Biotechnol 36(2):269-274
Guardini Z, Dall'Osto L, Barera S, Jaberi M, Cazzaniga S, Vitulo N, Bassi R (2021) High carotenoid mutants of *Chlorella vulgaris* show enhanced biomass yield under high irradiance. Plants 10(5):911
Gupta S, Jha A, Pal A, Venkateshwarlu G (2007) Use of natural carotenoids for pigmentation in fishes
Henley WJ, Litaker RW, Novoveská L, Duke CS, Quemada HD, Sayre RT (2013) Initial risk assessment of genetically modified (GM) microalgae for commodity-scale biofuel cultivation. Algal Res 2(1):66-77
Hlavova M, Turoczy Z, Bisova K (2015) Improving microalgae for biotechnology - From genetics to synthetic biology. Biotechnol Adv 33(6, Part 2):1194-1203. https://doi.org/10.1016/j.biotechadv.2015.01.009
Hu G, Fan Y, Zhang L, Yuan C, Wang J, Li W, Hu Q, Li F (2013) Enhanced lipid productivity and photosynthesis efficiency in a *Desmodesmus* sp. mutant induced by heavy carbon ions. PloS one 8(4):e60700
Hu W, Li W, Chen J (2017) Recent advances of microbial breeding via heavy-ion mutagenesis at IMP Lett Appl Microbiol 65(4):274-280. https://doi.org/10.1111/lam.12780
Huang W, Lin Y, He M, Gong Y, Huang J (2018) Induced high-yield production of zeaxanthin, lutein, and β-carotene by a mutant of *Chlorella zofingiensis*. J Agric Food Chem 66(4):891-897. https://doi.org/10.1021/acs.jafc.7b05400
Huesemann MH, Hausmann TS, Bartha R, Aksoy M, Weissman JC, Benemann JR (2009) Biomass productivities in wild type and pigment mutant of *Cyclotella* sp. (Diatom). Appl Biochem Biotechnol 157(3):507-526. https://doi.org/10.1007/s12010-008-8298-9
Iskandarov U, Khozin-Goldberg I, Cohen Z (2011) Selection of a Δ5 desaturase-defective mutant of the microalga *Parietochloris incisa*: the mutation site and effect on expression of LC-PUFA biosynthetic genes. Appl Microbiol Biotechnol 90:249-256
Jin E, Feth B, Melis A (2003) A mutant of the green alga *Dunaliella salina* constitutively accumulates zeaxanthin under all growth conditions. Biotechnol Bioeng 81(1):115-124. https://doi.org/https://doi.org/10.1002/bit.10459
John RP, Anisha G, Nampoothiri KM, Pandey A (2011) Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 102(1):186-193
Kato Y, Ho S-H, Vavricka CJ, Chang J-S, Hasunuma T, Kondo A (2017) Evolutionary engineering of salt-resistant *Chlamydomonas* sp. strains reveals salinity stress-activated starch-to-lipid biosynthesis switching. Bioresour Technol 245:1484-1490
Kawaroe M, Prartono T, Hwangbo J, Sunuddin A, Augustine D, Gustina AS (2015) Effect of ethyl methane sulfonate (EMS) on cell size, fatty acid content, growth rate, and antioxidant activities of microalgae *Dunaliella* sp. Aquacult Aqua Conserv Legislation 8(6):924-932
Khan S, Al-Qurainy F, Anwar F (2009) Sodium azide: a chemical mutagen for enhancement of agronomic traits of crop plants. Environ We Int J Sci Tech 4:1-21
Khoo KS, Ahmad I, Chew KW, Iwamoto K, Bhatnagar A, Show PL (2023) Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae: A review. Prog Energy Combust Sci 96:101071
Kim HS, Weiss TL, Thapa HR, Devarenne TP, Han A (2014) A microfluidic photobioreactor array demonstrating high-throughput screening for microalgal oil production. Lab Chip 14(8):1415-1425. https://doi.org/10.1039/C3LC51396C
Kim M, Ahn J, Jeon H, Jin E (2017) Development of a *Dunaliella tertiolecta* strain with increased zeaxanthin content using random mutagenesis. Mar Drugs 15(6):189
Klug WS, Cummings MR (2003) Concepts of genetics. Pearson Education, Inc, (7th edn). Snustard, DP
Krasovec M, Sanchez-Brosseau S, Grimsley N, Piganeau G (2018) Spontaneous mutation rate as a source of diversity for improving desirable traits in cultured microalgae. Algal Res 35:85-90
Kumar G, Shekh A, Jakhu S, Sharma Y, Kapoor R, Sharma TR (2020) Bioengineering of microalgae: recent advances, perspectives, and regulatory challenges for industrial application. Fronti Bioeng Biotechno 8:914
Kuo C-M, Lin T-H, Yang Y-C, Zhang W-X, Lai J-T, Wu H-T, Chang J-S, Lin C-S (2017) Ability of an alkali-tolerant mutant strain of the microalga *Chlorella* sp. AT1 to capture carbon dioxide for increasing carbon dioxide utilization efficiency. Bioresour Technol 244:243-251. https://doi.org/10.1016/j.biortech.2017.07.096
Lai Y-C, Chang C-H, Chen C-Y, Chang J-S, Ng IS (2019) Towards protein production and application by using *Chlorella* species as circular economy. Bioresour Technol 289:121625. https://doi.org/10.1016/j.biortech.2019.121625
Lambert S, Wagner M (2017) Environmental performance of bio-based and biodegradable plastics: the road ahead. Chem Soc Rev 46(22):6855-6871
Lee B, Choi G-G, Choi Y-E, Sung M, Park MS, Yang J-W (2014) Enhancement of lipid productivity by ethyl methane sulfonate-mediated random mutagenesis and proteomic analysis in *Chlamydomonas reinhardtii*. Korean J Chem Eng 31(6):1036-1042
Levine AJ (1993) The tumor suppressor genes. Annu Rev Biochem 62(1):623-651
Li P, Sun X, Sun Z, Huang F, Wei W, Liu X, Liu Y, Deng L, Cheng Z (2021) Biochemical and genetic changes revealing the enhanced lipid accumulation in *Desmodesmus* sp. mutated by atmospheric and room temperature plasma. Renewable Energy 172:368-381. https://doi.org/10.1016/j.renene.2021.03.048
Lian M, Huang H, Ren L, Ji X, Zhu J, Jin L (2010) Increase of docosahexaenoic acid production by *Schizochytrium* sp. through mutagenesis and enzyme assay. Appl Biochem Biotechnol 162(4):935-941
Lim DK, Schuhmann H, Sharma K, Schenk PM (2015) Isolation of high-lipid *Tetraselmis suecica* strains following repeated UV-C mutagenesis, facs, and high-throughput growth selection. BioEnergy Res 8(2):750-759
Liu B, Ma C, Xiao R, Xing D, Ren H, Ren N (2015a) The screening of microalgae mutant strain *Scenedesmus* sp. Z-4 with a rich lipid content obtained by 60 Co γ-ray mutation. RSC Adv 5(64):52057-52061
Liu B, Sun Z, Ma X, Yang B, Jiang Y, Wei D, Chen F (2015b) Mutation breeding of extracellular polysaccharide-producing microalga *Crypthecodinium cohnii* by a novel mutagenesis with atmospheric and room temperature plasma. Int J Mol Sci 16(4):8201-8212

Liu S, Zhao Y, Liu L, Ao X, Ma L, Wu M, Ma F (2015c) Improving cell growth and lipid accumulation in green microalgae *Chlorella* sp. via UV irradiation. Appl Biochem Biotechnol 175(7):3507-3518

Lo E, Arora N, Philippidis GP (2022) Physiological insights into enhanced lipid accumulation and temperature tolerance by *Tetraselmis suecica* ultraviolet mutants. Sci Total Environ 839:156361. https://doi.org/10.1016/j.scitotenv.2022.156361

Lopez-Rodas V, Agrelo M, Carrillo E, Ferrero LM, Larrauri A, Martín-Otero L, Costas E (2001) Resistance of microalgae to modern water contaminants as the result of rare spontaneous mutations. Eur J Phycol 36(2):179-190

Loppes R (1969) A new class of arginine-requiring mutants in *Chlamydomonas reinhardi*. Mol Gen Genet 104(2):172-177. https://doi.org/10.1007/BF00272799

Ma Y, Wang Z, Zhu M, Yu C, Cao Y, Zhang D, Zhou G (2013) Increased lipid productivity and TAG content in *Nannochloropsis* by heavy-ion irradiation mutagenesis. Bioresour Technol 136:360-367

Maadane A, Merghoub N, Ainane T, El Arroussi H, Benhima R, Amzazi S, Bakri Y, Wahby I (2015) Antioxidant activity of some Moroccan marine microalgae: Pufa profiles, carotenoids and phenolic content. J Biotechnol 215:13-19

Maltsev Y, Maltseva K (2021) Fatty acids of microalgae: Diversity and applications. Rev Environ Sci Bio/Technol 20(2):515-547

Mehtani J, Arora N, Patel A, Jain P, Pruthi PA, Poluri KM, Pruthi V (2017) Augmented lipid accumulation in ethyl methyl sulphonate mutants of oleaginous microalga for biodiesel production. Bioresour Technol 242:121-127. https://doi.org/10.1016/j.biortech.2017.03.108

Meireles LA, Guedes AC, Malcata FX (2003) Increase of the yields of eicosapentaenoic and docosahexaenoic acids by the microalga *Pavlova lutheri* following random mutagenesis. Biotechnol Bioeng 81(1):50-55

Metting F (1996) Biodiversity and application of microalgae. J Ind Microbiol 17(5):477-489

Michela C, Silvia B, Stefania P, Vigliante I, Barbara I, Barbara G, Maffei ME, Massimo D, Matteo B (2020) Improved lipid productivity in *Nannochloropsis gaditana* in nitrogen-replete conditions by selection of pale green mutants. Biotechnol Biofuel 13(1):1-14

Min J, Lee CW, Gu MB (2003) Gamma-radiation dose-rate effects on DNA damage and toxicity in bacterial cells. Radiat Environ Biophys 42(3):189-192

Moha-León JD, Pérez-Legaspi IA, Ortega-Clemente LA, Rubio-Franchini I, Ríos-Leal E (2019) Improving the lipid content of *Nannochloropsis oculata* by a mutation-selection program using UV radiation and quizalofop. J Appl Phycol 31(1):191-199

Morschett H, Loomba V, Huber G, Wiechert W, von Lieres E, Oldiges M (2017) Laboratory-scale photobiotechnology - current trends and future perspectives. FEMS Microbiol Lett 365(1). https://doi.org/10.1093/femsle/fnx238

Muthuraj M, Selvaraj B, Palabhanvi B, Kumar V, Das D (2019) Enhanced lipid content in *Chlorella* sp. FC2 IITG via high energy irradiation mutagenesis. Korean J Chem Eng 36(1):63-70

Nayak M, Suh WI, Oh YT, Ryu AJ, Jeong KJ, Kim M, Mohapatra RK, Lee B, Chang YK (2022) Directed evolution of *Chlorella* sp. HS2 towards enhanced lipid accumulation by ethyl methanesulfonate mutagenesis in conjunction with fluorescence-activated cell sorting based screening. Fuel 316:123410

Nečas J (1968) Influence of low doses of mutagens on growth of algae on a solid medium. Biol Plant 10(5):374-384. https://doi.org/10.1007/BF02921286

Noochanong W, Jirakranwong P, Chanprame S (2018) EMS-induced mutation followed by quizalofop-screening increased lipid productivity in *Chlorella* sp. Bioprocess Biosystems Eng 41(5):613-619

Ong S-C, Kao C-Y, Chiu S-Y, Tsai M-T, Lin C-S (2010) Characterization of the thermal-tolerant mutants of *Chlorella* sp. with high growth rate and application in outdoor photobioreactor cultivation. Bioresour Technol 101(8):2880-2883

Ouf SA, Alsarrani AQ, Al-Adly AA, Ibrahim MK (2012) Evaluation of low-intensity laser radiation on stimulating the cholesterol degrading activity: Part I. Microorganisms isolated from cholesterol-rich materials. Saudi J Biol Sci 19(2):185-193

Pamilo P, Pálsson S, Savolainen O (1999) Deleterious mutations can reduce differentiation in small, subdivided populations. Hereditas 130(3):257-264

Park S-B, Yun J-H, Ryu AJ, Yun J, Kim JW, Lee S, Choi S, Cho D-H, Choi D-Y, Lee YJ (2021) Development of a novel nannochloropsis strain with enhanced violaxanthin yield for large-scale production. Microb Cell Fact 20(1):1-11

Patel VK, Maji D, Pandey SS, Rout PK, Sundaram S, Kalra A (2016) Rapid budding EMS mutants of *Synechocystis* PCC 6803 producing carbohydrate or lipid enriched biomass. Algal Res 16:36-45

Patel VK, Soni N, Prasad V, Sapre A, Dasgupta S, Bhadra B (2019) CRISPR–Cas9 system for genome engineering of photosynthetic microalgae. Mol Biotechnol 61(8):541-561

Patil V, Reitan KI, Knutsen G, Mortensen LM, Källqvist T, Olsen E, Vogt G, Gislerød HR (2005) Microalgae as source of polyunsaturated fatty acids for aquaculture. Plant Biol 6(6):57-65

Perin G, Bellan A, Segalla A, Meneghesso A, Alboresi A, Morosinotto T (2015) Generation of random mutants to improve light-use efficiency of *Nannochloropsis gaditana* cultures for biofuel production. Biotechnol Biofuel 8(1):161. https://doi.org/10.1186/s13068-015-0337-5

Polat E, Altınbaş M (2022) Enhanced biodiesel properties of *Auxenochlorella* sp. using chemical mutagenesis and Tralkoxydim. Int J Green Energy 1-12

Priyadarshani I, Rath B (2012) Commercial and industrial applications of micro algae–A review. J Algal Biomass Utilization 3(4):89-100

Qu F, Jin W, Zhou X, Wang M, Chen C, Tu R, Han S-f, He Z, Li S-f (2020) Nitrogen ion beam implantation for enhanced lipid accumulation of *Scenedesmus obliquus* in municipal wastewater. Biomass Bioenergy 134:105483. https://doi.org/https://doi.org/10.1016/j.biombioe.2020.105483

Rachmayati R, Agustriana E, Rahman DY (2020) UV Mutagenesis as a strategy to enhance growth and lipid productivity of *Chlorella* sp. J Trop Biodivers Biotechnol 5(3):10. https://doi.org/10.22146/jtbb.56862

Rammuni M, Ariyadasa TU, Nimarshana P, Attalage R (2019) Comparative assessment on the extraction of carotenoids from microalgal sources: Astaxanthin from *H. pluvialis* and β-carotene from *D. salina*. Food Chem 277:128-134

Rao AV, Rao LG (2007) Carotenoids and human health. Pharmacol Res 55(3):207-216

Rastogi RP, Kumar A, Tyagi MB, Sinha RP (2010) Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. J Nucleic Acids. https://doi.org/10.4061/2010/592980

Richmond A (2008) Handbook of microalgal culture: biotechnology and applied phycology. John Wiley & Sons

Saadaoui I, Rasheed R, Aguilar A, Cherif M, Al Jabri H, Sayadi S, Manning SR (2021) Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. J Anim Sci Biotechnol 12(1):1-15

Sachdeva N, Gupta RP, Mathur AS, Tuli DK (2016) Enhanced lipid production in thermo-tolerant mutants of *Chlorella pyrenoidosa* NCIM 2738. Bioresour Technol 221:576-587. https://doi.org/10.1016/j.biortech.2016.09.049

Sandesh Kamath B, Vidhyavathi R, Sarada R, Ravishankar GA (2008) Enhancement of carotenoids by mutation and stress induced carotenogenic genes in *Haematococcus pluvialis* mutants. Bioresour Technol 99(18):8667-8673. https://doi.org/10.1016/j.biortech.2008.04.013

Sarayloo E, Simsek S, Unlu YS, Cevahir G, Erkey C, Kavakli IH (2018) Enhancement of the lipid productivity and fatty acid methyl ester profile of *Chlorella vulgaris* by two rounds of mutagenesis. Bioresour Technol 250:764-769

Sarayloo E, Tardu M, Unlu YS, Simsek S, Cevahir G, Erkey C, Kavakli IH (2017) Understanding lipid metabolism in high-lipid-producing *Chlorella vulgaris* mutants at the genome-wide level. Algal Res 28:244-252. https://doi.org/10.1016/j.algal.2017.11.009

Sauer U (2001) Evolutionary engineering of industrially important microbial phenotypes. Metab Eng 73:129-169

Schüler L, Greque de Morais E, Trovão M, Machado A, Carvalho B, Carneiro M, Maia I, Soares M, Duarte P, Barros A, Pereira H, Silva J, Varela J (2020) Isolation and characterization of novel *Chlorella Vulgaris* mutants with low chlorophyll and improved protein contents for food applications. Fronti Bioeng Biotechnol 8. https://doi.org/10.3389/fbioe.2020.00469

Senthamilselvi D, Kalaiselvi T (2022) Gamma ray mutants of oleaginous microalga *Chlorella* sp. KM504965 with enhanced biomass and lipid for biofuel production. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-022-02400-9

Shao Y, Fu Y, Chen Y, Abomohra A, He Q, Jin W, Liu J, Tan Z, Li X (2022) Enhancement of black and odorous water treatment coupled with accelerated lipid production by microalgae exposed to $^{12}C^{6+}$ heavy-ion beam irradiation. Chemosphere 305:135452. https://doi.org/10.1016/j.chemosphere.2022.135452

Shin W-S, Lee B, Jeong B-r, Chang YK, Kwon J-H (2016) Truncated light-harvesting chlorophyll antenna size in *Chlorella vulgaris* improves biomass productivity. J Appl Phycol 28(6):3193-3202. https://doi.org/10.1007/s10811-016-0874-8

Sikder S, Biswas P, Hazra P, Akhtar S, Chattopadhyay A, Badigannavar A, D'Souza S (2013) Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS. Indian J Genet 73(4):392-399

Singh J, Saxena RC (2015) An introduction to microalgae: diversity and significance. Handbook of marine microalgae, Elsevier pp 11-24

Sivaramakrishnan R, Incharoensakdi A (2017) Enhancement of lipid production in *Scenedesmus* sp. by UV mutagenesis and hydrogen peroxide treatment. Bioresour Technol 235:366-370

Smalley T, Fields FJ, Berndt AJE, Ostrand JT, Heredia V, Mayfield SP (2020) Improving biomass and lipid yields of *Desmodesmus armatus* and *Chlorella vulgaris* through mutagenesis and high-throughput screening. Biomass Bioenergy 142:105755. https://doi.org/10.1016/j.biombioe.2020.105755

Sohrabi N, Valizadeh A, Farkhani SM, Akbarzadeh A (2016) Basics of DNA biosensors and cancer diagnosis. Artif Cells Nanomed Biotechnol 44(2):654-663

Song M, Pei H (2018) The growth and lipid accumulation of *Scenedesmus quadricauda* during batch mixotrophic/heterotrophic cultivation using xylose as a carbon source. Bioresour Technol 263:525-531. https://doi.org/10.1016/j.biortech.2018.05.020

Soskine M, Tawfik DS (2010) Mutational effects and the evolution of new protein functions. Nat Rev Genet 11(8):572-582

Spicer A, Molnar A (2018) Gene editing of microalgae: scientific progress and regulatory challenges in europe. Biology 7(1):21

Sproles AE, Fields FJ, Smalley TN, Le CH, Badary A, Mayfield SP (2021) Recent advancements in the genetic engineering of microalgae. Algal Res 53:102158

Srinivas R, Ochs C (2012) Effect of UV-A irradiance on lipid accumulation in *Nannochloropsis oculata*. Photochem Photobiol 88(3):684-689

Sun X-M, Ren L-J, Zhao Q-Y, Ji X-J, Huang H (2018) Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation. Biotechnol Biofuel 11(1):1-16

Sun X, Li P, Liu X, Wang X, Liu Y, Turaib A, Cheng Z (2020) Strategies for enhanced lipid production of *Desmodesmus* sp. mutated by atmospheric and room temperature plasma with a new efficient screening method. J Cleaner Prod 250:119509. https://doi.org/10.1016/j.jclepro.2019.119509

Tamae C, Liu A, Kim K, Sitz D, Hong J, Becket E, Bui A, Solaimani P, Tran KP, Yang H (2008) Determination of antibiotic hypersensitivity among 4,000 single-gene-knockout mutants of *Escherichia coli*. J Bacteriol 190(17):5981-5988

Thurakit T, Pumas C, Pathom-Aree W, Pekkoh J, Peerapornpisal Y (2018) Enhancement of biomass, lipid and hydrocarbon production from green microalga, *Botryococcus braunii* AARL G037, by UV-C induction. Chiang Mai J Sci 45(7):2637-2651

Tillich UM, Lehmann S, Schulze K, Dühring U, Frohme M (2012) The optimal mutagen dosage to induce point-mutations in *Synechocystis* sp. PCC6803 and its application to promote temperature tolerance. Plos One 7(11):e49467. https://doi.org/10.1371/journal.pone.0049467

Tredici MR (2004) Mass production of microalgae: photobioreactors. Handbook of microalgal culture: Biotechnol appl phycol 1:178-214

Trovão M, Pereira H, Silva J, Páramo J, Quelhas P, Santos T, Silva JT, Machado A, Gouveia L, Barreira L (2019) Growth performance, biochemical composition and sedimentation velocity of *Tetraselmis* sp. CTP4 under different salinities using low-cost lab-and pilot-scale systems. Heliyon 5(5):e01553

Trovão M, Schüler LM, Machado A, Bombo G, Navalho S, Barros A, Pereira H, Silva J, Freitas F, Varela J (2022) Random mutagenesis as a promising tool for microalgal strain improvement towards industrial production. Mar Drugs 20(7):440

Tu R, Jin W, Wang M, Han S, Abomohra AE-F, Wu W-M (2016) Improving of lipid productivity of the biodiesel promising green microalga *Chlorella pyrenoidosa* via low-energy ion implantation. J Appl Phycol 28(4):2159-2166

Van Lijsebettens M, Vanderhaeghen R, Van Montagu M (1991) Insertional mutagenesis in *Arabidopsis thaliana*: isolation of a T-DNA-linked mutation that alters leaf morphology. Theor Appl Genet 81(2):277-284

Varshney P, Mikulic P, Vonshak A, Beardall J, Wangikar PP (2015) Extremophilic micro-algae and their potential contribution in biotechnology. Bioresour Technol 184:363-372

Wang N, Guan B, Kong Q, Sun H, Geng Z, Duan L (2016a) Enhancement of astaxanthin production from *Haematococcus pluvialis* mutants by three-stage mutagenesis breeding. J Biotechnol 236:71-77. https://doi.org/10.1016/j.jbiotec.2016.08.009

Wang S, Zhang L, Yang G, Han J, Thomsen L, Pan K (2016b) Breeding 3 elite strains of *Nannochloropsis oceanica* by nitrosoguanidine mutagenesis and robust screening. Algal Res 19:104-108

Wijffels RH, Barbosa MJ, Eppink MH (2010) Microalgae for the production of bulk chemicals and biofuels. Biofuels, bioproducts and biorefining: Innovation for a sustainable economy 4(3):287-295

Wu M, Zhang H, Sun W, Li Y, Hu Q, Zhou H, Han D (2019) Metabolic plasticity of the starchless mutant of *Chlorella sorokiniana* and mechanisms underlying its enhanced lipid production revealed by comparative metabolomics analysis. Algal Res 42:101587. https://doi.org/10.1016/j.algal.2019.101587

Xiaodong D, Yajun L, Xiaowen F (2011) Effects of selective medium on lipid accumulation of chlorellas and screening of high lipid mutants through ultraviolet mutagenesis. Afr J Agric Res 6(16):3768-3774

Xie B, Stessman D, Hart JH, Dong H, Wang Y, Wright DA, Nikolau BJ, Spalding MH, Halverson LJ (2014) High-throughput fluorescence-activated cell sorting for lipid hyperaccumulating *Chlamydomonas reinhardtii* mutants. Plant Biotechnol J 12(7):872-882

Xing W, Zhang R, Shao Q, Meng C, Wang X, Wei Z, Sun F, Wang C, Cao K, Zhu B (2021) Effects of laser mutagenesis on microalgae production and lipid accumulation in two economically important fresh *Chlorella* strains under heterotrophic conditions. Agronomy 11(5):961

Xue J, Balamurugan S, Li T, Cai J-X, Chen T-T, Wang X, Yang W-D, Li H-Y (2021) Biotechnological approaches to enhance biofuel producing potential of microalgae. Fuel 302:121169

Yamamoto J, Plaza P, Brettel K (2017) Repair of (6-4) lesions in DNA by (6-4) photolyase: 20 years of quest for the photoreaction mechanism. Photochem Photobiol 93(1):51-66. https://doi.org/10.1111/php.12696

Yi Z, Su Y, Xu M, Bergmann A, Ingthorsson S, Rolfsson O, Salehi-Ashtiani K, Brynjolfsson S, Fu W (2018) Chemical mutagenesis and fluorescence based high-throughput screening for enhanced accumulation of carotenoids in a model marine diatom *Phaeodactylum tricornutum*. Mar Drugs 16(8):272

Yi Z, Xu M, Magnusdottir M, Zhang Y, Brynjolfsson S, Fu W (2015) Photo-oxidative stress-driven mutagenesis and adaptive evolution on the marine diatom *Phaeodactylum tricornutum* for enhanced carotenoid accumulation. Mar Drugs 13(10):6138-6151

Zhang H, Gao Z, Li Z, Du H, Lin B, Cui M, Yin Y, Lei F, Yu C, Meng C (2017) Laser radiation induces growth and lipid accumulation in the seawater microalga *Chlorella pacifica*. Energies 10(10):1671

Zhang Q, Chang C, Bai J, Fang S, Zhuang X, Yuan Z (2018a) Mutants of *Scenedesmus* sp. for purifying highly concentrated cellulosic ethanol wastewater and producing biomass simultaneously. J Appl Phycol 30(2):969-978

Zhang X, Zhang X-F, Li H-P, Wang L-Y, Zhang C, Xing X-H, Bao C-Y (2014) Atmospheric and room temperature plasma (ARTP) as a new powerful mutagenesis tool. Appl Microbiol Biotechnol 98(12):5387-5396

Zhang X, Zhang X, Xu G, Zhang X, Shi J, Xu Z (2018b) Integration of ARTP mutagenesis with biosensor-mediated high-throughput screening to improve L-serine yield in *Corynebacterium glutamicum*. Appl Microbiol Biotechnol 102(14):5939-5951

Zhang Y, He M, Zou S, Fei C, Yan Y, Zheng S, Rajper AA, Wang C (2016) Breeding of high biomass and lipid producing *Desmodesmus* sp. by ethylmethane sulfonate-induced mutation. Bioresour Technol 207:268-275