Collection of Food Relevant Microscopic Fungi under the Czech National Programme of Protection of Genetic Resources of Economically Significant Microorganisms – A Short Report

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Abstract

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A unique project exists in the Czech Republic, namely the Czech National Programme of Protection of Genetic Resources of Economically Significant Microorganisms and Tiny Animals (NPPGR), which includes nineteen Czech collections of microorganisms (bacteria, fungi), viruses, and tiny animals. It is fully financed by the Ministry of Agriculture of the Czech Republic. Under this Programme, the Culture Collection of Fungi (CCF) in Prague maintains 293 fungal strains, 225 of which are food and feed relevant fungi (e.g. toxigenic *Aspergillus flavus, Penicillium verrucosum,* and *Fusarium sporotrichioides*). The main aims of the Programme are to provide adequate protection of the microbial genetic resources that are of importance to the agriculture and food industries, to provide strains (free of charge) for the research and educational purposes, and to support the cooperation between Czech and foreign institutions. A database of all microorganisms is accessible online.

Keywords: food contaminants; toxigenic fungi; Culture Collection of Fungi (CCF); fungal strains free of charge

The Culture Collection of Fungi (CCF) in Prague has existed for over 40 years. It was established in 1965 under the leadership of Dr. Olga Fassatiová by fusing the collections of the former Biological Institute of the Czechoslovak Academy of Science and of those held by the research staff of the Department of Botany, Charles University in Prague. It maintains saprotrophic microscopic fungi that have been isolated from soil, food, air, plant remains, etc. It currently contains ca 2400 fungal strains (mainly Ascomycota and Zygomycota).

In 2006, an important impetus for the Culture Collection of Fungi (CCF) came when part of the CCF was incorporated into the Czech National Programme of Protection of Genetic Resources of Economically Significant Microorganisms and Tiny Animals (NPPGR). This Programme was es-

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tablished in 1996 (Plesník & Roudná 2000) and is coordinated by the Research Institute of Crop Production, Prague-Ruzyně (Jaroslav Polák). It is fully financed by the Ministry of Agriculture of the Czech Republic. The Programme works under the legal framework of Act No. 148/2003, on the Conservation and Utilization of Plant and Microbial Genetic Resources for Food and Agriculture and Decree No. 458/2003 to the Act No. 148/2003, and according to the national strategy of the protection of biological diversity (Mládek 2006; Tošovská & Roudná 2006). At present, 19 Czech collections of microorganisms (bacteria, fungi), viruses, and tiny animals work under the Programme, all including biota of economical importance that is either beneficial or harmful for agriculture and the food industry. A central database of these organisms is accessible on the web.

The main aims of the Programme are to provide adequate protection of the microbial genetic resources that are important for agriculture, to provide strains (free of charge) for the research and educational purposes, and thus to support institutional cooperation and the exchange of information.

MATERIALS AND METHODS

At present, 293 fungal strains from the Culture Collection of Fungi (CCF) are maintained under the Czech National Programme of Protection of Genetic Resources of Economically Significant Microorganisms and Tiny Animals (NPPGR). The majority of these microfungi are food and feed relevant fungi (225 strains, 78% of fungi coming under NPPGR), and the rest are phytopathogenic fungi (12%), entomopathogenic fungi (4%), and other fungi (6%). All these fungi are unique Czech isolates. The data on their origin are accessible in the online central database of NPPGR (http://www.vurv.cz/collections/vurv.exe/search).

All fungi are preserved in tubes on slope agar media in a refrigerator at ca 5°C. Freeze-drying (lyophilisation) is applied for long-term storage. Skim milk is used as a protectant.

RESULTS AND DISCUSSION

During 2006–2009, several objectives were accomplished under the framework of the NPPGR.

Improving the pool of strains maintained under the NPPGR

To date, our fungal collection under the NPPGR contains 225 strains of food and feed relevant fungi belonging to 95 species. The majority of these microfungi are food and feed contaminants, and are often toxigenic. Several fungi cause rotting of fruits (e.g. *Penicillium expansum, P. digitatum*), and some of the strains belong to the fungi used in food technologies (e.g. *Penicillium camemberti, Botrytis cinerea*) or to those with biotechnological potential (e.g. *Monascus ruber*).

The genera *Penicillium*, *Aspergillus,* and *Fusarium* represent the most diverse groups (25 species, 12 species, and 7 species, respectively). *Aspergillus flavus*, an important toxigenic fungus, is the richest species, being represented by 35 strains isolated mainly from black pepper and black tea. Among the other toxigenic fungi are *Penicillium verrucosum* (potential producer of ochratoxin A), *Byssochlamys fulva, B. nivea, Paecilomyces variotii* and *Penicillium expansum* (patulin), *Aspergillus versicolor* and *Emericella nidulans* (sterigmatocystin*), Neosartorya fischeri* (verruculogen etc.), *Fusarium acuminatum, F. culmorum*, *F. equiseti, F. sporotrichioides* (trichothecenes), *Fusarium proliferatum* (fumonisins), and *Alternaria alternata* and *A. tenuissima* (alternariol etc.).

The fungal collection of food relevant fungi also includes a group of osmophilic fungi, which grow on substrates with low water activity, i.e. *Eurotium amstelodami, E. chevalieri, E. repens, E. rubrum, Wallemia sebi* (anamorph of *Basidiomycota*), and *Chrysosporium fastidium* (Kubátová & Muzikář 2007).

Chaetomium aureum is another interesting fungus. We maintain an isolate from fruit tea isolated by Dr. Vladimír Ostrý (Kubátová 2006b). This fungus is known from various substrates. Interestingly, it was also frequently found in soil and litter near Chernobyl and is considered a bioindicator of radionuclide-polluted soils (Zhdanova *et al.* 1995). In the Czech Republic, we also found it in the soil near the former Ralsko airport (Kubátová & Prášil 2008).

Our efforts focus on increasing the pool of fungal strains under the NPPGR and improving their quality. Old strains with a low viability will in turn be substituted; the interesting isolates will be characterised via molecular analyses.

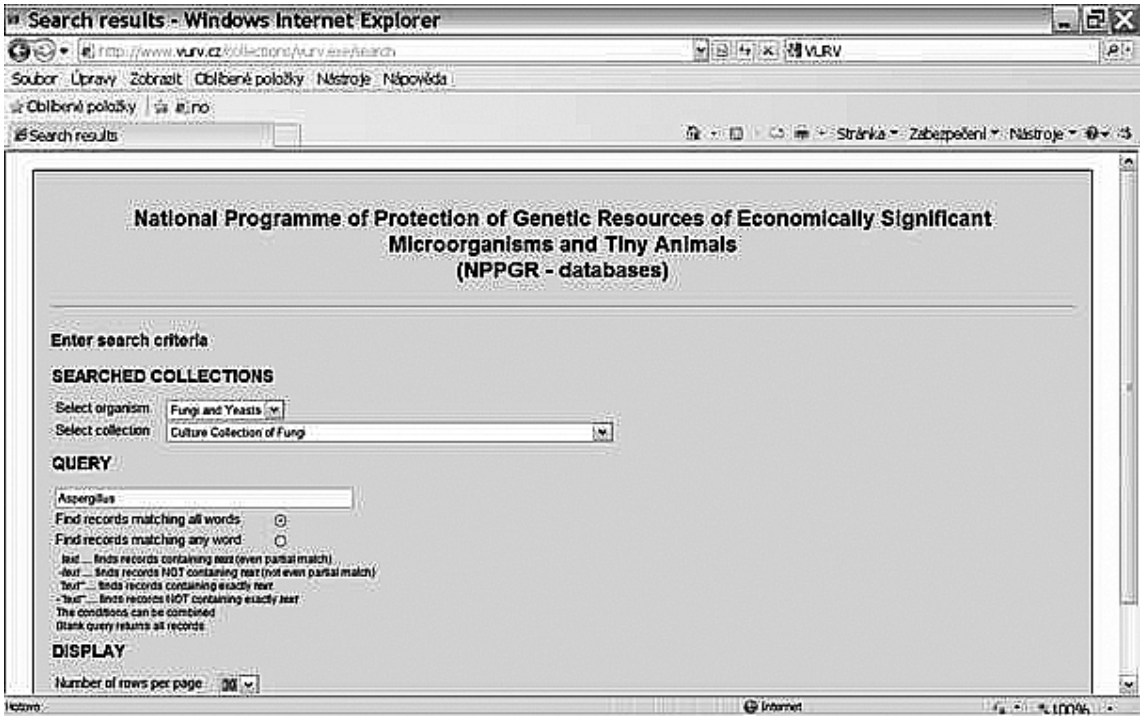

Figure 1. Online access to CCF fungal strains under "National Programme"

Improving the quality of fungal strains preservation

The majority of our food relevant fungi belong to the sporulating fungi, and therefore freeze-drying (lyophilisation) is the most suitable and relatively cheap method for long-term storage. The best and most recommended method for the storage of microorganisms is cryopreservation by liquid nitrogen (Smith & Onions 1994). Unfortunately, the optimal conditions for this method do not exist in our culture collection. In the future, we would like to test alternative methods of preservation, i.e. preservation in glycerol at –75°C in the deep-freezer, immobilisation in alginate pellets or preservation under mineral oil (Pereira & Roberts 1991; Smith & Onions 1994). These methods could at least partially substitute **subculturing on** solid agar media.

Updating the database

Data on CCF strains were input into the central database in 2006 (http://www.vurv.cz/collections/vurv.exe/search) (Figure 1). The database now contains the basic data on the 225 food and feed relevant fungal strains. The data include strains origins (substrate, locality, year of isolation, name of the specialist who isolated and determined the fungus, literature etc.), and in some cases macro- or microphotos. More detailed photographic documentation of some CCF strains is available on another website (Kubátová 2006a). Since 2007, we have been adding the data on new accessions, new literature references, and new photos. Any query is possible via the search option (Figure 1).

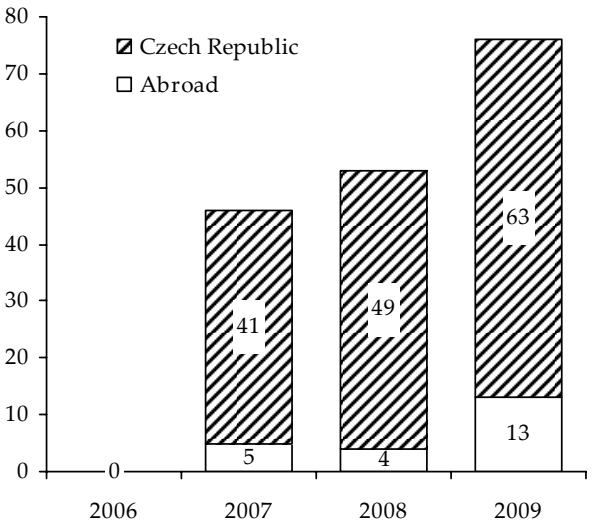

Figure 2. Growing count of CCF fungal strains sent in 2006–2009 to various institutions

Providing the fungal strains free of charge

As of 2009, the CCF has been part of the NPPGR for four years. During this time, an increasing number of requests for fungal strains has been recorded (Figure 2). To date, the CCF has received 29 requests and provided 175 strains, mainly to Czech institutions. The most frequently requested for were the following species: *Aspergillus flavus* (13 requests), *Botrytis cinerea* (8), *Aspergillus fumigatus* (6), *Penicillium verrucosum* (5), *Alternaria alternata* (5), *Aspergillus oryzae* (4), *A. tamarii* (4), and *Eurotium repens* (4). The strains were sent to universities, research institutes, laboratories, and culture collections and were used for research, education, and as reference materials. It is important that the provision of fungal cultures is free of charge for clients, being fully financed by the Ministry of Agriculture of the Czech Republic.

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