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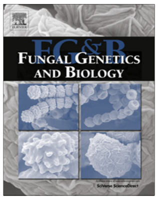

Microbial culture collections as pillars for promoting fungal diversity, conservation and exploitation

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A B S T R A C T

Fungi are a diverse group of organisms with an overall global number of 1.5 M up to 3.3 M species on Earth. Besides their ecological roles as decomposers, fungi are important in several aspects of applied research. Here, we review how culture collections may promote the knowledge on diversity, conservation and biotechnological exploitation of fungi. The impact of fungi diversity on biotechnological studies is discussed. We point out the major roles of microbial repositories, including fungal preservation, prospecting, identification, authentication and supply. A survey on the World Data Center for Microorganisms (WDCM) powered by the World Federation for Culture Collections and on the Genetic Heritage Management Council (CGEN) database revealed that 46 Brazilian culture collections registered in these databases are dedicate to preserving fungi. Most of these culture collections are located in the Southeast of Brazil. This scenario also demonstrates that Brazil has many collections focused on fungal strains, but the lack of up-to-date information in WDCM as well as of a solid national platform for culture collections registration do not allow accurate assessment of fungal preservation.

1. Introduction

Microbial culture collections vary in size form and function. They can be small, private, maintained by a single researcher and limited to a specific source/taxa. On the other hand, such collections may be extensive and be based in laboratories within large multifunctional organizations. Additionally, they may be funded by public policies with the aim to cover a wide range of microorganisms from many sources. Regardless of the size or institutional nature (public or private), they provide the resources for study, innovation and discovery (Smith, 2012).

The first recorded service culture collection was the Král Collection established in 1890 at the German University of Prague, Czech Republic (Sly et al., 1990), where cultures of fungi were made commercially available. However, the first independent center to endeavor to preserve and supply a wide range of fungi was the Centraalbureau voor Schimmelcultures (The Netherlands; CBS), established in 1904 (Hawksworth, 1985). According to Smith (2012) there are about 600 culture collections registered currently at the World Data Center for Microorganisms (WDCM) and which together they hold almost 1.8 million strains of a wide range of microorganisms (over half a million are fungi).

The use of nonconforming biological material (e.g. misidentified microorganisms and/or contaminated cultures) can cause serious problems in research as well as in industrial processes. Well-established collections can provide strains and services of high quality in agreement with the law and public policies. Service collections with high-quality operational standards are candidates to the post of Biological Resource Centres (BRCs).

Considering the tremendous developments of biotechnology and bio-economy the Organization for Economic Cooperation and Development (OECD) proposed the establishment of a Global Biological Resource Centre Network (GBRCN) (OECD, 2001), to be consolidated from service collections recognized as BRCs in their own countries. According to OECD (2007), BRCs are defined as "an essential part of the infrastructure underpinning biotechnology, consisting of service providers and repositories of the living cells, genomes of organisms, and information relating to heredity and the functions of biological systems... BRCs should meet the high standards of quality and expertise demanded by the international community of scientists and industry for the delivery of biological information and materials".

Additionally, as repositories and suppliers of microbial diversity and associated information, culture collections (and BRCs) have a relevant role in promoting the Convention on Biological Diversity, especially the Nagoya Protocol on Access and Benefit Sharing – ABS (CBD, 2011).

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2. Fungal diversity

The current magnitude of the fungal diversity is a matter of controversy. Efforts from the mycological community are now unraveling the tip of the iceberg but the actual size of this biodiversity is largely unknown (Scheffers et al., 2012). In recent years, modern taxonomic tools coupled with molecular analyses for the inventory of fungal diversity have signaled that the kingdom *Fungi* is a group of highly variable organisms in morphology, physiology, genetics and geographic distribution (Hibbett et al., 2011).

According to the 10th edition of the Dictionary of the Fungi about 100,000 fungal species were described until 2008 (Kirk et al., 2008). Although the exact number of extant fungal species is not known, mycologists have proposed several estimates to draw attention for this important group of organisms (Cannon, 1997; O'Brien et al., 2005; Schmit and Mueller, 2007; Blackwell, 2011; Bass and Richards, 2011, Bass and Richards, 2012; Mora et al., 2011). As a working hypothesis the global estimate of about 1.5 M species is generally adopted (Hawksworth, 1991). According to the author, for several reasons this figure should be considered an underestimation of the true number of extant fungal species (such an estimate does not include sampling in tropical regions or sampling of insect-associated fungi). However, after revisiting all current estimates of fungal species diversity, Hawksworth (2012) concluded that "at least 1.5, but probably as many as 3 million' be adopted for general use until some of the current uncertainties are resolved".

In fact, several uncertainties obscure the efforts towards a robust estimate of the number of fungal species in our planet. One of the major constraints that bias the estimates of missing fungal species is the problem of synonyms (Hawksworth, 1992). Due to the possibility of naming pleomorphic fungi in a dual nomenclature system (practiced for many years), names given to different morphs (teleomorph and anamorph) may correspond to the same fungal entity (the holomorph). This practice collaborated to inflate the number of described fungal species to the point that it was predicted that 65% of the published names are synonyms (Hawksworth, 1992). Fortunately, the dual nomenclature system is no longer in practice and now the quest of the mycological community is to stabilize fungal nomenclature (the one fungus = one name movement; Hawksworth et al., 2011).

Despite the wide views about fungal species estimates, all figures highlight the vast diversity of missing species to be unraveled in the kingdom *Fungi*. Blackwell (2011) pointed out that several undescribed fungal species are likely to be found in extreme and under explored environments. Thus, a tremendous task is ahead of the current and next generation of mycologists: to find and describe the missing fungal species.

Currently, the average number of fungal species described every year is about 1200 and Hibbett et al. (2011) predicted that at this rate it may take up to 4000 years to describe all the extant fungal species, considering the upper limit (5.1 M species) estimated by O'Brien et al. (2005). On the other hand, climatic changes and human activities have led to a loss of biodiversity and it was predicted that the fungal extinction rate might soon surpass the recovery rate of extant undescribed species.

Considerable impacts on the knowledge of fungal diversity come from next-generation sequencing (NGS) studies (Setaro et al., 2012). Several studies showed that NGS recovers a large number of undescribed species from environmental samples at faster rates than the discovery of new species by traditional methods (Hibbett et al., 2009, 2011). In addition, NGS has been used to assess new fungal lineages still unknown to science. One example is the recent discovery of a basal lineage of *Fungi* namely, *Cryptomycota*, known only from environmental sequences (Jones et al., 2011). Thus, molecular tools are helping mycologists to study and understand fungal diversity. To cope with the large amount of data generated from NGS approach, the urgent need therefore is to keep up the taxonomic studies of fungal cultures, to generate well-curated collections and databases that will support the NGS studies with correctly identified fungal DNA sequences.

When Brazilian biomes, especially those whose biodiversity are being endangered (for instance, the Atlantic Rain Forest, the Brazilian savannas and the Amazon Forest) are assessed with NGS, the mycological community will benefit with the putative new fungal taxa that will likely be discovered. Such studies will reinforce the need to further protect such biomes, the only way for the isolation and characterization of the missing fungal diversity. Large-scale programs to promote fungal conservation are still limited around the world but a joint international initiative has created the International Society for Fungal Conservation (www.fungal-conservation.org) with the aim to promote fungal conservation and to focus on probable endangered species.

3. Biotechnological potential of fungi

The properties of fungi have been exploited for thousands of years, mainly in brewing and baking. Fungal strains can be utilized in many social-economic areas, including the production of a wide range of commercially interesting compounds, such as enzymes, antibiotics, pigments, vitamins, alcohols, organic acids, pharmaceuticals, cosmetics, among others. Due to the fungal capacity to degrade, transform and mineralize organic chemicals, they are considered as a potential genetic resource for remediation and decontamination of environmental pollutants (Arun and Eyini, 2011; Purnomo et al., 2011; Bleve et al., 2011). Additionally, they have been used as efficient insect biocontrol agents (Bell et al., 2009; Barros et al., 2010; Balachander et al., 2013;), as protein sources (single cell protein) (Anupama, 2000) and are extremely useful in carrying out biotransformation processes, becoming essential to the fine-chemical industry in the production of single-isomer intermediates (Adrio and Demain, 2003), in oil refining industry (Borole and Ramírez-Corredores, 2006) and in the field of biofuel by the enzymatic production of simple sugars from polysaccharides (Baker et al., 2008; Octave and Thomas, 2009).

According to Borole and Ramírez-Corredores (2006), the driving forces in the life science marketplace impose a dynamics that have changed the appearance of the biotechnology industry continuously. The core activities of the business vary from basic research, commercial research and technology development, including general evaluation and testing, biology-based services, engineering services and manufacturing facilities.

The ability of yeasts and filamentous fungi to grow on rather simple and inexpensive substrates coupled with the advent of recombinant DNA technology and large scale genomics analysis has placed them in the forefront of contemporary commercial applications, highlighting the enormous impact of fungi on biotechnology (Bennett, 1998; Meyer, 2008) and resulting in the introduction of the term 'mycotechnology' (Bennett, 1998).

4. Major roles of fungal culture collections

Microbial culture collections contribute effectively in research and technological development when carrying out their main functions: screening, preservation, identification and certification of strains, making them readily available to users.

4.1. Fungal preservation

The *ex situ* preservation of microbiological resources is an indispensable practice for the knowledge and use of biodiversity. With-

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