

The Vanbreuseghem Mycotheque*

by

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KEYWORDS. — Tropical Mycology ; Conservation ; Mycological Patrimony.

SUMMARY. — The “Vanbreuseghem Mycotheque”, or more familiarly the “RV Collection”, is made up of 12,500 fungi collected during the last fifty years by professor Raymond Vanbreuseghem and collaborators at the Prince Leopold Institute of Tropical Medicine in Antwerp. Two years ago, this private collection was included in the official BCCM-IHEM Fungi Collection stored in the Laboratory of Mycology of Dr Nicole Nolard at the Scientific Institute of Public Health Louis Pasteur in Brussels. At that time, the collection listed more than 72,000 reference numbers related in majority to clinical samples from all over the world. The RV Mycotheque was initiated in 1947 when R. Vanbreuseghem, coming back from Central Africa after a ten-year stay in the former Belgian Congo, decided to devote himself to medical mycology. Rich of a large experience in tropical dermatology, he first collected dermatophytes but very quickly showed interest in the tropical agents of subcutaneous or deep mycoses. Those isolates, which constitute the ancestors of our contemporary strains, have all been conserved in good condition under lyophilization and are today absolutely unique reference fungi.

MOTS-CLES. — Mycologie tropicale ; Conservation ; Patrimoine mycologique.

RESUME. — La «Mycothèque Vanbreuseghem» ou plus familièrement la «R.V. Collection» se compose de 12 500 champignons récoltés pendant les cinquante dernières années par le professeur R. Vanbreuseghem et ses collaborateurs à l'Institut de Médecine Tropicale d'Anvers. Cette collection privée a été, voici deux ans, incorporée dans la collection officielle BCCM/IHEM entreposée au Laboratoire de Mycologie du Dr Nicole Nolard à l'Institut Scientifique de Santé Publique Louis Pasteur à Bruxelles. A ce moment, on y dénombrait 72000 numéros de référence relatifs dans la plupart des cas, à des échantillons d'origine clinique en provenance du monde entier. Cette mycothèque a pris naissance en 1947 quand R. Vanbreuseghem, revenant d'Afrique Centrale, après un séjour d'une dizaine d'années au Congo Belge décida de se consacrer à la mycologie médicale. Particulièrement expérimenté dans le domaine de la dermatologie tropicale, il récolta tout d'abord des dermatophytes, mais très rapidement s'intéressa aux agents tropicaux des mycoses sous-cutanées et profondes. Ces isolats qui constituent les ancêtres de nos souches contemporaines ont été parfaitement conservés par la lyophilisation et constituent aujourd'hui un matériel unique de référence.

* Paper read at the Fifth Raymond Vanbreuseghem Conference held on 10 March 2000. Text received on 26 April 2000.

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TREFWOORDEN. — Tropische mycologie ; Bewaring ; Mycologisch patrimonium.

SAMENVATTING. — *De Vanbreuseghemmycotheek*. — De Vanbreuseghemmycotheek, beter bekend als de „RV Collectie”, bestaat uit 12 500 zwammen die door professor R. Vanbreuseghem en zijn medewerkers aan het Antwerpse Instituut voor Tropische Geneeskunde de laatste vijftig jaar ingezameld werden. Twee jaar terug werd deze privé-collectie opgenomen in de officiële BCCM/IHEM collectie van het Laboratorium voor Mycologie van Dr. Nicole Nolard van het Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur te Brussel. De collectie omvatte toen 72 000 referentienummers die hoofdzakelijk betrekking hadden op monsters van klinische oorsprong afkomstig uit de hele wereld. De RV Collectie ontstond in 1947 toen R. Vanbreuseghem, na een verblijf van een tiental jaar in Belgisch Congo, uit Centraal-Afrika terugkeerde en besliste zich te wijden aan de medische mycologie. Uitzonderlijk bedreven op het gebied van de tropische dermatologie, verzamelde hij in de eerste plaats dermatofieten, om vrij snel belangstelling te krijgen voor tropische verwekkers van subcutane of diepe mycosen. Deze afgezonderde stammen, die de voorlopers zijn van de huidige stammen, werden uitstekend bewaard door lyofilisatie en vormen vandaag absoluut uniek referentiemateriaal.

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R. Vanbreuseghem was born in 1909 in Monceau-sur-Sambre, and graduated as Doctor of Medicine at the State University of Liège in 1934. After his diploma from the Prince Leopold Institute of Tropical Medicine in Antwerp, he worked from 1935 to 1946 in the former Belgian Congo.

Until 1942, his activities were essentially dedicated to leprosy, a logical way to explain his progressive interest in dermatology and later on in medical mycology.

Back to Europe at the end of the Second World War, he was encouraged by Professor A. Dubois, director of the Institute of Tropical Medicine, to further develop his interest in medical mycology. He decided to go to Paris where he had the opportunity to work with famous mycologists such as M. Langeron and his friend E. Rivalier.

It was during his stay in Paris that he collected the first isolates of a collection of 12,145 specimens named the “Vanbreuseghem Mycotheque” or “R.V. Collection”. This huge collection was built in forty-five years, with isolates coming from 72,000 material samples : either clinical and environmental samples or also cultures received from all over the world. All the samples bore “RV” numbers and the first isolates were dermatophytes obtained in Rivalier’s laboratory.

1961 was a turning point for Belgian medical mycology. Under the suggestion of Professor P. G. Janssens, director of the Institute of Tropical Medicine, a laboratory for medical mycology headed by R. Vanbreuseghem was created and special credits were allocated to maintain and increase his collection of fungi and an annual specialized course in human and veterinary mycology was organized.

At the same time, J. Bosmans, the technician in charge of the collection, began freeze-drying the isolates. Many years were necessary to polish up the procedure and determine the best conditions to keep the isolates.

In 1973, he made a first statement showing that 80 % of the isolates were still living after ten years except for some primitive fungi such as some Chromista, for example *Saprolegnia* and *Pythium* isolates, or even some primitive Eumycota, for example *Basidiobolus* isolates.

Between 1961 and 1997, a total of 12,145 isolates were freeze-dried including 6,539 filamentous fungi, 5,499 yeasts and 107 bacteria with a majority of actinomycetes responsible for mycetoma.

In 1997, an agreement signed by Professor B. Gryseels, director of the Institute of Tropical Medicine, and Doctor G. Thiers, director of the Scientific Institute of Public Health Louis Pasteur, has officialized a scientific collaboration between both institutes and it was decided that the "R.V. Collection" would be incorporated into the official BCCM / IHEM collection stored in the laboratory of mycology of the Scientific Institute of Public Health, headed by Doctor N. Nolard.

The first group of fungi which drew the attention is the dermatophytes including 3,393 specimens collected all over the world. Most of the isolates, *i.e.* 1,716 strains, are coming from Africa which is a proof of R. Vanbreuseghem's interest in that continent. It should be mentioned that 115 strains are "test-tube" fungi as they were obtained by *in vitro* crossing of single ascospores.

Although the five genera of dermatophytes are well represented with 33 *Keratinomyces*, 61 *Epidermophyton*, 346 *Microides* isolates, the genera *Microsporum* and *Trichophyton* are predominant.

The collection of *Microsporum* includes 1,289 strains distributed among 22 different species. *Microsporum langeroni*, an anthropophilic tropical species frequently responsible for tinea capitis in Central Africa, keeps the first position, followed by *Microsporum canis*, a worldwide zoophilic species more frequently encountered in our countries. However, the biggest group of dermatophytes consists of 1,664 *Trichophyton* isolates belonging to 21 different species. Again the two most frequently represented species are two tropical anthropophilic species mostly responsible for tinea capitis: *Trichophyton violaceum* and *Trichophyton soudanense*. They are followed by another anthropophilic but worldwide dermatophyte, *Trichophyton rubrum*: 293 isolates from all over the world. The collection of dermatophytes also holds rarer specimens, for example a unique isolate of *Microsporum vanbreuseghemii* isolated from soil in New Orleans in 1961, or still a few cultures of *Trichophyton kuriangei*, which is another species isolated and described by R. Vanbreuseghem in 1961 in a small village called Kuriange, situated not far from Bujumbura. So far, this species has never been found elsewhere.

Collections are regularly "reorganized" as certain isolates sometimes need to be reclassified when new species are described. With the description of

Trichophyton raubitschekii for example, some of our tropical *T. rubrum* isolates had to be transferred to this new species. Some old species such as *Microsporium gypseum* and *Microsporium langeroni* are complexes and will probably be reclassified in the future.

Yeasts too are regularly reclassified. *Pityrosporum* or *Malassezia* species are potentially pathogenic lipophilic yeasts living on the skin of warm-blooded animals. Traditionally, they were seen as two different species, one related to man, *Pityrosporum ovale* which has recently been renamed *Malassezia furfur*, while the other, *Pityrosporum canis*, more related to animals, has been renamed *Malassezia pachydermatis*. In 1996, molecular biological techniques proved that *P. ovale* was a complex and had to be subdivided into six different taxa (GUEHO *et al.* 1996). This must be applied to the 227 *P. ovale* of the "R.V. Collection".

Trichosporon species are also concerned : indeed, there was one species traditionally called *Trichosporum cutaneum* or *Trichosporum beigelii*. But in this case again, the genus was subdivided into 19 taxa, 6 of them associated with clinical cases (GUEHO *et al.* 1992). That means that the 123 *Trichosporon* isolates of the "R.V. Collection" must be reclassified.

Another kind of problems arose with *Candida albicans*. This is an endosaprophytic yeast living in the digestive tract of mammals and birds and candidiasis of the oral mucosa is a disease recognized since antiquity. This yeast has gained renewed significance more recently as an infection often seen in AIDS patients.

Until recently, the identification of this species could be performed on the basis of morphological characteristics, *i.e.* the presence of big round spores called chlamydo-spores on special media : a cheap and quick method used to identify all the *C. albicans* isolates of the "R.V. Collection". But in 1995, D. J. Sullivan using molecular criteria described a new species named *Candida dubliniensis* which showed the same morphological characteristics as *C. albicans* (SULLIVAN *et al.* 1995).

The consequence of this is that DNA studies must be used to control the 1,055 *C. albicans* isolates of the "R.V. Collection".

Let us hope that the checking of the collection will also bring new interesting data.

We know that the oldest isolate was sampled in the United Kingdom in 1957. In 1998, F. Odds had the opportunity to check 2,489 isolates from the yeast collection of the Janssen Research Foundation in which the oldest isolates date from 1973 (ODDS *et al.* 1998). He concluded that 55 of the *C. albicans* isolates, *i.e.* 2 %, had been misidentified and were *C. dubliniensis*. New information was collected about the distribution of the new species, for example that *C. dubliniensis* could also be found in healthy persons, new information that will certainly be enhanced by the results obtained with the specimens of our "R.V. Collection" including isolates dating from the fifties.

The "R.V. Collection" also includes the largest collection of *Cryptococcus neoformans* from all over the world with 2,549 isolates. Both varieties, *neoformans* and *gattii*, are well represented.

Although cryptococcosis is a worldwide mycotic disease, most cases nowadays are associated with AIDS. This probably explains why so many isolates are coming from Central Africa where AIDS is burning : 689 isolates from Rwanda, 374 from Congo and 129 from Burundi.

The first cryptococcal cases were described at the end of the nineteenth century, the diagnosis being established by direct examination of the cerebrospinal fluid which usually shows the presence of round encapsulated yeast cells. But in the sixties, F. Gatti, a physician working in Leopoldville, isolated from the cerebrospinal fluid of a seven-year-old child a *Cr. neoformans* strain showing a particular morphology *in vivo* : a mixture of round and elongated cells (GATTI & EECKELS 1970).

This case was published in 1970, and the isolate described by R. Vanbreuseghem and M. Takashio was included in the "R.V. Collection" (VANBREUSEGHEM & TAKASHIO 1970). It was forgotten until 1975 when the discovery of two different sexual reproductions corroborated the existence of two different varieties *Filobasidiella neoformans* var. *neoformans* corresponding to the asexual variety *neoformans*, and *Filobasidiella neoformans* var. *bacillispora* corresponding to the asexual variety *gattii*.

The type-strain is still living today and has been incorporated into the new collection.

Many problems were also solved thanks to the "R.V. Collection". With the emergence of AIDS, *Cr. neoformans* isolates were tracked down for further studies, and it could, for example, be demonstrated that, from the early fifties until 1970, the variety *gattii* seemed predominant in Congo but that later this variety disappeared from that country and gave way to the variety *neoformans* which is the variety associated with AIDS.

By serotyping the African isolates, it could be demonstrated that serotype D, like the other serotypes A, B and C, could be responsible for cryptococcosis in Africa, a question that was shelved for a long time.

Cr. neoformans var. *neoformans* and var. *gattii* are both exosaprophytic yeasts. The variety *neoformans*, however, is most often recovered from soil mixed with pigeon droppings, which constitute the main source of contamination for patients. Apart from isolates from clinical cases, the "R.V. Collection" also includes 640 strains from diverse saprophytic origins and indeed also from pigeon droppings. Moreover, and this is more original, some strains were isolated in Africa from dust collected in houses of AIDS and non-AIDS-associated cryptococcosis patients. This is thus the ideal material to compare strains from patients with the isolates recovered from their environment.

Finally, the collection also includes numerous other fungi, many of them dimorphic with, for example, isolates of *Histoplasma duboisii*, the agent of

African histoplasmosis, a species described by R. Vanbreuseghem and dedicated to Professor Albert Dubois, more than one hundred *Sporothrix schenckii*, the agent of sporotrichosis, received from South American colleagues, and so on.

And if we do not have any isolate of *Rhinosporidium seeberi*, the agent of rhinosporidiosis, nor *Loboa lobo*, the agent of lobomycosis, it is only because those two organisms cannot be maintained in culture and are only known as parasites.

In conclusion, I am convinced that this collection is not only the witness of the past, but also biological material for the present and future, and that it is part of the world patrimonium.

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