BioResource Now !
Issue Number 9 May 2013

Introduction to Resource Center No. 46
Masahiro Fujishima
(Graduate School of Science and Engineering, Yamaguchi University)
Characteristics of *Paramecium* species as Bioresources ..... P1 - 2

Database of This Month
National BioResource Project "Bacillus subtilis" P2

Download the PDF version of this newsletter at
http://www.shigen.nig.ac.jp/shigen/news/

Characteristics of *Paramecium* species as Bioresources

Masahiro Fujishima, Professor
Graduate School of Science and Engineering, Yamaguchi University

Important paradigms in the history of biology, such as the observations that (1) cells can be electrically stimulated (Kamata, 1934), (2) cells can be distinguished by sex (Sonneborn, 1937), (3) each type of cell has its own life span (Sonneborn, 1953), and (4) deviated codons exist in a cell nucleus (Preer *et al.*, 1985) were first discovered in *Paramecium* species. At present, *Paramecium* species are used in research on intracellular symbiosis, ciliary movement nuclear differentiation, mating substances, aging, genome rearrangement in the developmental process, deviated codons*1, circadian rhythm*2, phylaxis, osmoregulation, environmental adaptation, phagocytosis, mechanoreceptors*3, photoreceptors, ionic channels*4, effects of gravity, taxis, learning, water purification, and the environmental pollution index.

Although *Paramecium* species are sufficiently described in senior high school textbooks, senior high school students have seldom seen living *Paramecium* species. The number of researchers using *Paramecium* species, the best-known ciliate, is much smaller than those using *Tetrahymena pyriformis*, which is also a ciliate. The main reason for this is that no system has been established to stably conserve and distribute the strains of *Paramecium* species. This problem was solved when *Paramecium* species were adopted in the third term of the National BioResource Project (NBRP); the project was able to manage detailed information on strains of *Paramecium* species using a uniform format, and these strains could be distributed, which satisfied users' purposes. Because an applicable cryopreservation technology was not developed, strains of *Paramecium* species were difficult to conserve for a long time; consequently, the number of researchers studying *Paramecium* species did not increase. This situation has gradually improved.

In Japan, strains of *Paramecium* species have been conserved since the Second World War. The situation at that time was described in a book titled "Investigation of the Origin of Sex — the World of *Paramecium* species" (Iwanami Shinsho 345) written by the late Koichi Hiwatashi (Emeritus Professor, Tohoku University). Strains of *Paramecium* species had been conserved in Tohoku University for a while with the aid of the Ministry of Education at that time. After this system of conserving strains was terminated, many strains aged and became extinct. The number of the strains conserved by Yamaguchi University when the third term of the NBRP adopted *Paramecium* species in June 2012 was approximately one-tenth that at the heyday. Since then, strains conserved in foreign and domestic institutes have been deposited at Yamaguchi University. Consequently, mutant strains, syngens (genetically isolated groups), and complementary mating types of *P. caudatum* were prepared. At present, the strains of *Paramecium* species conserved in the NBRP-Paramecium are world-class bioresources in Japan.

Although 45 *Paramecium* species have been described to exist, presently, only 27 species can be collected from the field while other species are thought to have become extinct or to live secretly in exclusive environments. The 27 species include 2 species (*P. dubosqui* and *P. chlorelligerum*) that were thought to have been extinct but have been rediscovered, and a new species (*P. schewiakoffi*), which was recently collected by Fujishima et al. Yamaguchi University conserves 24 species; this number is larger than the number of species conserved in either the ATCC*5 or CCAP*6, and is the largest in the world. Yamaguchi University conserves strains in 2 different buildings, and a backup system has been established, in which important strains are conserved by multiple users in other universities. In collaboration with the Dresden University of Technology (Germany), the University of Stuttgart (Germany), the Polish Academy of Learning (Poland), and Saint Petersburg State University (Russia), Yamaguchi University distributes the conserved strains.

Many *Paramecium* species possess syngens, and complementary mating type cells exist in each syngen. When these cells are mixed under appropriate conditions, adhesion occurs between mating substances exposed on the ciliary membrane at the cytostome side, clumps of cells are formed, and mating pair formation is induced in a short time. Cell aggregation due to the gender difference between complementary mating cells is called the mating reaction, and this reaction occurs due to adhesion between mating substances in the same syngen. However, the mating reaction is not induced between mating type cells in different syngens regardless of the combination of the cells.

Mating pair formation occurs in a manner that is not specific to the mating type and syngen. For example, when the O-type and E-type, which are complementary mating types of syngen 1 in *P. caudatum*, are mixed, the mating reaction is induced and 3 types of mating pairs (O-O, O-E, and E-E) are formed after approximately 45 min at 25°C. When complementary mating type cells with genetic markers of vital staining or ciliary movement are mixed, these three types of mating pairs can be distinguished and collected; consequently, these descendants can be obtained. When cells of each complementary mating type of multiple syngens are mixed and the mating reaction is induced before mating pairs of cells of the same syngen are formed, mating pairs of cells of different syngens can be formed and these descendants can be obtained. Thus, syngens are genetically isolated groups only due to the existence of the mating reaction and are thought to be in the transient stage of species differentiation. In the future, if mating type substances are purified or their genes cloned*7, the mechanism(s) underlying the differentiation of syngens can be elucidated.

↳To the next page

*1 These codons differ from general codons that are common to many species (codon: a combination of 3 bases that determines an amino acid).
*2 This physiological phenomenon varies in cycles of approximately 24 h and it exists in almost all organisms, such as animals, plants, fungi, and algae. It is a day-and-night rhythm possessed by almost all organisms.
*3 This is a general term used to denote receptors that receive a mechanical stimulus (a physical force or deformation due to a force), convert the stimulus into an electrical signal, and transmit the signal to the central nerve. In other words, these are receptors for the senses of touch, hearing, gravity, equilibrium, pressure, tension, and vibration.
*4 These are transmembrane proteins existing in the biomembranes of cells (cell membrane, lining membrane, etc.) and a general term for proteins that are passively permeable to ions.
*5 American Type Culture Collection
*6 Culture and Collection of Algae and Protozoa
*7 Cloning is defined as creating copies of an organism possessing the same genotype. In molecular biology, cloning means to increase the amount of a specific gene: i.e., isolation of a specific gene.
*8 This is an antibody (immunoglobulin) molecule obtained from a clone derived from a single antibody-forming cell.

Since *Paramecium* species live in freshwater, they have been geographically isolated by seas. Therefore, the differentiation of syngens related to the differentiation of the continental crust is expected to be elucidated.

In *Paramecium* species, complementary mating type cells or syngens cannot be differentiated using morphology but can only be differentiated using the existence of the mating reaction. Therefore, the conservation of syngens and their complementary mating type strains is extremely important. Regarding *P. caudatum*, 16 syngens were once reported to exist. However, researchers in the USA accidentally failed to maintain these syngens. Consequently, 4 syngens (syngen numbers 1, 3, 12, and 13) conserved in Tohoku University remained as the type strains. Following this, between 1980 and 1985, Fujishima et al. collected complementary mating type cells of 4 new syngens in Japan, which differed from the 4 syngens conserved in Tohoku University. However, no methods could investigate whether these 4 newly collected syngens were parts of syngens lost in the USA. The numbers of syngens lost in the USA were then reassigned to these 4 newly collected syngens as 2, 4, 5, and 6. These 8 syngens (1, 2, 3, 4, 5, 6, 12, and 13) have been conserved in Yamaguchi University as the global type strains.

The aging of *Paramecium* species proceeds as the number of cell divisions after mating increases. However, since a practicable method for cryopreservation has not yet been developed, *Paramecium* species are conserved for a long time in such a way that the species slowly divides in a test tube kept at a low temperature (10°C) and a culture solution is added every month. Using this method, strains can be conserved for several years. However, the aging of a *Paramecium* species due to cell division cannot be avoided unless its descendant is obtained. When a *Paramecium* species is used for experiments, some of its strains that have been conserved at 10°C are cultured at 20°C− 25°C. When the cultured strains become aged, young strains conserved at 10°C are cultured again at 20°C−25°C. By repeating this procedure, strains with similar degrees of aging can be used for experiments for a long time. The life span differs according to the species. In the case of *P. caudatum*, it divides approximately 3 times a day, and its clone death occurs when the number of cell divisions after mating reaches approximately 700 (about 6 months). The life span of *P. tetraurelia*, the genome of which has been sequenced in the macronucleus, is equivalent to approximately 400 divisions. The life span of *P. bursaria* is long, which possesses symbiotic chlorella in its cytoplasm, and the number of cell divisions when it dies is still unknown. Similar to humans, the aging of *Paramecium* species proceeds as the number of cell divisions increases. Therefore, *Paramecium* species are excellent materials to study aging.

When using a particular *Paramecium* species for an experiment, its degree of aging is considered essential to reproduce the experimental results.
The NBRP-Paramecium distributes strains to researchers, the degree of aging (the number of subcultures in test tubes) of which is known.

The NBRP-Paramecium also advises users regarding the culture and experimental methods used for *Paramecium* species. The project also maintains various monoclonal antibody*8 -producing hybridomas for *Paramecium* species and their intracellular symbionts. Therefore, the project has made preparations for distributing these monoclonal antibodies. The NBRP-Paramecium management committee meets once a year in October at Yamaguchi University. At present, Associate Prof. Yuki Kodama of Shimane University is the chair of the committee, and the committee consists of 5 members from the core- and sub- facilities and 8 members from the user institutions. At the committee meeting, the activities of the core- and sub-facilities are reported, and the members from the user institutions evaluate these activities and propose ideas for improvement, which are then discussed by all the members.

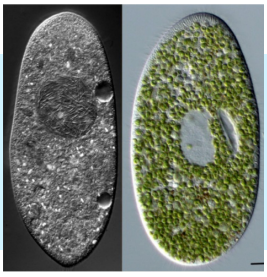

Fig. 1: *Paramecium* species possessing intracellular symbionts
Left : *P. caudatum* possessing *Holospora obtusa*, a macronucleus-specific symbiont
Right : *P. bursaria*, in the cytoplasm of which *Chlorella variabilis* coexists

References

Fujishima M., Kodama Y. Endosymbionts in *Paramecium*. *European Journal of Protistology*, 48, 124–137, 2012.

Kodama Y., Fujishima M. Chapter 2. Secondary symbiosis between *Paramecium* and *Chlorella* cells. In, "*International Review of Cell and Molecular Biology*", (Ed) Jeon K. W., Vol. 279, pp. 33–77, Elsevier Inc. San Diego, Burlington, London, Amsterdam: Academic Press, 2010.

Fujishima, M. Chapter 8. Infection and maintenance of *Holospora* species in *Paramecium caudatum*. In, "Endosymbionts in *Paramecium*". *Microbiology Monographs* vol. 12, (Ed) Fujishima M., Springer Dordrecht Heidelberg London New York, pp. 201–225. 2009.

Database of This Month

National BioResource Project "Bacillus subtilis"

Number of strains : 2,908
Number of genes : 4,423
(as of May 2013)

DB name : National BioResource Project "Bacillus subtilis"
URL : http://www.shigen.nig.ac.jp/bsub/
Language : Japanese, English
Original contents :
- Information on *Bacillus subtilis* resources for research (wild, gene-disrupted, and large-scale chromosomal deletion strains)
- Genetic information
- Various tools including GenomeViewer, BLAST, and SequenceCutter

Features :
① Various gene-disrupted strains can be ordered from the website.
② This database adopts a simple and easy-to-search system.

Cooperative DB : BSORF
DB construction group :
NBRP-Bacillus subtilis, NBRP Information
Management organization : Genetic Resource Center, NIG
Year of DB publication: 2008 Year of last DB update: 2013

Comment from a practicing developer : The NBRP-Bacillus subtilis is a relatively new database among the databases in the NBRP. Previously, we handled only strains in which a gene or a small number of genes had been disrupted. At present, we can also distribute large-scale chromosomal deletion strains. Since pages for genetic information have been added to the database, information on genes and genetic lines can be easily obtained. We are preparing to publish a genome map of large-scale chromosomal deletion strains. We will continuously enrich information on genes and genetic lines to improve the database. Please feel free to use the database and do not hesitate to express your comments, questions, or opinions.

Contact Address

Genetic Resource Center, National Institute of Genetics
1111 Yata, Mishima-shi, Shizuoka 411-8540, Japan
Tel.: 055-981-6885 (Yamazaki)
E-mail : brnews@shigen.info

Editor's Note

Some days ago, I observed real *Paramecium* species in Prof. Fujishima' s laboratory for the first time. My first impression was that the swimming motion of *Paramecium* species was faster and more elegant than I had imagined. As I listened to Prof. Fujishima, it was a wonder to hear how the *Paramecium* grew bigger and bigger in his eyes until they were as important as humans. In order to explain many unknown vital phenomena, *Paramecium* species may become essential model organisms. Resources on *Paramecium* species can be seen at the following website:
http://www.shigen.nig.ac.jp/paramecium/ (Y.Y.)

BioResource Information

(NBRP) www.nbrp.jp/
(SHIGEN) www.shigen.nig.ac.jp/
(WGR) www.shigen.nig.ac.jp/wgr/
(JGR) www.shigen.nig.ac.jp/wgr/jgr/jgrUrlList.jsp

BioResource Now !
Issue Number 9 May 2013
"translated by ASL translatiaon service and proofread by Sharoh Yip"