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Maintaining fungal diversity — integration of a herbarium and living collection

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INTRODUCTION

Canadian culture collections are specialized by organism type or function. They often contain unique resources which are not duplicated in other international collections and which may have particular properties of strategic value to Canadian research. However, communication and cooperation flow within a discipline rather than within national boundaries and some of our service collections have broad mandates and sytematic expertise of importance not only to Canadian scientists but also to the global research community. There are several Canadian fungal resource centers, the two largest being the Canadian Collection of Fungus Cultures, Agriculture Canada, Ottawa and the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton [12]. These two collections are complementary since each is associated with systematics expertise on different groups of fungi. Established in 1960, the UAMH now contains more than 7300 strains of filamentous fungi and yeasts [9] which ranks it about eighth largest in the world among fungal collections. Its coverage includes all major taxonomic groups, but particular emphasis has been placed on mold fungi, especially Hyphomycetes. Fungi associated with human and animal disease are particularly well represented because the collection was originally associated with a diagnostic service for medical mycology. Medically important fungi continue to be a special interest and we often receive unusual or uncommon organisms for identification from sources worldwide. Other areas of interest include fungi related to the Onygenales and/or soil fungi associated with keratinous substrates [2,4,10], arthroconidial fungi [7,10], and specialized collections from specific habitats or ecosystems including ericaceous and orchidaceous endophytes [6,7], microflora associated with alfalfa leafcutter bees [8], and fungi ectomycorrhizal with Canadian forest trees.

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UAMH CURATORIAL PROCEDURES

The curatorial procedures in use at UAMH [5] have been developed to preserve microfungi in a manner which allows optimum use by the scientific community. The components of the collection include living cultures, herbarium specimens, records on the strain history and properties stored in an online database, and an extensive library. The maintenance of a herbarium, in which specimens are readily accessible and accurately represented, provides a useful adjunct to a culture collection since it provides a convenient mechanism for confirming the identity of strains sent for deposit or identification, and valuable information for systematic studies. We receive between 200 and 400 cultures annually, the majority for identification. Upon arrival, the fungus is grown on various types of culture media and under different growing conditions to determine optimum growth conditions for sporulation and to test for contamination. When the organism is identified, the sender is notified of its name and UAMH accession number if the strain is selected for deposit into the permanent collection. An organism sent for deposit is tested for contamination and the identification verified since redistribution of a misnamed culture can do serious damage to the reputation of the collection. Strain verification is an ongoing process beginning at the time of deposit and continuing whenever it is regrown. This process requires an enormous amount of time by staff who need to be well trained in the techniques of handling fungi and discriminating among them. Once assigned an accession number, strain data are entered into the database. Key elements in the strain file include species name, UAMH accession number, isolation details, sender's name and number, cross-reference to the same strain held in other collections, metabolites produced and literature reports (e.g. antibiotics, enzymes, toxins), other strain properties (e.g. thermophilic, human/animal pathogen, mycorrhizal), and preservation information. A variety of printed reports can be produced including a *Catalogue of Strains* [9], pages for the accession record book, inventories of preserved material, herbarium folder labels and information on strains to accompany cultures being despatched. The next step in the accession process involves growing the fungus on a cellophane membrane layered over agar [3]. When the colony is fully

developed, the cellophane with fungus growth is transferred to a specially designed drying rack. After air-drying in a biological safety cabinet, the cellophane-mounted colonies are transferred to polypropylene sleeves. Dried colonies and additional records of strain characteristics including photomicrographs, spore measurements and *camera lucida* drawings of microscopic morphology, are stored in conveniently accessible file drawers [5]. These materials allow for examination of strain characteristics without the requirement for regrowing the strain. It is well known that characteristics of a strain may change over the time it is held in a collection. Although modern methods of long-term preservation allow for good maintenance of phenotypic characters, the availability of herbarium specimens prepared at the time of deposit allow the taxonomist to monitor changes over time, to quickly determine problems of contamination, and to have ready access to strain characteristics for systematic studies. Since the majority of fungi maintained at UAMH sporulate readily in culture, most isolates have been preserved well by freeze-drying and freezing at −20 °C [1]. In 1989, we began using cryopreservation for storage of mycelial ectomycorrhizal fungi which are particularly difficult to maintain, and all strains are gradually being transferred to liquid nitrogen storage, using a straw technique modified by Stalpers et al. [11]. The use of small diameter (4 mm) polypropylene drinking straws greatly increases the capacity of the cryofreezer and reduces costs. Straws are cut into lengths of 5 cm, autoclaved and sealed at one end using a heat sealer (Audion Automaster AM400, Audion Electronic, Amsterdam, The Netherlands). Six straw vials are prepared for each strain, either by transferring 6–8 plugs from the colony of a mycelial fungus using a specially designed corkborer with pushpin, or by transferring several drops of a suspension of spores prepared in 10% glycerol as cryoprotectant. The straws are then sealed and moved to the cryofreezer. One vial is placed into a separate holding area for viability check after one week. When a straw is removed, it is thawed for 5–10 min in water warmed to 30–35 °C, one end snipped off, and the contents aseptically transferred to an appropriate medium. Fungi which cannot be recovered in viability tests following lyophilization and cryopreservation are then stored under oil, in water or by continuous subculture. Cultures are distributed worldwide to scientists.

All of these activities, in addition to consulting and advisory services, and education and training, are natural extensions of a well developed systematics program. Individual strain characterizations are the building blocks of systematics research. The carefully designed system for integrating the herbarium and culture collection at UAMH provides ready access to both specimens and cultures for evaluating 'relatedness' of a given strain, for establishing range of variation within a species, and for comparisons between species.

A culture collection is analogous to a library: it is more than a center in which stock cultures are held. While a user can simply request a culture listed in a catalogue, consultation with the collection curator may result in selection of a more appropriate strain or species. A broad collection of microbes provides the basis for study on their taxonomy, chemistry or genetics and for improving methods of propagating and preserving them, and the knowledge and experience of the curatorial staff can be drawn upon to best access this information.

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