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TAXONOMIC WORK IN A CULTURE COLLECTION OF FUNGI

By J. A. von ARX [1]

RESUMEN

Este trabajo señala las ventajas que involucra para el conocimiento taxonómico el trabajar con materiales vivos, tales como los que ofrece una colección de cultivos de hongos. El manejo de los "Normkultur" o "Hochkultur" permite una mejor delimitación de las especies, y la disponibilidal de un gran número de cepas permite someterlas a diversas condiciones de ambiente (luz, temperatura, medios, etc.). así como ensayos de apareamiento sexual, todo lo cual contribuye a componer el ciclo de vida completo.

In the classical taxonomy of fungi, the delimitation of taxa was based on morphological characters as visible by the naked eye and under the light microscope. Fresh collections or usually dried herbarium specimens were studied. For fungi growing on plants and insects it was supposed that a species was specialized to a single or a restricted number of related hosts. A fungus collected on a new host was described as a new species without comparison with similar fungi earlier described from other hosts. It was often supposed that a species growing on a leaf had to be different from a similar fungus growing on other parts of the same host plant. Different states, for example summer- and winter-forms of the same fungus, have been described under different names. The same is the case with the ascigerous and the conidial states of pleomorphic species, which have their own names. This without doubt still has practical value.

The study of dead specimens is static, based on a "snap shot", no observations being possible of the life cycle or of the variability and the influence of growing conditions. Working with material on the natural substrate, especially in microfungi, a reliable delimitation of species often proved to be impossible. The morphological characters

[1] Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

may have been influenced by the nature of the substrate (mostly a higher plant or an insect) and by external circumstances such as temperature or humidity during development.

More information became available when experimental work was carried out on living fungi growing on their respective hosts or on their natural substrates. The life cycle was discoveed by cultivation and observation on the hosts and by carrying out inoculation experiments. The pioneer in this field of study was A. de Bary (1831-1888), often called the father of mycology. Biotrophic, obligate parasitic fungi such as rusts, powdery or downy mildews, many ascomycetes and most of the basidiomycetes seen to the present day can be studied nearly exclusively on their natural substrate. Facultative parasitic and most of the saprophytic fungi, however, con also be grown in pure culture under well defined conditions. One of the first mycologists to use this method extensively was O. Brefeld (1839-1925).

Culture have the great advantage over herbarium specimens that they are living and can be subcultured and thus propagated to any desired quantity. A fungus in culture can be studied over a larger period, under well defined conditions such as medium, temperature, humidity and light, Many important fungi, especially most of these growing in soil, causing biodegradation of materials or diseases in man and animals were described from pure cultures and can be determined only after isolation. On the other hand many fungi, especially those parasitic on plants, often only form sterile mycelia in pure culture and identification is not possible. Numerous ascomycetes form only their conidial state in pure culture. In many other cases freshly isolated strains show only poor growth and poor sporulation.

For a reliable study cultures should be grown under optimal conditions, which can be realized by the choice of medium, temperature and light. Suitable media for plant-parasitic fungi are in particular cornmeal, oatmeal or potato-carrot agar; that is media containing starch and nearly no sugars. For other fungi a diversity of different media are available, many being enriched with yeast extracts. Osmophilic fungi sporulate best on media containing sugars (or salt) in high concentration. The optimal temperature for growth and sporulation has to be determined; the temperature intervals should not be more than 3°C and should cover a range from 0°–45°C. A fungus may be psychrophilic, mesophilic, thermotolerant or thermophilic. The optimal temperature and the most suitable medium should be chosen for further studies.

Cultures with good development showing typical characteristics are called NORMKULTUR, or HOCHKULTUR if they show good, typical sporulation when mature. These terms were introduced by Appel and Wollenweber (1910) in their pioneering studies on *Fusarium*, but equally apply to other fungi in culture. Hochkulturen give the most information for species delimitation. As many strains as possible should

be available for a comparative study. The characters of the colonies are described and different development states of the fungus are examined microscopically.

The overall taxonomic position of a fungus culture can easily be determined by any mycologist with some experience. Sterile or only conidia-forming strains may represent mating partners of heterothallic species of Oomycetes, Mucorales, Endomycetes, Ustomycetes, Ascomycetes or Basidiomycetes. Such strains can often only be identified by mating experiments. *Pythium sylvaticum* and *P. heterothallicum*, for example, have been proved to be very common soil fungi, but were previously only isolated as sterile *Pythium*-like mycelia. They have only been recently described by Campbell & Hendrix (1967, 1968) after mating experiments revealed the oogonia.

In many Mucorales, especially in the genus *Mucor*, a suitable species delimitation could only be achieved after extended mating experiments (Schipper, 1973, 1975, 1976). *Mucor* strains can sometimes only be identified with surety by mating them with identified partners.

Many imperfect yeasts and yeast-like hyphomycetes have been mated recently resulting in a restricted number of sexual forms belonging to the Endomycetes or Ustomycetes. New Ascomycetes, mainly Gymnoascaceae and Sordariaceae were formed in mating experiments with *Chrysosporium*-like Hyphomycetes. In matings between sterile mycelia, hyphae with clamp connections can be produced which allow the identification of the basidiomycete.

A large number of the fungi known to develop in pure culture are Fungi Imperfecti, mainly Hyphomycetes. Many are very common and wide-spread in nature and are often of economic importance. A good survey of the genera of Hyphomycetes from soil is given by Barron (1968). Extensive monographic studies of most of the larger genera are, for example, of *Fusarium* (Wollenweber & Reinking, 1935; Booth, 1971; Gerlach, 1970), *Penicillium* (Raper & Thom, 1949; Samson, Stolk & Hadlok, 1976); *Aspergillus* (Raper & Fennell, 1965), *Acremonium* (=*Cephalosporium* auct.) (Gams, 1971); *Paecilomyces* (Samson, 1974), *Phialophora* (Schol-Schwarz, 1970) or *Sporothrix* (de Hoog, 1974). Revisions of some other important genera such as *Verticillium*, *Gliocladium* and of the "black yeasts" are in preparation.

In the Coelomycetes revisions based mainly on cultural studies have been carried out by Boerema & co-workers (e.g. 1973) on *Phoma* or by van der Aa (1973) on *Phyllosticta*. As yet unpublished data show that hundreds of species described as *Phyllosticta* and *Phoma* belong to a limited number of *Phoma* species, mainly to *Phoma exigua* Desm. and *Phoma macrostoma* Mont.

In *Colletotrichum* the number of species was reduced by von Arx (1957) from approximately 800 to 12 after herbarium and cultural studies. Mainly the *Colletotrichum* conidial state of *Glomerella cingulata* was proved to have been described under several hundred names as

Gloeosporium and *Colletotrichum*. Similar comparative studies with cultural experiments would show that, for example, in *Pestalotia* (including *Pestalotiopsis* and *Truncatella*), in *Coniothyrium* (sensu Saccardo) or in the *Dothiorella-Botryodiplodia* –complex the number of distinguishable species can be reduced to a great extent.

Ascomycetes can in general best be studied from fresh collections on the natural substrate. Those fungi studied in pure culture are mainly isolated from soil or litter. Common isolates especially from soil from warmer areas, are species of *Eupenicillium* and *Talaromyces*, ascigerous states of *Penicillium* (e.g. Stolk & Samson, 1972; Scott, 1968). Other genera, studied mainly or exclusively in pure culture, are *Chaetomium* (Ames, 1963), *Melanospora* (Doguet, 1955), *Thielavia* and its relatives (Malloch & Cain, 1973; von Arx, 1975) the genera of the Microasceae (Malloch, 1970; von Arx, 1973, 1975) or *Ceratocystis* and *Ophiostoma* (e.g. Hunt, 1956; de Hoog, 1974).

Basidiomycetes in pure culture in general only develop sterile mycelia. Identification is often not possible or has to be based on the mycelial characters (Nobles, 1948, 1965).

In addition to morphological study, chemical and serological techniques often give valuable information about the taxonomy of smaller and larger groups. Staining reactions are well known tests, one of the most useful reagents still being iodine. No Ascomycetes or Basidiomycetes should be described without information on staining with Lugol or Melzer reagents because the amyloidy or dextrinoidy of ascus or spore wall are essential characters in the taxonomy of these groups.

The classification of larger groups, as proposed by Bartnicki-Garcia (1968) or Moore (1972), was based on the chemical constitution of the cell wall and on electron microscope work respectively. Consequently the yeasts will have to be excluded from the Ascomycetes and be classified in 2 separate classes: the ENDOMYCETES for the ascosporogenous yeasts with cell walls containing mainly mannan and glucan (von Arx, 1967, 1974) and the USTOMYCETES (Ustomycota sensu Moore, 1972) for ustilaginaceous (basidiomycetous) yeasts with cell walls containing mannan and chitine. The latter group is represented by genera such as *Rhodotorula*, *Sporobolomyces*, *Aessosporon*, *Rhodosporidium* on *Leucosporidium*. Chemical and serological characters can also be used to delimit genera and species and are well known in yeast taxonomy. Weijman & de Hoog (1975) recently showed that the ascomycetous genera *Ceratocystis* Ellis & Halst. (*s.str.*) and *Ophiostoma* Syd. can be distinguished not only by different conidial states, but also by the presence or absence of rhamnose in the cell wall. The two genera show similarities in the structure of their ascigerous states and in their ecology (both include ambrosia fungi and are dependent on insects for dispersal), but are not closely related. No intermediate forms between Ascomycetes and yeasts (Endomycetes)

have yet been found. *Cephaloascus fragrans* Hanawa, which was suggested by Cain (1972) to be such a form, proved to be unrelated to *Ophiostoma*, but has the same chemical composition of the cell wall as, for example, *Sacharomycopsis fibuliger* (Weijman, personal communication).

Future taxonomy cannot be based solely on morphology but also on mating experiments and on chemical, serological and submicroscopical characters. We need revisions of the described taxa, based on experimental study and reexamination of type specimens, with resulting reliable, phylogenetically natural taxa and a stable nomenclature. Such studies have to be preferably carried out with the aid of well equipped culture collections.

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