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Morphological properties of *Cyanidium caldarium* and related algae in Japan

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Two strains of a unicellular alga *Cyanidium caldarium* (TILDEN) GEITLER cultured in a laboratory are compared with each other concerning their structures. The cells of RK-1 strain isolated from Yumoto-spa, Nikko, Japan, are blue-green coloured and are spherical, 2.3 μm (av.) up to 5 μm in diameter, and multiply through 4 endospore formation. On the other hand, the cells of M-8 strain isolated from Noboribetsu-spa, Japan, are blue-green coloured and are spherical, 4.1 μm (av.) up to 10.5 μm in diameter, and multiply through 4, 8 and 16 endospore formation. Electron micrographs show that a cell of RK-1 strain has a nucleus, a chloroplast, a mitochondrion and many starch granules, while a cell of M-8 strain has a nucleus, a chloroplast(s), many mitochondria and a vacuole. From these results, the authors propose that RK-1 strain must belong to *Cyanidium caldarium* (TILDEN) GEITLER as identified previously elsewhere, and that M-8 strain may belong to the genus *Chroococcidiopsis* GEITLER.

Key Index Words: Cyanidium caldarium; Chroococcidiopsis *hot spring algae*; *taxonomy*; *ultrastructure*.

A unicellular alga, *Cyanidium caldarium* (TILDEN) GEITLER is widely distributed in acid hot springs (DOEMEL and BROCK 1970). This alga has been referred to various phyla, such as Cyanophyta (GEITLER and RUTTNER 1936), Chlorophyta (ALLEN 1959), Rhodophyta (HIROSE 1958) and others (FREDRICK 1971), and there is still a controversy on the subject of its systematic position. Besides this point, it has been pointed by DE LUCA and TADDEI (1970) that two algae belonging to different taxa may be mixed in the "*Cyanidium*" strain from Italian acidic fumaroles. Namely, cells of *C. caldarium* forma A are about 3.4 μm in diameter and multiply through 4 endospore formation, and the cells of *C. caldarium* forma B are about 6.6 μm in diameter and multiply through 4-32 endospore formation. The same alga as *C. caldarium* forma B was later isolated from Yellowstone (U. S. A.) and identified as *Protococcus sulphurarius* GALDIERI (DE LUCA *et al* 1979).

In the present paper, the ultrastructural features of two strains of *C. caldarium* isolated from acid hot springs in Japan are compared with each other and the taxonomical position of them is discussed. A brief report on this work was made previously (NAGASHIMA and FUKUDA 1979).

Materials and Methods

Cyanidium caldarium strain RK-1 was isolated by FUKUDA (1958) from Yumoto-spa, Nikkô, Japan. This strain has been cultured continuously in the laboratory. The strain M-8 originally isolated from the acid hot springs, Noboribetsu, Hokkaidô, Japan, was kindly provided by the algal collection of the Institute of Applied Microbiology, The University of Tokyo, Japan. RK-1 and M-8 strains were grown autotrophically for 8 days in an inorganic medium of pH 3.0 (ALLEN 1959) by shaking flasks at 35°C under fluorescent light (1500 lux). Cell numbers were counted with Thoma's haemacytometers and their sizes were measured with micrometers. For electron microscopy, the cells were fixed at 4°C for overnight in 2.5% glutaraldehyde in 0.1 M phosphate buffer of pH 7.0, then washed in the same buffer and post-fixed for 30 min in 1% $KMnO_4$ solution at room temperature. The fixed cells were washed, dehydrated and embedded in Epon 812 resin. The sections were stained in uranyl acetate and lead acetate, and examined with an Hitachi HU 125E electron microscope.

Observations

Optical microscopic observations: *C. caldarium* RK-1 cells are spherical and their sizes are 1.0 to 5.0 μm (av. 2.3 μm, N=200) in diameter, and multiply through 4 endospore formation, with an exception of two ones (Fig. 1A). A blue-green chloroplast occupies the greater part of the cell. *C. caldarium* M-8 cells are also spherical and their sizes are 2.0 to 8.5 μm (av. 4.1 μm, N=200) in diameter. In rare cases, the maximal cell size is 10.5 μm in diameter. The blue-green chloroplast is found in the cell. The cells multiply through 4, 8 and 16 endospore formation (Fig. 1B). In some cases, one or two unidentified dark particles were found to move to and fro in the cytoplasm.

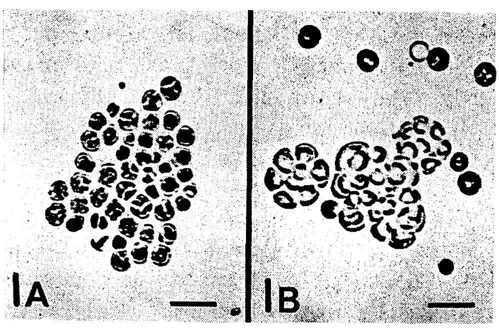

Fig. 1. Endospore formation in two strains of *Cyanidium caldarium* by optical microscopy. Scale=10 μm. A. RK-1 strain cells showing four endospores (two ones in a few cases); B. M-8 strain cells showing eight endospores.

Fine structure of C. caldarium RK-1: The mature cell shows a double layered cell wall by electron microscope (Fig. 2). When the outer cell wall is adjacent to the inner cell wall as shown in the figure, it may be the stage in the cell cycle just before the endospore formation. The outer cell wall is liable to be broken during the preparation of samples for electron microscopy (ENAMI *et al.* 1975). Only immature cells are enclosed by a single layered wall. A nucleus, a chloroplast, and starch granules are seen in the cell, but no vacuole. The chloroplast occupies a large part of the cell and contains no pyrenoid. It shows mostly many layers of single concentric thylakoids at intervals of about 0.08 μm. The chloroplast envelope looks like only one layer (Fig. 3). The nuclear envelope is obscure, but may be composed of double membranes. Only one mitochondrion is always recognized between the nucleus and the chloroplast (Figs. 3, 4). Round vesicles (0.25 μm in diameter) which may be microbodies are found near the nucleus (Fig. 4). In some cases, electron dense bodies are also found in the cytoplasm (Fig. 5). The strain which was grown in the light on agar culture containing the basal inorganic medium has many starch granules around the chloroplast (Fig. 6). Fig. 7 shows the feature that the chloroplast are ready to divide into two parts. In this stage the outer layer of the cell wall has been already formed. This chloroplast division may be followed by 4 endospore formation (Fig. 8).

Fine structure of C. caldarium M-8: Fig. 9 shows the electron micrograph of a mature cell of M-8 strain. The electron dense thick cell wall (ca. 0.07 μm in width) is not liable to be broken during the preparation of the samples. A vacuole occupies the center of the cell. In contrast to a RK-1 strain cell which has only one mitochondrion, a M-8 strain cell contains many mitochondria (Fig. 10). Several chloroplasts are apparently present in each cell (Figs. 9,

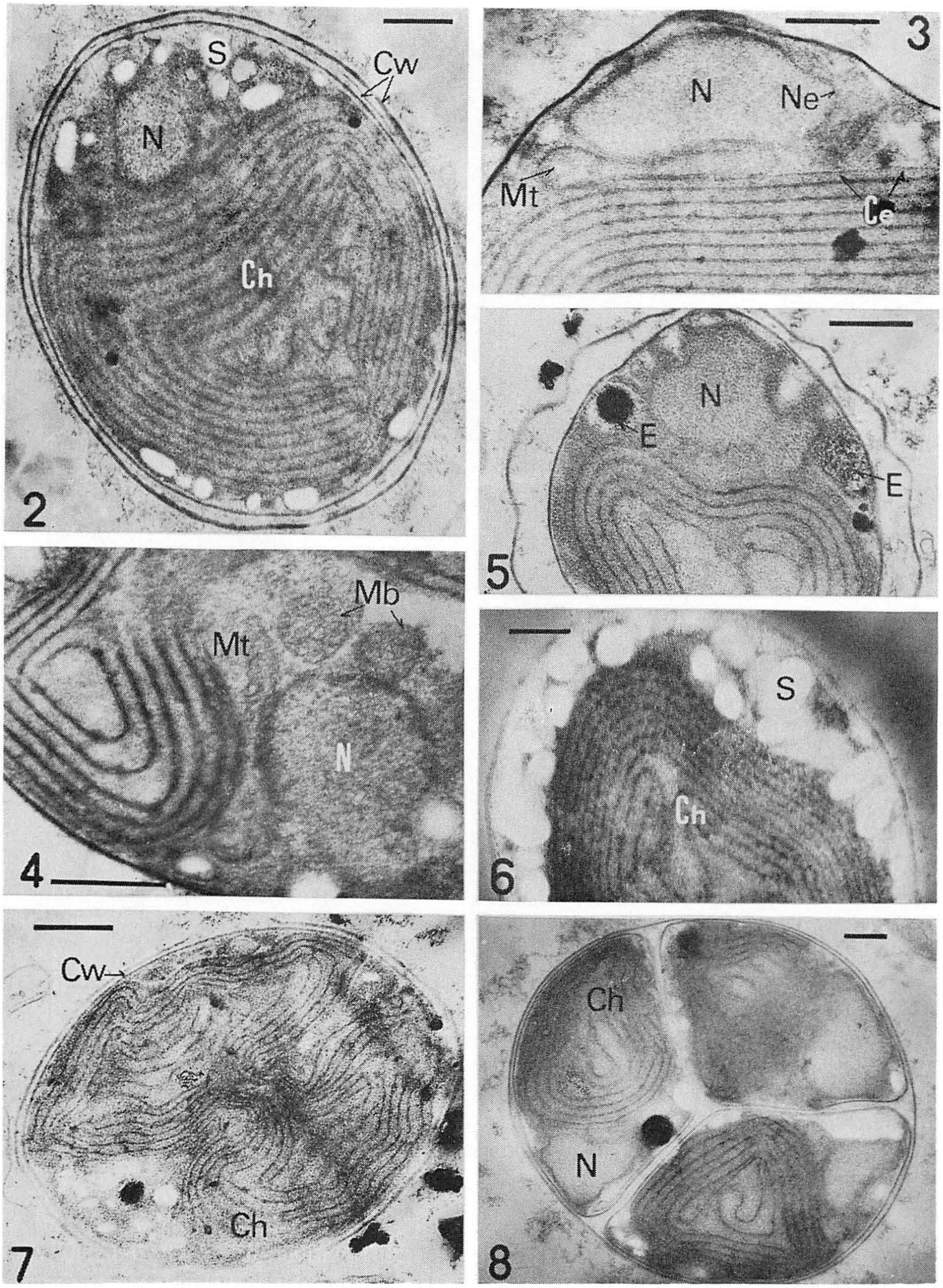

Fig. 2-8. Electron micrographs of thin sections of RK-1 strain cells. Scale=0.5 μm. 2. The mature cell is enclosed by double layered cell wall (Cw). A nucleus (N) and a well developed chloroplast (Ch) are seen. Starch granules (S) are present outside the chloroplast; 3. A single mitochondrion (Mt) is present between the nucleus (N) enclosed by a nuclear envelope (Ne) and the chloroplast whose envelope (Ce) seems to be a single membrane; 4. A nucleus (N), a mitochondrion (Mt) and two vesicles which may be microbodies (Mb) are seen; 5. Electron dense bodies (E) are deposited near the nucleus (N); 6. Many starch granules (S) are reserved outside the chloroplast (Ch) in the cell cultured on an agar plate; 7. The chloroplast (Ch) is ready to divide into two parts. The cell is enclosed by double layered cell wall (Cw); 8. Four endospores may be formed. Each spore has a chloroplast (Ch) and a nucleus (N).

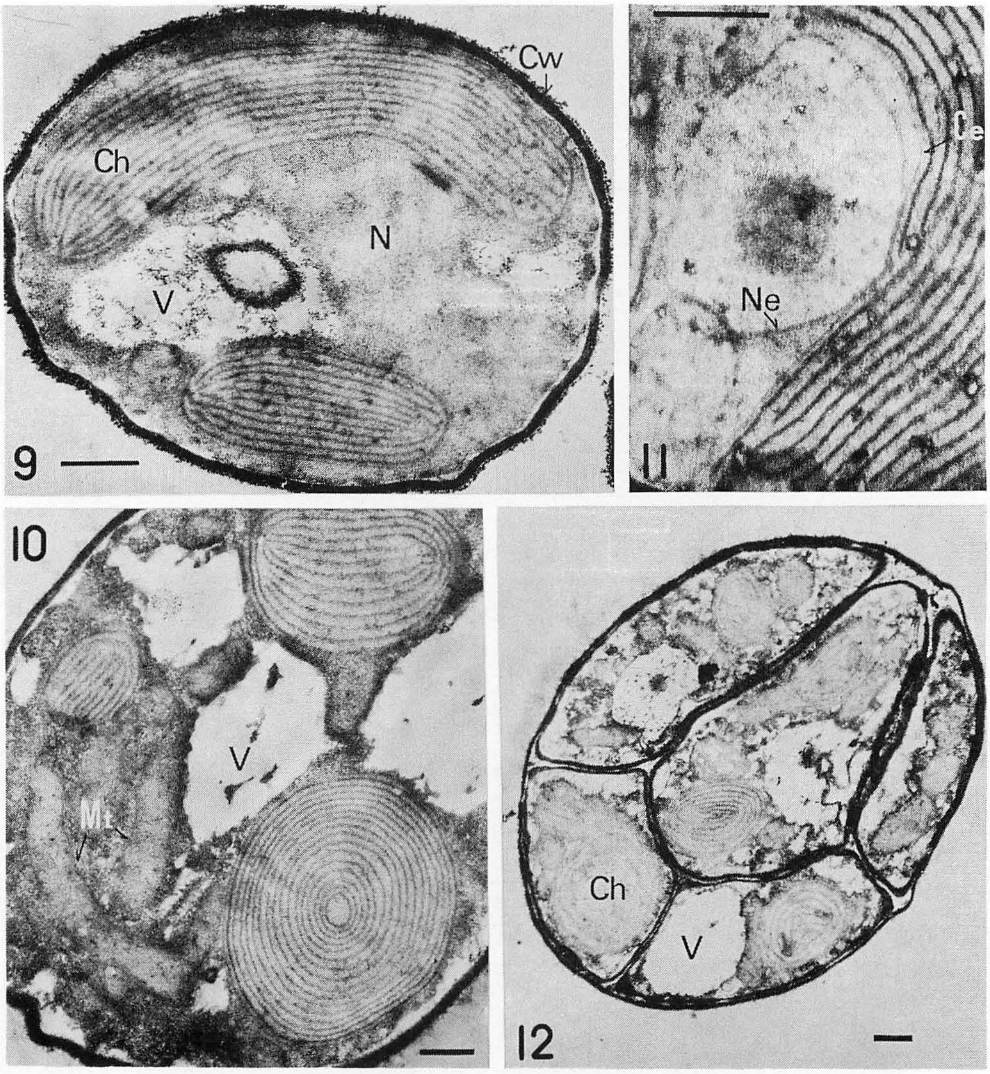

Fig. 9-12. Electron micrographs of thin sections of M-8 strain cells. Scale=0.5 μm. 9. A mature cell showing thick cell wall (Cw), chloroplasts (Ch), a nucleus (N) and a vacuole (V) ; 10. The cell showing many mitochondria (Mt), several types of chloroplasts and a vacuole (V) ; 11. The chloroplast envelope (Ce) seems to be a single membrane. A nuclear envelope (Ne) is also seen ; 12. Eight endospores may be formed in the cell. Each spore has a chloroplast (Ch) and a vacuole (V).

10). In a longitudinal section of it, parallel single thylakoids are present at intervals of about 0.09 μm and they are surrounded by a peripheral thylakoid characteristic of some red algae (BISALPUTRA 1974). In some cases, concentric arranged thylakoids are recognized. No pyrenoid is found in the chloroplast. Its envelope seems to be a single membrane (Fig. 11) as in the case of RK-1 strain. A nuclear envelope is shown in Fig. 11, but the double membrane structure of it is obscure. It is to be noted that starch granules which are contained in the cytoplasm of RK-1 strain (Figs. 2, 6) were not found anywhere within the cell of M-8 strain. The strain multiplies by 4, 8 and 16 endospore formation (Fig. 12). In the endospores vacuoles have been already formed.

Table 1. Comparison of the structure of two different strains of *Cyanidium caldarium*.

Structure	RK-1 strain	M-8 strain
Size	2.3 μm (av.)	4.1 μm (av.)
Endospore	4, (2)	4, 8 and 16
Cell wall	single or double	single-thick
Nucleus	present	present
Mitochondrion	one	many
Chloroplast	one	one (plural)*
-envelope	single membrane	single membrane
-lamella	single thylakoids	single thylakoids
Vacuole	absent	present
Starch granule	present	absent

*Plural chloroplasts are apparently present in a mature cell.

Discussion

Cyanidium caldarium RK-1 and M-8 strains isolated from the hot springs in Japan have some common properties. The two strains are, however, considerably different from each other concerning several properties as shown in Table 1. This suggests that these materials called "two strains" should be called "different species" of algae. The natures of RK-1 strain in cell colour, cell size and endospore number observed by optical microscopy are exactly consistent with those of *Cyanidium caldarium* (TILDEN) GEITLER (GEITLER and RUTTNER 1936, NEGORO 1943, HIROSE 1958). It may be, therefore, valid that strain RK-1 is so far named *Cyanidium caldarium*. The ultrastructural features of the alga are similar to those of *C. caldarium* forma A isolated from Italy (CASTALDO 1970), the small algal strains isolated from Yellowstone, U. S. A. (SECKBACH 1972, DE LUCA *et al.* 1979) and also the alga isolated from Kusatsu-spa, Japan (TÔYAMA 1980).

The facts that a chloroplast of the alga contains single thylakoids and starch granules are present out of the chloroplast resemble well the ones recognized in red algae. Moreover, floridoside which is an assimilatory product characteristic of red algae was found in the alga (NAGASHIMA and FUKUDA 1981). These facts support the idea that *Cyanidium caldarium* may belong to the Rhodophyta.

Fine structure of M-8 strain is similar to that of "*Cyanidium caldarium*" (ROSEN and SIEGESMUND 1961, MERCER *et al.* 1962, CASTALDO 1968). However, M-8 strain is clearly different from *C. caldarium*, as shown in Table 1. The present authors propose that M-8 strain must be an alga belonging to the genus different from *Cyanidium*. The structure of M-8 strain may be similar to that of *C. caldarium* forma B (DE LUCA and TADDEI 1970) and that of *Protococcus sulphurarius* (DE LUCA *et al.* 1979). However, the genus *Protococcus* belongs to the Chlorophyta to which M-8 strain does not belong clearly, because of the presence of blue-green pigments. In addition, the lamella structure of the chloroplast in the alga is quite similar to that of red algae.

The feature of M-8 strain is generally the same as *Chroococcidiopsis thermalis* GEITLER *var. nipponica* NEGORO (NEGORO 1943) in cell size, cell colour and endospore number. The genus *Chroococcidiopsis* GEITLER originally belonged in the Cyanophyta (GEITLER and RUTTNER 1936), but, recently, its assignment to the Rhodophyta has been proposed by KUMANO and HIROSE

(1977). According to this view, it may be a more suitable idea that M-8 strain should belong to the genus *Chroococcidiopsis* and that it may be one of the primitive Rhodophyta. Further studies concerning species names for it are now in progress.

References

ALLEN, M. B. 1959. Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. Archiv Mikrobiol. **32**: 270-277.

BISALPUTRA, T. 1974. Plastids, *In* W. D. P. STEWART (ed.), Algal Physiology and Biochemistry. Blackwell Sci. Pub. London. p. 124-160.

CASTALDO, R. 1968. Ricerche sull'ultrastruttura del *Cyanidium caldarium* (TILDEN) GEITLER dei Campi Flegrei (Napoli). Delpinoa **8-9**: 135-147.

CASTALDO, R. 1970. Ultrastruttura di due forme isolate dalle popolazioni di *Cyanidium caldarium* (TILDEN) GEITLER. Delpinoa **10-11**: 91-109.

DE LUCA, P. and TADDEI, R. 1970. Due alghe delle fumarole acide dei Campi Flegrei (Napoli): *Cyanidium caldarium*? Delpinoa **10-11**: 79-89.

DE LUCA, P., GAMBARDELLA, R. and MEROLA, A. 1979. Thermoacidophilic algae of north and central America. Bot. Gaz. **140**: 418-427.

DOEMEL, W. N. and BROCK, T. D. 1970. The upper temperature limit of *Cyanidium caldarium*. Archiv Mikrobiol. **72**: 326-332.

ENAMI, I., NAGASHIMA, H. and FUKUDA, I. 1975. Mechanisms of the acido- and thermo-phily of *Cyanidium caldarium* GEITLER. II. Physiological role of the cell wall. Plant & Cell Physiol. **16**: 221-231.

FREDRICK, J. F. 1971. Storage polyglucan-synthesizing isozyme patterns in the Cyanophyceae. Phytochemistry **10**: 395-398.

FUKUDA, I. 1958. Physiological studies on a thermophilic blue green alga, *Cyanidium caldarium* GEITLER. Bot. Mag., Tokyo **71**: 79-86.

GEITLER, L. and RUTTNER, F. 1936. Die Cyanophyceen der Deutschen Limnologischen Sunda-Expedition, ihre Morphologie, Systematik und Ökologie. Archiv für Hydrobiol. suppl-Bd. **14**: 371-483.

HIROSE, H. 1958. Rearrangement of the systematic position of a thermal alga, *Cyanidium caldarium*. Bot. Mag., Tokyo **71**: 347-352.

KUMANO, S. and HIROSE, H. 1977. Class Rhodophyceae, *In* H. HIROSE and T. YAMAGISHI (eds.), Illustrations of the Japanese freshwater algae. Uchidarokakuho-shinsha Tokyo. p. 157-175.

MERCER, F. V., BOGORAD, L. and MULLENS, R. 1962. Studies with *Cyanidium caldarium*. I. The fine structure and systematic position of the organism. J. Cell Biol. **13**: 393-403.

NAGASHIMA, H. and FUKUDA, I. 1979. Organotrophic properties in two strains of a hot spring alga *Cyanidium caldarium*, *In* Proceedings of the 44th annual meeting of the Botanical Society of Japan, Hiroshima, p. 176.

NAGASHIMA, H. and FUKUDA, I. 1981. Low molecular weight carbohydrates in *Cyanidium caldarium* and some related algae. Phytochemistry **20**: 439-442.

NEGORO, K. 1943. Über die Algenvegetation der Thermen von Kusatu, Gunma Präfektur, Japan. Bot. Mag., Tokyo **57**: 302-312.

ROSEN, W. G. and SIEGESMUND, K. A. 1961. Some observations on the fine structure of a thermophilic, acidophilic alga. J. Biophys. Biochem. Cytol. **9**: 910-914.

SECKBACH, J. 1972. On the fine structure of the acidophilic hot-spring alga *Cyanidium caldarium*: a taxonomic approach. Microbios **5**: 133-142.

TÔYAMA, S. 1980. Electron microscope studies on the morphogenesis of plastids. VIII. Further studies on the fine structure of *Cyanidium caldarium* with special regard to the photosynthetic apparatus. Cytologia **45**: 779-790.

長島秀行・福田育二郎：日本産イデユコゴメおよび近縁藻の形態について

日光湯元温泉産の単細胞藻イデユコゴメ *Cyanidium caldarium* (TILDEN) GEITLER RK-1 株と北海道登別温泉産の M-8 株の形態を比較した。RK-1 株の細胞は球形で平均直径 2.3 μm で，5 μm に達し，内生胞子は 4 個であるが，M-8 株の細胞の平均直径は 4.1 μm で，10.5 μm に達し，内生胞子は 4 個から 16 個まで存在する。RK-1 株は 1 個の核，葉緑体，ミトコンドリアと，ふつう多数のデンプン粒をもつが，M-8 株は 1 個の核と 1 個と思われる葉緑体の他に複数のミトコンドリアと大きな液胞をもつ。これらの結果などから，RK-1 株はこれまで通り *Cyanidium caldarium* (TILDEN) GEITLER であるが，M-8 株は *Chroococcidiopsis* GEITLER に所属すべきであろうと提案した。(162 新宿区神楽坂 1-3 東京理科大学理学部生物学教室)。