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Forum

The protist cultural renaissance

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Protists are key players in the biosphere. Here, we provide a perspective on integrating protist culturing with omics approaches, imaging, and high-throughput single-cell manipulation strategies, concluding with actions required for a successful return of the golden age of protist culturing.

> M'exalta el nou i m'enamora el vell (I get carried away by the new and I fall in love with the old). J.V. Foix, Sol, i de dol (1947)

Protists, a hotchpotch of the eukaryotic tree of life

Protists can be defined as all eukaryotes that are not animals nor land plants, nor fungi. These abundant and omnipresent organisms represent the bulk of extant eukaryotic genetic and functional diversity [1] and account for an estimated biomass of 4 gigatons of carbon, doubling the animal biomass [2]. Consequently, they are crucial players in all ecosystems on Earth, interacting both with prokaryotes and other eukaryotes. The Last Eukaryotic Common Ancestor (LECA) was a protist. This renders protists key to unravelling the evolution of eukaryotes and the rise of their functional and morphological diversity. Protists have served for decades as model organisms of countless biological processes, exemplified by the ciliate *Tetrahymena*, which has enabled two Nobel Prize-winning discoveries [3] (Box 1). Despite their importance, our knowledge of these organisms is greatly hampered by the limited number of stable laboratory cultures. This, in combination with generally larger genomes which are thereby difficult to assemble from environmental sequencing, constrains our capacity to obtain high-quality genome sequences. In consequence, protists currently represent the main pool of unexplored genomic information within the global biome. Besides enabling access to its genome, bringing an organism into culture takes the research of its ecology and physiology to a whole new level, which, not surprisingly, will facilitate exciting discoveries.

350 years of protist culturing

Scientists have been culturing protists since the very early days of microbiology. Initial cultures were multispecies enrichments obtained by infusing pepper or straw into water. These 'protocultures' allowed Antoine Van Leeuwenhoek to observe microbes under the microscope as early as 1673. Indeed, the first microbe ever reported was a protist [4]. The most popular way of culturing protists has been, and probably still is, the enrichment method. In this approach, environmental samples are first 'enriched' with nutrients that promote cell growth. Cells of interest are then isolated either by serial dilution or single-cell picking and finally placed into a rich medium. The composition of growth media varies depending on the physiological properties of the organism and its natural environment (i.e., phototrophs, marine or freshwater phagotrophs, parasites, etc.). Antibiotics are sometimes used to suppress the growth of prokaryotes and fungi and, in rare cases, to help establish axenic cultures. However, these strategies limit our culturing abilities in several ways. First, the enrichment approach introduces biases towards certain taxa, because it selects for organisms that are ready to grow in nutrient-rich conditions [5]. Second, these isolation methods are very low throughput, in particular single-cell picking. Third, many protists require being co-cultured with other microorganisms that are essential for their growth as prey or to provide essential nutrients. Fourth, to maximise the diversity of cultured taxa, the targeted cells should preferably be organisms that are not already available in culture. Unfortunately, only well-trained taxonomists have the required knowledge for an efficient identification of protists. Because new generations of protistologists have largely shifted their interest from exploratory to experimental research, we are rapidly losing the necessary taxonomic expertise. Furthermore, the aforementioned cultivation approaches are mostly suitable for free-living protists, additional challenges arise for symbiotic protists, particularly intracellular symbionts that are adapted to live within their host cell. However, many parasitic protists are readily cultivable, as their impact on our health and economy has bolstered their study by the scientific community. These successes in parasite cultivation indicate that, if the necessary resources, humanpower, and commitment are in place, the manifold challenges of protist culturing can potentially be overcome.

An old craft that slowly evolves

The protistology community is developing and employing various approaches to solve the biases and limitations inherent in traditional culturing methods. Oligotrophic media are being developed to mimic the living conditions of dominant organisms that live in nutrient-limited environments (e.g., the open ocean), with notable results [6]. Methods that allow fast isolation of thousands of cells, such as fluorescence-activated cytometry sorting (FACS), are successfully used to increase the yield of culturing efforts [7]. Finally, the taxonomic expertise needed to isolate certain groups of organisms into culture is being substituted

Box 1. Protists' contribution to biology

The study of protists has made countless contributions to scientific knowledge, in fields ranging from biochemistry to palaeontology. Here, we highlight six prominent examples of how research on protists has enhanced our understanding of the natural world.

Biomedicine

Trypanosoma brucei (Kinetoplastea) and related trypanosomatids are highly diverse flagellates capable of parasitising invertebrate and vertebrate hosts. *T. brucei* is amenable to all forward and reverse genetic methods, and is among the best-studied protists. Almost all of its ~9000 genes have been knocked down, and subcellular localisation has been established for their protein products. Trypanosomes have been at the forefront of the discoveries of polycistronic transcription, *trans*-splicing, RNA editing, RNA interference, variant surface proteins, quorum sensing, endocytosis, drug metabolism, drug resistance, and others [11].

Biotechnology

Thraustochytrids (Stramenopiles) are a group of fungus-like protists that are common in marine systems. These organisms have acquired importance in biotechnology due to the usefulness of the secondary metabolites they produce. Among them, *Aurantiochytrium* spp. are well known for the production of pharmacology-relevant molecules, such as docosahexaenoic acid (DHA), carotene, sterols, or squalene. They can also be harnessed to sustainably produce high amounts of poly-unsaturated fatty acids (PUFAs), such as Omega-3, for use as a food complement (typically obtained from fish) [12].

Cell biology

Tetrahymena thermophila (Ciliophora) is a heterotrophic free-living protist, which can be easily grown axenically in chemically defined media. It has been used as a model organism in various fields of biology, in particular, to reveal universally conserved cellular processes. Among the most striking examples of *Tetrahymena*'s impact on cell biology are the discovery of dynein, the first known molecular motor associated with microtubules (1965); the discovery of the first histone-modifying enzyme (1996); the structure and function of telomeres (1978); and the catalytic activity of RNA (1981). The last two discoveries were awarded Nobel Prizes [3].

Ecology

Symbiodiniaceae (Dinoflagellata) are a diverse group of dinoflagellates that form a symbiotic relationship with corals that is essential for the survival of coral reefs, as well as the rich diversity of species that depend on them. Research in the past decade, based on isolated cultures, has not only revealed tremendous diversity at the species and genus levels, but also highlighted subtle interspecific differences related to ecological niches, host-range diversity, and biogeographic distribution. These findings may have important implications for coral reef restoration strategies, such as assisted genetics and experimental evolution to improve coral adaptation and resistance to bleaching [13].

Evolution

Salpingoeca rosetta (Choanoflagellatea), named for its rosette-shaped flagellate colonies, is one of the youngest protistan models. Choanoflagellates are the closest known living relatives of animals, and comparisons between choanoflagellates and animals have been used to reconstruct the earliest events in animal evolution. Analysis of its 55 Mbp genome, together with the genomes of other choanoflagellates, has also revealed a surprising complement of genes that were previously thought to be animal-specific, such as those involved in cell–cell adhesion, signalling, innate immunity, and development in animals. The study of these closest animal relatives helps us to understand the evolution and biological foundations of metazoan multicellularity [14].

Genetics

Blastocrithidia nonstop (Kinetoplastea) was initially an uncultured flagellate for which a unique genetic code was predicted from *in silico* data. This species was hypothesised to reassign all three stop codons as sense codons, challenging the basic principles of the genetic code. Only its subsequent introduction into culture allowed an in-depth insight into how departures from the canonical genetic code and the molecular mechanisms involved in the process have evolved [15].

in some cases with the use of specific stains for certain organisms, physiologies, or structures [7].

The field of protistology also profits from the most recent advances in environmental genomics. Metabarcoding and metagenomic data have revealed that there is little overlap among lineages reported as abundant in nature by molecular methods and lineages available in culture [5]. This discrepancy has guided recent efforts in strain isolation and cultivation towards lineages revealed as abundant in molecular surveys but that have eluded cultivation efforts so far. However, despite the insight gained by molecular techniques, full integration between environmental diversity, as retrieved with molecular approaches, and the cultivation of this diversity has not yet been achieved for protists. This stands in contrast to bacteriology, where culturomics [8] has become a game changer, for example in the study of the human gut microbiota. Culturomics is characterised by a combination of high-throughput isolation capacity and the quick and easy identification of isolates that allows the recovery of a broad diversity of organisms [8]. Although this approach cannot be directly adopted for protists, its philosophy should be fully embraced to push the field forward.

Protist culturomics

In contrast to bacteria, protists have a wide variety of morphological features that can be exploited by image recognition methods coupled with artificial intelligence. Consequently, this approach allows the selection of cells that markedly differ from already cultured organisms. Image-based isolation will be particularly suitable for photosynthetic microorganisms with high variability in pigment content. If coupled with cell-sorting methods – that is, FACS or microfluidics – distinct cell types can be isolated in a high-throughput and sensitive manner, in turn greatly increasing culturing capacity and efficiency (Figure 1). Microfluidics also has the potential to increase the number of testable culture conditions and to facilitate the subsampling of cultures for molecular characterization. In bacteriology, the implementation of even relatively simple culturing methods in a high-throughput manner has already been proven to be successful for cultivating previously uncultured

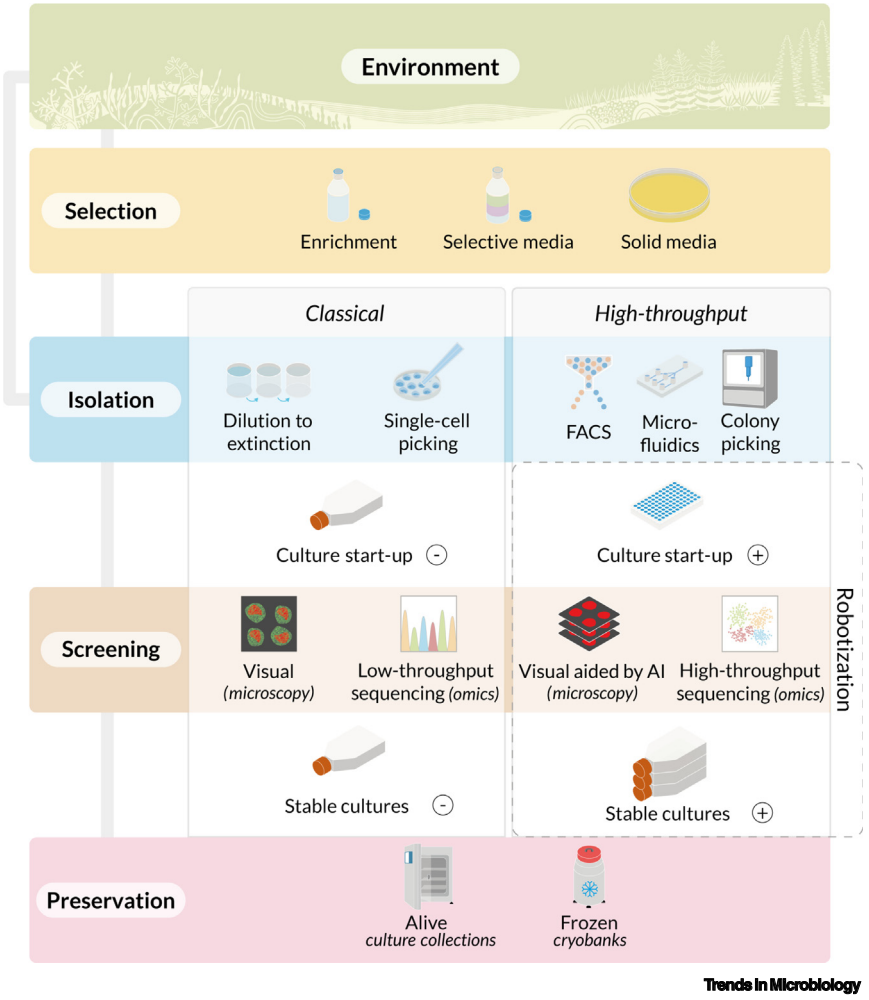

Figure 1. Classical versus innovative methods for the isolation and cultivation of protists. The classical ways of protists' culturing and the most innovative ones differ mainly in the use of high-throughput methods and automatisation which allows for increasing the number of stable cultures that we can retrieve.

marine bacterioplankton [9]. For the successful application of a culturomics approach in protistology, particular challenges in cultivation methodology need to be tackled. This includes the unification of media composition to increase reproducibility, or the creation of laboratory-scale co-culture systems to help fill the gap between single-species studies and field samples. In general, the aim of protist culturomics should be to fill the enormous gaps of available cultures that currently exist in the evolutionary tree of eukaryotes. Community efforts should focus largely on retrieving organisms that can deepen our knowledge of the evolution of eukaryotes, are ecologically relevant, have biomedical and biotechnological potential, or can serve as new models in cell and molecular biology research. However, independently of the broader scientific impact it has, every protist culture is a treasure and we must keep isolating, culturing, and preserving them all.

Culturing is a risky business, but it is worth the effort

Culturing protists is neither fast nor easy, yet the cultures that have been established to date have allowed us to dramatically expand our knowledge of the eukaryotic tree of life and the biology of the eukaryotic cell (Box 1). As pointed out above, this is because culturing facilitates access to genomic information and also allows biochemical and physiological experiments. In consequence, the availability of protists in culture has led to the discovery of new cellular compartments, processes, and metabolic capabilities that differ greatly from the textbook biology of eukaryotes that is based on currently established multicellular models and baker's yeast [10]. Protists in culture have also made critical contributions to increasing our knowledge of fundamental evolutionary events, such as the evolution of animal multicellularity, and to improving our understanding of global ecological processes and the impacts of climate change (Box 1).

Therefore, the goal of the proposed culturomics strategy is to help protists' culturing become faster and easier. We are convinced that accompanied by the necessary resources, the roadmap that we are proposing could lead to changing our current understanding of protistan ecology and evolution. As one example, the high-throughput nature of the strategy, the possibility to use multiple media in parallel, and the rapid taxonomic identification of isolates represent a good opportunity to culture one of the most abundant, widespread, diverse and elusive heterotrophic protists of the oligotrophic ocean, the 'Marine Stramenopiles' (MAST). They were described on the basis of environmental 18S rRNA gene metabarcoding data from water-column samples and also have been reported with environmental single-cell genomics [7]. Despite their abundance and prevalence, only two out of the 18 MAST lineages have cultured species. Having a representative diversity of MAST in culture will allow us to have a better understanding not only of the evolution of the Stramenopiles but, more importantly, also of the trophic dynamics of the ocean, as MAST are known to play a key role as bacterial consumers in marine ecosystems

connecting the microbial loop to upper levels of the trophic network.

Bringing back the golden age of culturing

Culturing protists has made important advances in medicine possible and has greatly improved our understanding of ecology, evolution, and cell biology (Box 1). To continue this progress we need to increase the number of protists in culture. This can be achieved by the development of new methodologies that allow high-throughput approaches for the identification, isolation, cultivation, and long-term maintenance of protists. However, methodological progress is not the only way to move forward. Equally relevant is a grassroots effort to raise public awareness of the importance of this task. Indeed, sampling and culturing protists, describing them, and their taxonomic classification have nowadays acquired a tinge of old-school faunistics. It is likely for this reason that this field of research per se is not considered cutting-edge science and thus not properly funded by research institutions and funding agencies. However, this attitude will invariably lead to losing know-how on protist culturing and taxonomic expertise, thus squandering the opportunity to bring countless interesting and potentially valuable organisms into the laboratory spotlight. For these reasons, we need to support those who have the necessary knowledge to isolate and culture protists from the environment and to train new generations of scientists who could find this field of research attractive, both intellectually and financially, so they are ready to take over and lead the protist culturing renaissance.

Declaration of interests

No interests are declared.

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References

1. Burki, F. *et al.* (2020) The new tree of eukaryotes. *Trends Ecol. Evol.* 35, 43–55
2. Bar-On, Y.M. *et al.* (2018) The biomass distribution on Earth. *Proc. Natl. Acad. Sci.* 115, 6506–6511
3. Ruehle, M.D. *et al.* (2016) *Tetrahymena* as a unicellular model eukaryote: genetic and genomic tools. *Genetics* 203, 649–665
4. Leeuwenhoek, A.V. (1997) Observations, communicated to the publisher by Mr. Antony van Leewenhoeck, in a Dutch letter of the 9th Octob. 1676. here English'd: concerning little animals by him observed in rain-well-sea- and snow water; as also in water wherein pepper had lain infused. *Philos. Trans. R. Soc. Lond.* 12, 821–831
5. del Campo, J. *et al.* (2013) Culturing bias in marine heterotrophic flagellates analyzed through seawater enrichment incubations. *Microb. Ecol.* 66, 489–499
6. del Campo, J. *et al.* (2013) Taming the smallest predators of the oceans. *ISME J.* 7, 351–358
7. del Campo, J. *et al.* (2014) The others: our biased perspective of eukaryotic genomes. *Trends Ecol. Evol.* 29, 252–259
8. Lagier, J.-C. *et al.* (2018) Culturing the human microbiota and culturomics. *Nat. Rev. Microbiol.* 16, 540–550
9. Connon, S.A. and Giovannoni, S.J. (2002) High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Appl. Environ. Microbiol.* 68, 3878–3885
10. Faktorová, D. *et al.* (2020) Genetic tool development in marine protists: emerging model organisms for experimental cell biology. *Nat. Methods* 17, 481–494
11. Lukeš, J. *et al.* (2023) Trypanosomes as a magnifying glass for cell and molecular biology. *Trends Parasitol.* 39, 902–912
12. Raghukumar, S. (2008) Thraustochytrid marine protists: production of PUFAs and other emerging technologies. *Mar. Biotechnol.* 10, 631–640
13. LaJeunesse, T.C. *et al.* (2018) Systematic revision of symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr. Biol.* 28, 2570–2580.e6
14. Booth, D.S. and King, N. (2022) The history of *Salpingoeca rosetta* as a model for reconstructing animal origins. *Curr. Top. Dev. Biol.* 147, 73–91
15. Kachale, A. *et al.* (2023) Short tRNA anticodon stem and mutant eRF1 allow stop codon reassignment. *Nature* 613, 751–758