Protistology 8 (2), 71–75 (2014)

Protistology

Collection of the proteus-type amoebae at the Institute of Cytology, Russian Academy of Sciences. I. History, goals and research fields

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Summary

Amoebae Culture Collection at the Institute of Cytology RAS in St. Petersburg (Russia) – ACCIC (SPb) – was founded in 1960; it includes strains (clones) of large free living freshwater amoebae of *Amoeba proteus*-type (family Amoebidae). These protists have been extensively used as model organisms in cell biology for almost two centuries. The amoebae strains were originally received from a number of laboratories and collections of cell cultures in many countries; some strains were raised from the samples isolated directly from nature in various regions of the world. Many of the strains in the ACCIC have been thoroughly characterized. In this paper, history of the Collection and its application in different scientific research fields are briefly presented.

Key words: Amoebae, *Amoeba*, free living freshwater amoebae, strains collection

It is well known that the collections of living organisms play a key role in various modern experimental biological investigations. In addition to huge national collections – for example, such as the American Type Culture Collection (ATCC), there exist also many specialized zoological, botanical, and microbiological collections that are maintained in some scientific institutions or laboratories; the examples are: Culture Collection of Algae and Protozoa (CCAP), National Center for Marine Algae and Microbiota (NCMA), Canadian Center for the Culture of Microorganisms (CCCM), All-Russian Collection of Microorganisms (VKM), and many others. They are extremely important when different taxonomic groups of organisms are studied.

Amoebae of the *Amoeba proteus*–type are the classic biological objects, one might say, the model ones, during already about two centuries (Ord, 1973; Yudin, 1982, 1990; Jeon, 1995; Goodkov et al., 2010, etc.). At the very initial stage of usage of these lower eukaryotes for biological research, the necessity emerged to establish the collections of different amoebae species, and different strains of one species, for their certification and comparison. The proteus-type amoebae collection established and maintained in the Laboratory of Cytology of Unicellular Organisms at the Institute of Cytology, Russian Academy of Sciences is an example of such collection.

The Collection (Amoebae Cultures Collection of the Institute of Cytology at St. Petersburg –

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ACCIC) was founded in 1960 when professor Yu.M. Olenov began to develop and expand the researches that had started in the 1950-s by the group of British scientists under J.F. Danielli leadership (Danielli, 1952). The direction of these researches (the study of comparative role of nucleus and cytoplasm in cell heredity), as well as the basic method applied – the micrurgical transplantation of cell nuclei and cytoplasm (Commandon and de Fonbrune, 1939) had determined the range of cells of amoeboid protists that were selected for the Collection (Yudin, 1979, 1982, 1990). The amoebae had to be large enough, able to withstand micrurgical procedures, and may be cultured and cloned in the strictly controlled laboratory conditions to reveal interstrain hereditary differences, if any.

Among many methods described by now, the method suggested by David Prescott was at once unambiguously selected for amoebae culturing (Prescott and James, 1955; Prescott and Carrier, 1964). This method allows to multiply numerous *Amoeba proteus* strains (=clones) of different origin under the same conditions to achieve 80–100% of cloning efficiency and to obtain amoeba cells in amounts sufficient for biochemical studies without contaminations.

The same method appeared to be suitable for culturing of another species and genera of uni- and multinuclear amoebae of Amoebidae family – *Polychaos dubium* (Schaeffer, 1916), *Chaos carolinense* (Wilson, 1900), *Ch. illinoisense* (Kudo, 1950), *Ch. nobile* (Penard, 1902), *Amoeba borokensis* Kalinina, Afon'kin, Gromov, Khrebtukova et Page, 1987, *Metamoeba leningradensis* (Page et Kalinina, 1984) Friz, 1992, *Amoeba indica* (Rao, 1971), *Amoeba amazonas* (Flickinger, 1974) Friz, 1992, and numerous unidentified clones derived from the "*proteus*-like" amoebae isolated from nature.

Alongside with the cultures maintained according to the method mentioned above, the ACCIC also maintains the duplicates of these cultures which are cultivated at Prescott medium with rice seeds and mixture of ciliates *Colpidium* sp. and flagellates *Chilomonas* sp. inoculated there. Such microsystems can exist without any renewal up to 1.5–2 months, thus representing the emergency depository of strains.

All strains of the collection are subcloned from time to time; the technique of amoebae feeding applied in the ACCIC excludes the contamination of one strain by the amoebae of another strain.

Detailed description of methods and techniques used in maintaining the collection, as well as the media compositions are published in several special articles (Yudin, 1975, 1990; Kalinina and Page, 1992).

In the course of experimental work with amoebae, sometimes it is necessary to synchronize the culture – in such a culture the cells have to be at the same stage of cell cycle. To some extent, it may be achieved by use of special regime of feeding and medium replacement (Makhlin, 1993). Recently, the new reliable method to synchronize the amoebae culture was developed – the feeding by pinocytosis which results in synchronization up to 90% of the cells by their nuclear cycle, depending upon the strain and experimental conditions (Podlipaeva et al., 2013).

The amoebae strains in the collection were received at different time from various laboratories and collections of cell cultures. We greatly acknowledge Dr. M.M. Isakova-Keo (Invertebrate Zoology Department of Leningrad State University), Prof. Miklosh Müller (Medical University, Budapest, Hungary), Sister M. Taylor (Notre Dame Training College, Glasgow, Scotland), Dr. M.J. Ord (Southampton University, UK), Prof. D.M. Prescott (University of Colorado, Boulder, USA), Dr. F.C. Page (Institute of Terrestrial Ecology, Cambridge University, UK) and others who have placed their amoebae strains at our disposal.

The collection also includes many amoebae strains based on the samples isolated directly from nature in various regions of the world.

Being initially founded for genetic research purposes, the collection had served and still serves as a rich source of material for different investigations. Four main research directions based on biodiversity of the collection material are distinguished and may be conventionally designated as "genetic", "physiological", "biochemical" and "morphological" research fields.

Genetic investigations were mainly devoted to research into the comparable role of nucleus and cytoplasm. They also dealt with the role of nuclear-cytoplasmic relationships and the role of epigenetic mechanisms in cell heredity. In these works, the so-called natural (i.e. those that were not experimentally induced) hereditary differences between strains of one species – *A. proteus* – were used as genetic markers. From time to time while cultivating the strains, or in the course of experimental work with the strains, the "mutant" forms were distinguished. Such forms differed from the initial type by some inherited features (Gorjunova and Kalinina, 1977; Nikolaeva and Selivanova, 1979; Nikolaeva et al., 1979). Main results concerning nuclear-cytoplasmic relationships, cell here-

dity and variability in these obligatory agamic unicellular microorganisms were presented in the monograph by Alexander Yudin (1982).

Within the frame of the same research field, the studies were carried out to compare the DNA content in the nuclei of different amoebae strains and to study the spontaneous and induced nuclear "polyploidization". The peculiarities of DNA synthesis during cell cycle were studied by the method of cytofluorimetry (Afon'kin, 1983, 1989; Makhlin, 1987, 1993; etc.). Also, an attempt was made to apply the confocal microscopy for studying the structure of the interphase chromatin in *A. proteus* nucleus and to determine the relation between DNA hyperreplication and DNA content in nucleoli (Borisenko et al., 2010).

The search of genetic markers has resulted in data acquisition concerning natural and induced inherited variability within the *A. proteus* species. Mainly, there were the data about physiological difference between the strains belonging to this species. In particular, considerable information was obtained about dependence of various physiological activities of the cell on cultivation temperature (Sopina, 1968, 1976, 1978; etc.). Later on, the focus was transferred to biochemical characters – first of all to protein polymorphism (isoenzyme spectrum of some ferments), surface antigens, etc. (Sikora and Kalinina, 1975; Kalinina et al., 1980; Sopina, 1986, 1991, 2000; Sopina and Yudin, 1994; Podlipaeva and Yudin, 2001; etc.). Similar data were also obtained for different strains of multinuclear amoebae species *Chaos carolinense, Ch. illinoisense* and others (Sopina, 1993, 1999).

The material from the collection was used to carry out comparative morphological and ultrastructural studies of the amoebae cells of different *A. proteus* strains and related species and genera of the family Amoebidae, their interphase nuclei organization and mitotic apparatus (Gromov, 1985, 1986a, 1986b; Page, 1986). These data mainly served as a basis for modern classification of the representatives of the family Amoebidae (Page, 1986, 1988).

It is important that all the data were obtained in standard conditions identical for the amoebae forms under study, thus providing an opportunity to discriminate the genetic differences of these forms from the modifications. On the base of such complex investigations two strains from the collection – Bor (established in 1974) and Sh (established in 1967) – were distinguished and described as new species – *A. borokensis* (Kalinina et al., 1986) and *Metamoeba leningradensis* (Page and Kalinina, 1984; Friz, 1992), respectively. At present, there are some strains in the collection which are likely to have the similar destiny.

Various amoebae strains from the ACCIC were used as model objects when study the mechanisms of cell adaptation to the environmental changes, namely temperature and salinity. In the cells of all strains, the heat shock protein of 70 kDa family (so-called Hsp70, widespread in nature and known by its high chaperone activity) was revealed by the method of immunoblotting. It was shown that intact (nonstressed) cells of practically all species and strains of *Amoeba* genus contain rather high constitutive Hsp70 level; this phenomenon was presumed to be a preadaptation of these unicellular organisms to the negative environmental factors (Podlipaeva, 2001; Plekhanov et al., 2006; Podlipaeva and Goodkov, 2009; Goodkov et al., 2010).

One more research direction must be mentioned which had appeared to be possible to develop only due to availability of the unique collection of amoebae strains. It is known that in natural conditions the symbiosis between *A. proteus* (and allied species of the *Amoeba* genus) and the photosynthetic eukaryotic organisms, *Chlorella* sp. among them, have not been found. There were numerous attempts to create such an association artificially. These attempts have been made repeatedly since the very initial experimental works on symbiotic associations in protists, but all of them failed (see: Karpov, 1993). Nevertheless, the investigation of our collection has resulted in the possibility to infect experimentally some amoebae strains by *Chlorella* – the symbionts of ciliate *Paramecium bursaria* (Afon'kin and Goodkov, 1989; Karpov et al., 1991). In the cytoplasm of the Amazonas strain cells, the chlorellae can be stably saved during several years of the protists' cultivation. Such an artificially created neogenic symbiotic association bears all the main features of the typical intracellular symbiosis (Karpov, 1993; Tchistyakova et al., 1997).

Besides, the collection gives an opportunity to introduce the comparative aspect even into the particular cytological researches where the "*proteus*-like" amoebae are used only as a convenient model of a large eukaryotic cell. It concerns the research of the cell motility mechanisms (amoeboid movement), phagocytosis, temperature and salinity adaptations – and all these investigations may be carried out on standard and comparable material due to the Collection available at Institute of Cytology RAS.

Today, the ACCIC is the unique collection containing numerous strains (clones) of free living

freshwater amoebae of the family Amoebidae; many of these clones have been thoroughly characterized. Regretfully, some of the old strains have been lost, and some eliminated. However, nowadays the collection continues to enlarge by new cultures, mainly by natural isolates.

In Part II, we are going to present the full index of the amoebae strains of the ACCIC and the list of publications dealing with these strains.

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