The importance of *ex situ* preservation for the utilization of microbiological resources

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Summary

Historically, Biological Resource Centres (BRCs) have been of utmost importance for the progress of knowledge of the living world. This is a tradition that may be tracked back to the 18th century, when the first Botanic Gardens were installed in Europe. After pointing the most important landmarks in the evolution of the concept of BRCs, several new issues are identified, related with the new questions that are currently being addressed by new discoveries in the microbial world in the fields of biodiversity, environment, medicine and pharmacology. The new tools coming from Molecular Biology gave birth to new problems and readdressed old questions, pushing Microbiology to the frontiers of Science and presenting new challenges to BRCs researchers.

Keywords: Biological resource centres; Biogeochemistry; Uncultivable microorganisms; Emerging pathogens; Lateral gene transfer

Introduction

As a consequence of the planetary trade that was established after the travels of the Iberian navigators in the end of the 15th century, many exotic plants began to be imported to Europe. Among them several medicinal herbs were brought to the knowledge of European physicians and the traditional pharmacopoeia was enriched. The influence of this planetary trade is far from being totally evaluated.

Garcia da Orta was a Portuguese physician who embarked to Goa to escape from Inquisition in 1534. He lived in Goa between 1534 and 1568, the year of his death. Working as a doctor, he contacted regularly with Hindu and Arabian physicians, and learned to cultivate medicinal herbs in his garden. In 1563, he published a book entitled "Colloquies

on the Simples, Drugs and *Materia Medica* of India and Some of the Fruits Found There". By 1602 there were already 6 Editions of this book in Latin. The work was also translated to French, Italian and Spanish and became known throughout Europe.

Meanwhile, Portuguese and Spanish aristocrats populated their gardens with exotic species. Particularly famous in Portugal were the gardens of the Dukes of Palmela, and, later on, the gardens of the Royal Palace of Ajuda.

In 1560s, Jean Nicot was the French ambassador to the Portuguese Court and sent dried tobacco leaves to Catherine of Medicis. Using tobacco snuff became immediately fashioned among French aristocrats.

The name of Nicot became forever linked with the tobacco plants, giving birth to the word nicotine and to the Latin name of tobacco, *Nicotiana*. When Nicot went back to Paris, he took with him whole tobacco plants, which were eventually grown in the *Jardin du Roi*. This botanic garden was called *Jardin des Plantes* after the French Revolution and eventually gave birth to the *Musée National d'Histoire Naturelle* in 1795.

On his return to France, Jean Nicot devoted the rest of his life to humanities and is nowadays more famous in his homeland as the father of the first French Dictionary, published in 1606, than for his connection with tobacco. Whole plant trade kept on being practised all over Europe.

One century later, Carolus Linnaeus (1707-1778) was appointed in 1741 to reorganize and improve the Uppsala botanic garden, which he naturally enriched with plants brought from distant regions, many of them firstly described by Garcia da Orta. He profited from his long-term assignment to develop his monumental work on taxonomy, publishing in 1758 the book *Species Plantarum*, followed later on the book *Systema Naturae*. Botanic gardens became quite popular around Europe and were constantly enriched with species coming from all over the world.

In 1772 the Botanic Garden of Coimbra was finally put in place, with the help of the private collections established in Portugal.

Botanic gardens became real life sciences schools. Notably, the *Jardin des Plantes*, firstly led by Buffon, still with its ancient name *Jardin du Roi*, later on named *Musée National d'Histoire Naturelle* by 1795,

became a renowned school for naturalists. There worked men of the calibre of Geoffroy Saint-Hilaire, Cuvier and Lamarck.

It is worth noting that, when Humboldt (1769-1859) decided to leave on expedition to the American Continent, he visited the *Musée National d'Histoire Naturelle* in 1798, where he got acquainted with the botanist Aimé Bonpland, whom he convinced to join the expedition and became his close friend.

In the midst of the 19th century, everything seemed stable. Political and social convulsions appeared to have been eradicated. Together with the *Musée National d'Histoire Naturelle*, the Natural History Museum was definitely installed at South Kensington, London, in 1856. The Smithsonian Institution was open to visitors in 1855, in Washington, DC. And linked to these institutions, researchers were exploiting the living world mankind apparently was ruling, invested by the mighty God as Master of Nature. Darwin was already at home, after his travels around the world, painfully writing "The Origin of Species", which came to light only in 1859. As tranquillity precedes a storm, another strike on man's assurance was about to come.

The follow-up

During the 18th century Leuwenhoek (1632-1723) reported the first observations on living beings that eye could not see. Lately, Robert Hooke's *Micrographia*, issued in 1665, was a bestseller. Nevertheless, the compound microscope, invented by Hooke, served mainly to observe minerals and crystals. People did not pay attention for nearly a century to the reports on tiny beings presented by Hooke and Leuwenhoek.

It was only with Pasteur, on the one hand, and with Koch, on the other, that microscopic life was reckoned to belong to a Continent waiting to be unveiled.

Again, as had happened before with the macroscopic life and with the creation of *ex situ* preservation facilities such as Natural Museums and Botanic Gardens, the need for *ex situ* preservation became evident.

In 1925, a committee of scientists recognised the need for a collection of microorganisms to serve researchers all over the world. After a number of years spent at the McCormick Institute in Chicago, the

collection, known as ATCC (American Type Culture Collection), moved to Georgetown University, in Washington DC, in 1937.

In the 1940s, the NRRL (Northern Regional Research Laboratory) had also established its own microbial collection in Peoria. And gradually, many other microbial collections were settled around the world, some of them highly specialised, whereas others maintained a horizontal profile and kept on harbouring organisms of many different kinds.

By the end of the 20th century, it was finally recognised that all the institutions harbouring whole organisms like bacteria, archaea, fungi, phages, but also those harbouring biological material such as plasmids, or even pluricellular animals such as nematodes, should share the same basic principles regarding handling and storage, safety and security, data-bases, research, identification, supplies to third parties.

The *ex situ* collections began to be called Biological Resource Centres (BRC). As soon as BRCs realised they shared the same problems, a movement towards networking began and is still taking place. At first, the movement was national (viz. the UKNCC – United Kingdom National Culture Collections) and now is predominantly international, the first example having been the UNESCO's Microbial Resource Centres, MIRCEN.

The challenges

Today, BRCs are facing new and exciting challenges. On the one hand, molecular biology provided BRCs with powerful means for identification and for taxonomical studies.

On the other hand, the very same molecular biology raised such a number of questions that our ignorance about the microbial world soon became evident.

First, let us mention the microbial diversity. It will be enough to mention two examples. Many of us still come from the time when we were taught that, in the microbial world, viruses came first as the tiniest organisms, followed by prokaryotes and by eukaryotes, in that order.

This certainty, comforted by many and many microscopic observations, collapsed in recent years. In 1988, Montgomery and Pollak reported

the presence of an endosymbiont living in the gut of a coral reef fish, the surgeonfish, and considered it as a protist. Ten years later, the same authors, together with Bresler (1998) proved that the endosymbiont *Epulopiscium fishelsoni* was in fact a prokaryote, 600 microns long and 50 microns wide, that is, 125000 fold bigger than the "big" bacterium *Bacillus megaterium*.

Another surprise was still to come. In 1994, Courties *et al.* reported the identification of the smallest eukaryotic organism. They called it *Ostreococcus tauri*. It is a nearly spherical alga, 0.8 microns in diameter, smaller than many well-known bacteria. Even so, it was proven that it has a nucleus, with 14 linear chromosomes, one chloroplast and several mitochondria (Derelle *et al.*, 2002).

Following this work, research on pico- and nano-eukaryotes is now very active (Baldauf, 2003).

Recent reports on microorganisms living in solid rocks 1500 meters down in ancient lavas, or 3.2 kilometers deep in South African gold mines, raise the question on how ubiquitous life might be (Kerr, 2002). Even more interesting, the involvement of microorganisms of the *Geobacter* genus in the formation of magnetite was recently proven (Pennisi, 2002). Knowing how important magnetite is for the magnetic field of Earth, one may wonder how crucial this microbial activity might be for the sustainability of the terrestrial macroscopic life, since the magnetic field holds away from Earth crust most of the cosmic rays permanently bombarding our planet.

In the past 20 years, thanks to molecular biology, several human diseases were proven to be associated with microbial pathogens. These were the cases of Hepatitis C, bacillary angiomatosis, Whiple's disease, hantavirus pulmonary syndrome, Kaposi's sarcoma (Relman, 1999) and, even more recently, the Severe Acute Respiratory Syndrome, SARS (Rota *et al.*, 2003; Marra *et al.*, 2003).

Furthermore, several species of *Chlamydia* are insistently being associated with cardiovascular pathology (Fang *et al.*, 1999).

Finally, there is an extensive list of chronic inflammatory diseases, including rheumatoid arthritis, Wegener granulomatosis, diabetes mellitus and primary biliary cirrhosis with possible microbial ethiologies (Relman, 1998).

This set of examples poses quite a number of challenges to BRCs researchers.

For instance, every human being harbours *Archaea* in his intestinal gut, most of which methanogens. Why there are no known archaeal pathogens? This is an intriguing question. Indeed, since it is known that a persistently delayed faeces transit may be linked to intestinal pathologies, and since a delayed transit also enhances anaerobic populations, it should be at least plausible that some pathological states might be connected with some methanogenic species. It is possible that the absence of identification of pathogenic archaea might be explained by the absence of reliable *in vitro* cultivation methods.

The issue of uncultivable microorganisms is turning out to be recurrent. This is again a challenge to BRC's researchers. Timid progress has been made on this matter (Kaeberlein, 2002). However, either a real thrust is made on *in vitro* cultivation methods – and, concomitantly, on *in vitro* preservation – in the next few years, or the gap between the identification of extraneous genetic material in a sample, made possible by genotypic methods, and the effective isolation of the pertaining strain, will remain unfilled and may become wider.

Cultivation and preservation methods are desperately needed for protozoa, cyanobacteria, archaea, filamentous bacteria as well as for many diatoms.

It is known that several recently described pathogens are transmitted to humans from small animal reservoirs, through airborne or vector-borne routes. Such pathogens include rickettsiae, babesiae, borreliae, bartonellae, hantaviruses (Relman, 1998) and SARS-coronaviruses (Enserink, 2003).

SARS epidemics raised a number of questions. The first coronavirus was isolated in 1937 and was associated with avian infectious bronchitis. Many coronaviruses were thereafter connected with infections of cattle, pigs, and many other animals. Only in the 1960s were isolated the first two known coronaviruses that caused human cold infections.

However, in 1999, Spanish researchers showed that just 2-point mutations could alter significantly the virus entry routes into the animal hosts (Enserink, 2003).

Since some of recent epidemics and disease threats have been connected to the consumption of animal food sources – avian flu, BSE, SARS are among the more recent cases – it is all but prudent to increase surveillance on animal viruses prone to change their virulence characteristics (hosts, routes) through simple point mutations. These viruses should be closely surveyed and rapid diagnostic tests must be developed, as these viruses will be the most plausible candidates to new human infections. Again, this should be a task for BRCs researchers.

Related to this issue is gene transfer. The importance of gene transfer to evolution and virulence acquisition is nowadays completely accepted. In the 12 September 2000 issue of the *Proceedings of the National Academy of Sciences*, Reeves *et al.* showed that *Shigella* is not a genus in itself. *Shigella* appeared as a new strain when innocuous *E. coli* strains acquired genetic material that enabled them to invade intestinal cells (Zimmer, 2001).

The process of gene acquisition is so common that species-specific chromosomal regions containing virulence genes are now classed as "pathogenicity islands" (Ochman and Moran, 2001). These regions can encompass as much as 100 Kb, and their proximity to tRNA *loci* suggests they are probably being introduced via phage transfer.

Microbes can even steal genes from higher organisms. For instance, the bacterium *Deinococcus radiodurans* contains genes previously found only in plants. Other researchers found several human genes integrated in *Mycobacterium tuberculosis* (Pennisi, 1999).

All these gene transfer mechanisms, especially those that might elicit infection through new host cell-receptors or enhance the resistance to natural defences should be surveyed. A huge challenge is before those BRC researchers who deal with traditional bacterial pathogens.

Another important task has to be committed to BRCs researchers. At this moment, BRC have a gold mine waiting to be deeply exploited. Nearly all the strains stored in BRCs are still waiting to be studied as far as bioactivity is concerned.

It is well known that many non-edible mushrooms display neuro-physiologic activity. Therefore it is probable that similar properties may be found in their cousins, the filamentous fungi. Mycotoxins are likely to display many interesting bioactive features. The potential bioactivity of metabolites - immuno-inducers, chemotherapeutic drugs,

immuno-suppressors, anti-inflammatory drugs, cardio tonics, etc. – produced by the millions of strains stored in BRCs around the world remains to be uncovered. This will be the last challenge we would like to leave to BRC researchers.

As was stated by Barry Bloom in the Editorial of *Science* of May 2, 2003, "Infectious diseases do not respect national borders. One important implication of September 11, 2001, is that the security of the United States increasingly depends on expertise around the world in identifying potential health threats and in having the scientific capability to address those threats locally. In the 1980s, it took 2 years to identify HIV as the cause of AIDS. In 2003, WHO created an extraordinary network of 13 laboratories in 10 countries, including the CDC, which identified a virus associated with SARS in 2 weeks and had its entire genome sequenced in 2 more." In the globalisation era, networking must be the keyword.

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