Mitsubishi Corporation Life Sciences Limited

Press release

March 30, 2022

Mitsubishi Corporation Life Sciences Limited

Developing the technology TAQing2.0 for reorganizing the genome structure without introducing foreign DNA and RNA

Research on “TAQing2.0 for genome reorganization of asexual industrial yeasts by direct protein transfection” was published in *Communications Biology*. This study has been performed by a collaborative research group led by Mitsubishi Corporation Life Sciences Limited (head office: Chiyoda-ku, Tokyo; representative director & president: Hiroshi Fujiki) and Professor Kunihiro Ohta and coworkers at the University of Tokyo (address: Bunkyo-ku, Tokyo; president: Teruo Fujii).

[Highlights]

- We developed the breeding method TAQing2.0 without introducing DNA and RNA by updating the existing technology of the TAQing system, which induces large-scale genome rearrangement and thereby improves rapidly quantitative traits controlled by a complex gene network.
- This technology is characterized by delivering endonuclease *Taq*I directly into the cell nucleus by using a commercially available cell-penetrating peptide. We can markedly enhance the frequency of genome restructuring events, which naturally occur in the process of crossbreeding, in a simple manner where the cells are gently warmed at a specific temperature to regulate the activity of *Taq*I.
- By using TAQing2.0 for torula yeast, one of the non-sporulating industrial yeasts, we confirmed the occurrence of large-scale chromosomal rearrangements at multiple sites using computational genome analyses. Moreover, we succeeded in generating a more accurate reference genome sequence of the wild type torula yeast.
- We demonstrated the potential capability of TAQing2.0, which renders increased genetic diversity within a species, contributing to the efficient acquisition of microorganisms of high industrial utility. Strains, obtained by TAQing2.0, can be handled in the same manner as naturally occurring mutants because any incoming DNA and RNA have not been contained in the strains, as well as in this technical process. Therefore, we are speculating that this may relieve the difficulties of public acceptance toward genetically modified organisms.

[Announcement document]

Since ancient times, people have adopted a crossbreeding technique, the representative utility for sexual reproduction, for domestic animals or plants, and then improved their traits so as to obtain more useful ones for themselves. In this method, genomic DNA(1) happened to mutate through generational change not through

intentionally introducing an exogenous gene. For improvement of species, therefore, fundamental issues must be overcome, such as the need for a very long period. Although problems have arisen in applying crossbreeding methods to asexual organisms, we induced mutagenesis by using radiation or drugs. In these approaches, nevertheless, the same problems mentioned above, in other words, the need for time-consuming processes, remained to be addressed.

Professor Kunihiro Ohta and coworkers recently developed a large-scale genome restructuring technology called the TAQing system[(2)] (Muramoto et al., *Nat. Commun.* 2018) and applied the technology to cells of budding yeast and the plant *Arabidopsis thaliana*. In this technology, gene coding for the restriction enzyme[(3)], *Taq*I or *Mbo*I, is genetically transfected into cells using vector-mediated tools, and then up-regulated transiently under the control of an inducible promoter. Subsequently, the cells are gently warmed to partially and temporarily activate *Taq*I, which simultaneously induces multiple random DNA double-strand breaks (DSBs) at TCGA sites throughout the genome and rejoins them via DNA repair. As a result, large-scale genomic rearrangements are rapidly induced, and variants with altered and improved phenotypes can be obtained in a very short period, such as the yeast mutant with higher bioethanol fermentative ability.

In this original TAQing system, however, the genetic engineering approach is necessary to deliver exogenous plasmid harboring *Taq*I gene into cells. Therefore, in the practical fields where we seek to apply the TAQing system, there remain several issues to resolve; the TAQing system cannot be applied to species for which a gene recombination assay has not been established or where a newly generated variant could be subject to recombinant DNA regulation if it contains incoming DNA.

Now researchers in this study have succeeded in resolving these challenges explained above by submitting the established technology of TAQing2.0.

In this experiments, we used *torula* yeast[(4)] (Cu), which is one of the non-sporulating industrial yeasts. A cell-penetrating peptide (CPP, commercially available) and the *Taq*I complex were introduced into live Cu cells in a buffer, and then the cells were warmed at mild temperatures for a constant time to temporarily and partially activate *Taq*I. According to these simple procedures, we confirmed that the intracellular events, random DNA DSBs and DNA repairs throughout the genome, efficiently took place, contributing to large-scale chromosomal restructurings (Figure 1). Here, we also established an easy and versatile method for transfecting various foreign active proteins in a minimally invasive and highly efficient manner by using CPP for budding yeasts. As the result of the various trials, we optimized the transfection condition in which two factors turned out to be important: the use of cells in the early exponential growth phase and the employment of a buffer condition.

By using the TAQing2.0 method one time, Cu mutants with altered phenotypes, for example, aggregability or stress resistance (unpublished data), were generated, and genome resequencing demonstrated that the hyper-flocculated mutants have multiple rearrangements with the *Taq*I recognition sequence (TCGA) at break

points (Figure 2). In this study, moreover, we generated a more accurate reference genome sequence of wild type Cu that has been roughly unveiled and rather controversial.

Co-researchers in this group named this updated method TAQing2.0 and are now attempting to evaluate the practicality of this technology in practical and industrial fields. At our company, TAQing2.0 would be an additional and an effective breeding approach to obtaining naturally occurring strains, and we aim to submit a high-order microbial platform to flexibly meet customer and market requests.

(Notes 1) Genomic DNA
It is the smallest unit of DNA information that determines a specific organism. One genomic DNA, involved in all genetic information, is encapsulated in one cell.

(Notes 2) TAQing system
This technology was reported by Muramoto et al. in *Nat. Commun.* 2018 and the process simultaneously induces multiple random DNA double-strand breaks and rejoins them in the genome by using *Taq*I-plasmid transfection, resulting in large-scale genomic rearrangements. This method enables rapid improvement of phenotypes, such as the fermentative capacity of budding yeast to accumulate bioethanol or the increased stress resistance and biomass production for *Arabidopsis thaliana*.

(Notes 3) Restriction enzyme
This endonuclease recognizes specific 4-, 6- or more bases of DNA sequences and cleaves. Present in some bacteria, the enzyme functions to restrict propagation of infectious phages (viruses infect host bacteria), and so the enzyme has come to be called a restriction enzyme. Because a restriction enzyme has the property of cleaving DNA in a specific sequence, it has made an indispensable contribution to the development of genetic engineering technology.

(Notes 4)
Since Germany started production of torula yeast (*Cyberlindnera jadinii*, *Candida utilis*) as a source of protein nutrients in the 1910s, and now this yeast has been widely used as edible/fodder yeast and a dietary supplement in many countries. The Food and Drug Administration (FDA) of the United States certified GRAS (generally recognized as safe) status to torula yeast.

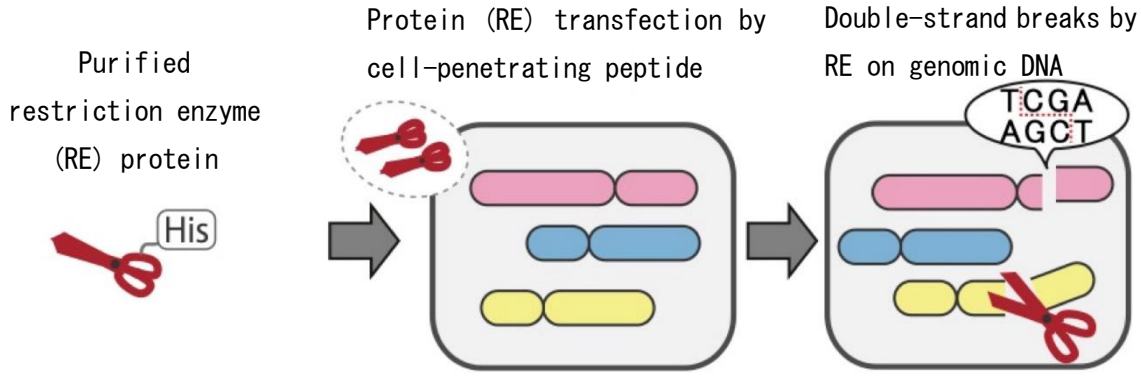

Figure 1 A schematic diagram describing the procedure for TAQing2.0. In the original TAQing system, a plasmid containing restriction enzyme (RE) gene is transfected into cells, where RE is expressed and partially activated, resulting in the induction of double-strand breaks at the RE recognition site. In TAQing2.0, a purified RE is mixed with cell-penetrating peptide and then delivered directly into intact cells. TAQing2.0 thus enables the induction of genome rearrangement. In addition, this technology is easy to apply toward species for which a gene recombination assay has not been established, so we can use the TAQing2.0 method for a wider range of organisms.

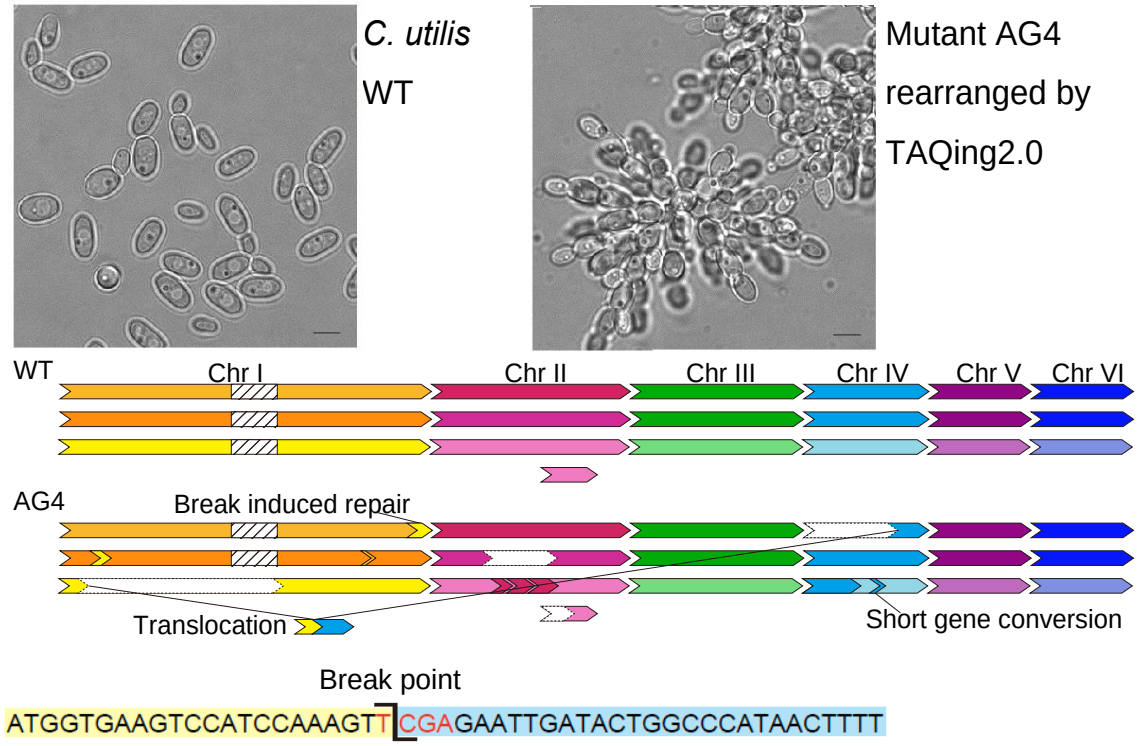

Figure 2 Microscopic images for the torula yeast (Cu) mutants obtained by TAQing2.0 (upper left; Wild Type (WT), upper right; mutant AG4, scale bar; 5 μm) and the chromosomal structure of Cu and mutant AG4 (lower;

schematic diagram). Concerning the developed method in this study, we can introduce an active protein into yeast cells with intact cell walls and obtain altered phenotypic Cu variants with flocculation or stress resistance. Note that a translocation event (double-strand break and rejoin) between Chr I and Chr IV occurred in AG4, and RE recognition sequencing TCGA at the break point in translocation is shown.

[Reference]

Magazine: *Communications Biology* (Online version: February 17)
Title: TAQing2.0 for genome reorganization of asexual industrial yeasts by direct protein transfection
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DOI number: 10.1038/s42003-022-03093-6
URL: https://www.nature.com/articles/s42003-022-03093-6

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