(Press Release)

2024.07.04
NTT Corporation
Euglena Co., Ltd.

Established the world's first algal breeding technology using neutron beam irradiation

Successfully increased oil production of biofuel feedstock by up to 1.3 times

News Highlights:

- We have clarified the irradiation conditions for the neutron beam[1], which are optimal for breeding[2] of algae
- Using neutron beam irradiation under these optimized conditions, we succeeded for the first time in the world in breeding algae that can increase the amount of oil, a raw material for biofuel[3]
- The new algal breeding technology established in this study is expected to be widely used to tackle climate change issues, such as reducing greenhouse gas emissions and generating new energy resources

Tokyo –July 4, 2024 – NTT Corporation (Headquarters: Chiyoda Ward, Tokyo; Representative Member of the Board and President: Akira Shimada, hereinafter "NTT") and Euglena Co., Ltd. (Headquarters: Minato Ward, Tokyo; Founder and President CEO: Mitsuru Izumo, hereinafter "Euglena Co.") have succeeded for the first time in the world in breeding of algae by mutagenesis[4] using neutron beam irradiation. This achievement is expected to be a fundamental technology that will solve various issues related to climate change by improving the amount of CO_2 absorbed by algae and by breeding and producing algae that are useful for various purposes.

The results were published in the scientific journal *Scientific Reports* on July 3, 2024.

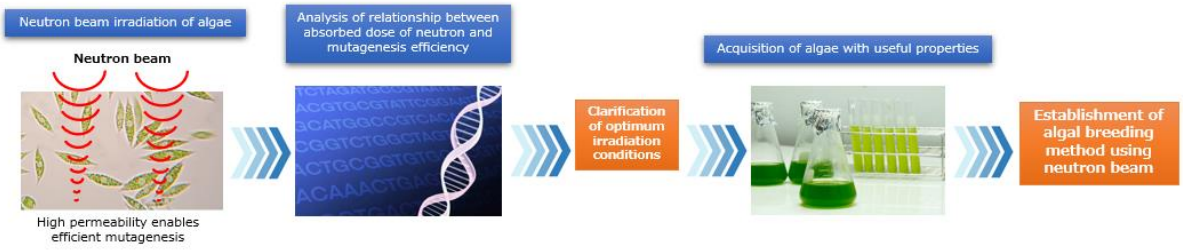

Figure 1 *Development of Algae Breeding Technology Using Neutron Beam*

1. Background

According to the Intergovernmental Panel on Climate Change (IPCC) Sixth Assessment Report[5], it states "It is unequivocal that human influence has warmed the atmosphere, ocean and land."

Therefore, there is an urgent need to reduce greenhouse gases such as CO_2 caused by human activities. Algae, which photosynthesize like plants and have a fast growth rate, are attracting attention as a means to address the climate issue. To effectively utilize the functions of algae, breeding technology that can maximize the characteristics of the desired algae is required.

In the past, algal breeding methods have attempted mutagenesis using electromagnetic waves and heavy particle beam[6], which have low permeability to substances containing water such as culture medium has not been effective against most algal cells growing in culture medium. Instead, NTT and Euglena Co. focused on neutron beams that have no electric charge and are highly permeable to substances containing water such as culture medium. Consequently, since 2022, NTT and Euglena Co. have been conducting a joint research project aiming to establish algal breeding technology by using two types of neutron beams; high-energy neutron beam[7] and thermal neutron beam[8] (Figure 1)[9].

2. Key points of technology and experiment overview

(1) Optimization of neutron beam irradiation conditions

For the first time, we clarified the relationship between the type of neutron beam (high-energy neutron beam or thermal neutron beam) and the absorbed dose[10] and the mutagenesis efficiency of algal genes.

The mutation was evaluated using the unicellular alga *Cyanidioschyzon merolae*[11] (Cyzon), based on a mechanism in which a mutation in a gene that synthesizes nucleic acids allows growth on agar medium containing a drug that inhibits growth. The analysis revealed that mutations were most effectively introduced at 20 Gy[12] irradiation for high-energy neutron beams and at 13 Gy irradiation for thermal neutron beams (Figure 2).

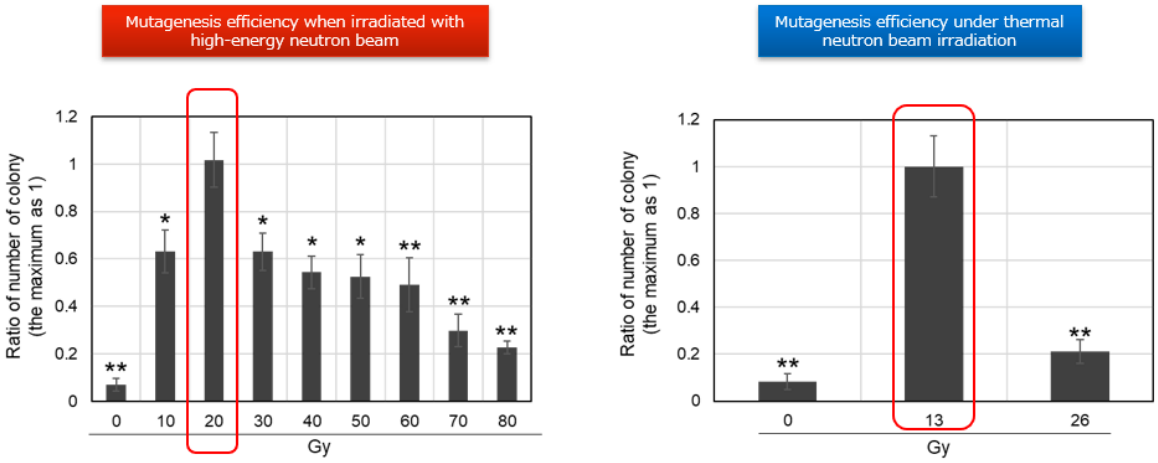

Figure 2 *Clarification of the relationship between absorbed dose of neutron beam and mutagenesis efficiency*

Each type of neutron beam was irradiated to the absorbed dose (Gy) shown in the figure. The maximum number of colonies that appeared at that time is shown as a relative value when 1 is set.

(2) Elucidation of mutation patterns caused by optimized irradiation conditions

Next, we analyzed the mutation patterns caused by the optimized irradiation conditions. As a result, about 90% of the mutation patterns of the genes in which mutations were introduced were substitutions, deletions, and insertions of the 1 nucleotide sequence[13], and about 10% were changes of 2 or more nucleotide sequences (Figure 3). There is a report[14] that the change of 2 nucleotide sequence or more is about 30% by gamma ray (γ-ray), suggesting that the mutation pattern caused by neutron beam irradiation is different from the current method.

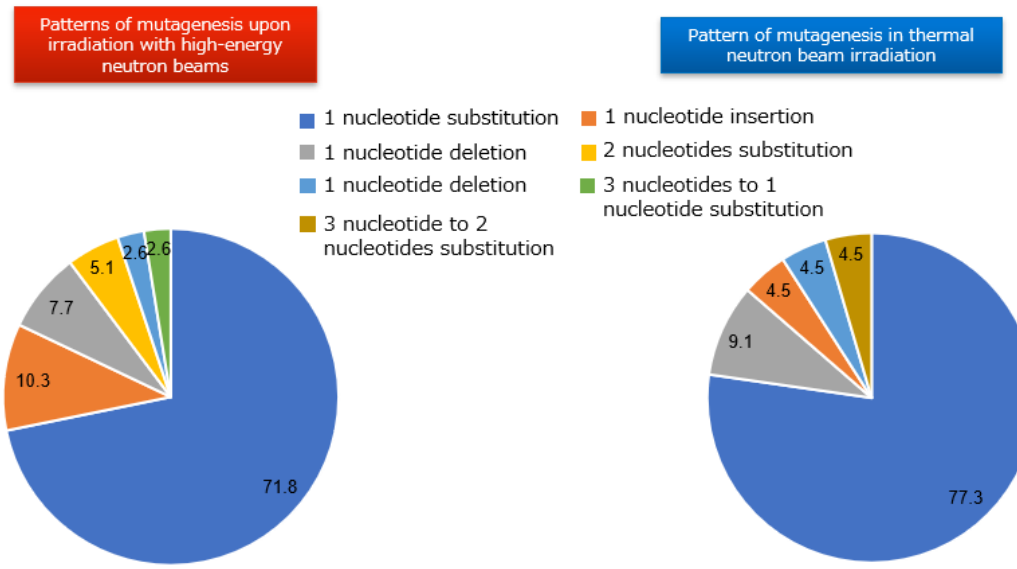

Figure 3 *Mutation pattern detected in the gene into which the mutation was introduced Numbers in the figure indicate the percentage of total mutations detected.*

(3) Isolation of algae with improved oil production

By applying the optimum neutron beam irradiation conditions to *Euglena gracilis*[15] (Euglena), we succeeded in breeding strains that produce up to 1.2 to 1.3 times more oil than the wild type strain.

The optimum neutron beam irradiation conditions identified by Cyzon were applied to Euglena, one of the commercial algae that produce biofuels (equivalent to jet fuel or diesel fuel), to acquire cells with improved oil production. Fluorescent dye, which specifically stains oils, was added to cells irradiated with neutron beams to stain the oils, and the strength of the amount of fluorescence emitted by each cell was used as an indicator for selection. As a result, we succeeded in acquiring four strains[16] with higher oil production than the wild type strain (Figure 4).

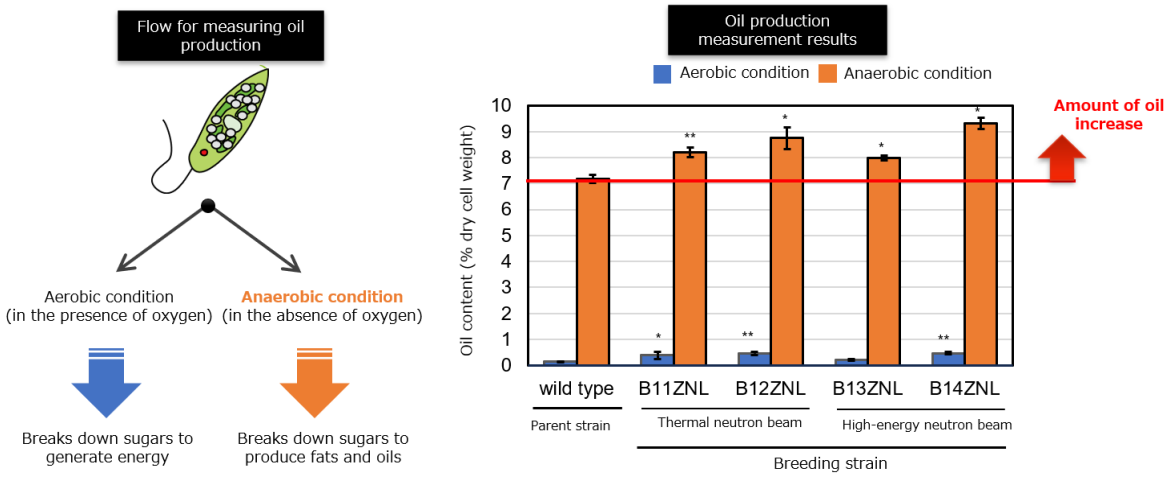

Figure 4 *Amount of oils by the acquired breeding strains*

Left: description of aerobic conditions and anaerobic conditions under which oils are produced (anaerobic conditions cause cells to break down sugars to produce oils)

Right: Quantitative measurement of the amount of oil (B11ZNL and B12ZNL were obtained by thermal neutron beam irradiation, and B13ZNL and B14ZNL were obtained by high-energy neutron beam irradiation.)

3. Roles of each company

NTT: Using the knowledge on neutron beam irradiation accumulated in the test, which is a soft error[17] caused by neutron beams originating from cosmic ray[18] in semiconductors used on the ground, we clarified the relationship between the type of neutron beam and the absorbed dose and the mutagenesis efficiency and mutation pattern.

Euglena Co.: Utilizing technology to evaluate cells suitable for production of oils and the functional substance paramylon[19] that can be used as a raw material for biofuels, we acquired strains from algae after neutron beam irradiation that produced higher amounts of oils than the original strain.

4. Outlook

These results confirm the applicability of neutron breeding to two types of algae. NTT will increase the variety of algae that have improved the amount of CO_2 absorbed and analyze the gene responsible for the improvement, as well as verify the effectiveness of expanding the scope of application of this technology beyond the two types of algae. We aim to provide solutions to various issues related to climate issues, such as the reduction of greenhouse gases and the production of energy resources, as well as the creation of agricultural, forestry and fishery feeds, by improving the variety and production of algae that are useful for the various utilization cases.

[1] *Neutron beam: Neutrons are the particles that make up the nucleus. When an atomic nucleus undergoes nuclear reaction, neutrons fly out of the nucleus with kinetic energy. Neutrons moving in one direction are called neutron beam.*

[2] *Breeding: This refers to the creation of a breed that is more useful to humans by taking advantage of the fact that genetic changes change its properties.*

[3] *Biofuels are fuels made from biological resources (biomass). As a measure to reduce CO_2 emissions, the use of fossil fuels as an alternative fuel is expected to increase.*

[4] *Mutagenesis: A change in the base sequence of the DNA that makes up a gene from its original sequence. As a result of mutagenesis, the function of the protein made from the gene is changed.*

[5] *https://www.ipcc.ch/*

[6] *Heavy particle beam: A heavy particle beam. This includes particles such as helium, carbon, neon, and argon.*

[7] *High-energy neutron beam: A neutron with a high kinetic energy, that is, traveling at a high speed. Here, the neutron is about 10% of the speed of light.*

[8] *Thermal neutron beam: Neutrons with energy around 25 meV (2200 m/s). When a neutron scatters repeatedly in a material, it is called a "thermal" neutron because it is equal on average to the thermal kinetic energy of the atoms of the material.*

[9] *https://group.ntt/en/newsrelease/2022/09/13/220913a.html*

[10] *Absorbed Dose: The energy absorbed by a substance by irradiation and is a measure of the effect of radiation on a cell.*

[11] *Cyanidioschyzon merolae: a unicellular red alga (member of the nori family) found in an Italian hot spring. It is used as a model alga and a model photosynthetic eukaryote, with 100% of the nuclear genome determined for the first time as a eukaryote.*

[12] *Gy: A metric unit of energy imparted to an object by radiation, defined as gray (Gy) for an absorbed dose of energy equivalent to 1 J of work per kilogram of material.*

[13] *nucleotide sequence: indicates the order of binding of the constituent nucleotides in a nucleic acid, such as DNA or RNA. In this case, it is DNA and consists of adenine, guanine, cytosine, and thymine.*

[14] *https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1009979*

[15] *Euglena gracilis: a freshwater species of single-celled alga in the genus Euglena that is considered to be the most suitable for industrial use among Euglena species due to its established mass culture method.*

[16] *Strain: A group of the same strain in micromatter or microalgae.*

[17] *Soft error: Unlike a hard error that permanently causes a device to fail, this is a temporary failure that can be recovered by restarting the device or overwriting data.*

[18] *Cosmic Rays: High-energy particles that travel through space, mainly consisting of protons and other nuclei such as alpha particles, lithium, and beryllium. Cosmic rays also affect electronics and the human body in space. On the ground, neutrons generated by cosmic rays reacting with the Earth's atmosphere can cause electronic devices to malfunction in rare cases.*

[19] *Paramylon: A type of dietary fiber, a polysaccharide produced by the genus Euglena as an intracellular storage substance. Recent research has shown that dietary fiber has new functions in health care that are different from those of conventional dietary fiber, such as affecting immune function.*

About NTT

NTT contributes to a sustainable society through the power of innovation. We are a leading global technology company providing services to consumers and businesses as a mobile operator, infrastructure, networks, applications, and consulting provider. Our offerings include digital business consulting, managed application services, workplace and cloud solutions, data center and edge computing, all supported by our deep global industry expertise. We are over $97B in revenue and 330,000 employees, with $3.6B in annual R&D investments. Our operations span across 80+ countries and regions, allowing us to serve clients in over 190 of them. We serve over 75% of Fortune Global 100 companies, thousands of other enterprise and government clients and millions of consumers.

About Euglena

Euglena Co., Ltd. is a Japan-based biotechnology-driven and sustainability-oriented growth company, starting from the world's first success in outdoor mass cultivation of the edible microalgae *Euglena* in 2005. With "Sustainability First" as its corporate philosophy and its advanced R&D capabilities for proposing sustainable solutions to social problems, the Company has operated the Healthcare Business for marketing functional foods/cosmetics, the Biofuel Business for producing biofuels, and the Other Business in such areas as fertilizer, bioinformatics, farming, social business, etc. The Company went public in 2012 and is currently listed on the Prime Market of the Tokyo Stock Exchange.

Media contacts

NTT Information Network Laboratory Group
Public Relations

Euglena Co., Ltd.
Public Relations and Publicity Department