ISOLATED MICROMYCETES FROM SOME SOILS OF MOLDOVA PLATEAU

MICROMICETE IZOLATE DIN UNELE SOLURI ALE PODIȘULUI MOLDOVEI

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Abstract. *Micromycetes are distributed on a large geographical scale and have been observed in a wide range of habitats mainly located in soil and decaying vegetation. This study shows wich are the most common genera and species of micromycetes isolated and identified from soil. Soil samples were collected from different types of soil in terms of their composition and their importance in agriculture. The study shows the most common species of micromycetes occurring in the soil of mold greyic, solonchak, cambic chernozem and cambic chernozem psamic. The research led to the description of a number of ten fungal species. Six of them belong to genus Aspergillus, two to genus Penicillium, one to genus Stysanus (Stysanus medius Sacc.) and the last on to genus Verticillium (Verticillium cellulosae Dasz.). Based on the fact that many species of Aspergillus have been reported in over 250 studies that have as main topic micromycetes from soil and litter, we found wich are the most common species of Aspergillus and what other types of predominant fungi are in the different studied soils types.*
Key words: *micromycetes, soil, isolation*

Rezumat. *Micromicetele sunt distribuite pe scară geografică largă şi au fost observate în numeroase habitate, în principal soluri şi vegetația aflată în descompunere. Acest studiu arată care sunt cele mai frecvente genuri şi specii de ciuperci izolate şi identificate din sol. Probele au fost prelevate din soluri de diferite tipuri, diferențiate din punct de vedere al alcătuirii cât şi a importanței lor agricole. Studiul arată care sunt cele mai frecvente specii de fungi prezente în solurile de tip cernoziom gleic, solonceac, psamic şi cernoziom cambic. Cercetările efectuate au condus la descrierea unui număr de 10 micromicete dintre care șase speciii aparțin genului Aspergillus, două specii genului Penicillium şi speciile Stysanus medius Sacc. şi Verticillium cellulosae Dasz. Bazându-ne pe faptul că numeroase specii de Aspergillus au fost menționate în peste 250 de studii ce au ca principal subiect microfungii din sol şi litieră, am identificat care sunt cele mai frecvente specii de Aspergillus, şi ce alte genuri de fungi predomină în solurile luate în studiu.*
Cuvinte cheie*: micromicete, sol, izolare*

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INTRODUCTION

The role of micromycetes in soil is an extremely complex one and is fundamental (Suhail et al., 2006). Soil microorganisms have an important role in the decomposition of organic matter, increase nutrient uptake by some plants, improv plant resistance and their hyphae bind together the soil particles to make water-stable aggregates. Aggregates in turn create pore spaces in the soil which improves water drainage.

Studies regarding soil micromycetes are not evenly distributed worldwide (Klich, 2002). Most published studies considering the distribution of soil micromycetes from subtropical and temperate climate areas are focused on forest and cultivated soil.

The present study is an attempt to isolate and identify which species of fungi dominate some of the Moldavian Plateau soils.

MATERIAL AND METHOD

Studied soil samples were collected from Iasi-Ezareni and Vanatori, Vaslui-Rosiesti and Galați-Matca and are from the following types of soil: mold greyic, solonchak, cambic chernozem and cambic chernozem psamic

Soil samples were collected from a depth of 10-15cm after the surface plant remains have been removed. Soil samples were gathered in paper bags, by means of a metallic spatula and the used material was previously sterilized (Ulea et al., 2012).

The soil samples were preserved at 4^0C until analysis. For the isolation and determination of micromycetes from soil we have used the culture method in Petri dishes. Species isolated from soil were then transferred on Czapek medium for identification.

RESULTS AND DISCUSSIONS

During the macroscopic and microscopic investigations several species of micromycetes were isolated and identified.

1. *Aspergillus candidus* Link, in Observationes, p.16 (1809); Thom and Church, A Manual of the Aspergillus, p.157, (1926) Thom and Raper, A Manual of Aspergilli, p.207-210, Plate 6A, Figs. 59, 60 (1945); Gilman, A Manual of Soil Fungi, p.231 (1957); Raper and Fennell, The Genus Aspergillus, p.347, figs. 72, 73 (1965).

Developed micromycete on Czapek medium is white at start and after the appearance of the sporifere heads the color becomes cream but the fungus are colorless on the back. Conidiophores height is approximately of 900µm with smooth walls and thickness between 6-8µm. Terminal vesicle of the conidiophor is spherical, 25-35µm in diameter and is lined by two series of sterigme: the former has 12.5µm in height and the latter 5x2.5µm. Conidias that occur in long chains are globular, smooth and have 2.5µm in diameter.

The micromycete forms brown sclerotia with diameter by 62.5-70µm.

The micromycete was isolated from psamic soil collected from Matca-Galati and solonchak from Vanatori-Iasi.

2. *Aspergillus funiculosus* Smith, in Brit. Mycol. Soc. Trans. XXXIX, p.111-114, fig. 1 (1956); Gilman, A Manual of Soil Fungi, p.220 (1957); Raper and Fennell, The Genus Aspergillus, p.440, fig. 91, f-h (1965).

The colony isolated on Czapek medium grows with difficulty at 20-24^{0}C reaching 3cm only after 10-14 days. The colony surface is smooth and the color is olive green. The radial sporophore heads measure 300µm and are supported by smooth, colorless conidiophores, or lightly colored in pale green under the globular vesicle. It is up to 1µm in diameter and supports a single row of sterigma by 5-7.5µm, lightly colored in green-brown. The spherical conidias are rough and have a diameter by 2.5-3µm.

The micromycete was isolated from gleyic chernozem soil collected from Rosiesti-Vaslui.

3. *Aspergillus niger* van Tieghem, Ann. Sci. Nat. Botan., Ser. 5, VIII, p.240 (1867); Thom and Church, The Aspergilli, p.167-170 (1926); Thom and Raper, A Manual of Aspergilli, p.216-219, Figs. 61A, 62A (1945); Gilman, A Manual of Soil Fungi, p.228 (1957); Raper and Fennell, The Genus Aspergillus, p.309, Fig. 68, 69 (1965).

Micromycete colonies on Czapek medium held at 24-26^{0}C reach 3cm in diameter after 10 days. Mycelium is smooth, hyaline, the colony reverse is white and with many black sporophore heads. These sporophore heads are typical, globular between 700-800µm, supported by conidiophores of 1500-1520µm in height with smooth walls and 2.5µm in width. Under the sporophore head the conidiophor is lightly colored in brown. The vesicle is globular, by 50µm in diameter, with sterigme arranged in two series: the former has 30x5µm and the latter 7x3µm that eliminate 4-5µm conidia that in the beginning have smooth walls that later on become lightly rough.

The micromicete was isolated from cambic chernozem soil collected from Ezareni, Iasi.

4. *Aspergillus okazaki* Okazaki, Fungi isolated from Manitoba soil by the plate method, Canadian Jour. Res. VIII, p.253-275 (1933); Gilman, A Manual of Soil Fungi, p.232 (1957); Raper and Fennell, The Genus Aspergillus, p.350-356 (1965).

The grown colonies on Czapek medium are white, soft, with numerous sclerotia, but on the reverse the colony is yellow-sulfur. The mycelia are hyaline and from it numerous smooth conidiophores grow 2-3µm in height supporting sporophore heads up to 100µm in diameter. The vesicle is 35-40µm in diameter and has two rows of sterigme: the former 10-12µm in height and the latter 5µm. Conidias are smoot and small by1.5µm.

The micromycete was taken from Canadian soil in 1933. It is hard to separated *Aspergillus candidus* from *Aspergillus okazaki* because of the small differences in the spores size, but the color on the reverse of mature colony clearly distinguishes between them.

The micromycete was isolated from cambic chernozem from Ezareni-Iasi.

5. *Aspergillus proliferans* G. Smith, Brit. Mycol. Soc. Trans., XXVI, fig. 26, Plate III (1943); Thom and Raper, A Manual of Aspergilli, p.117 (1945); Gilman, A Manual of Soil Fungi, p.218 (1957); Raper and Fennell, The Genus Aspergillus, p.186 (1965).

In 1957 Gilman cites this micromycete as a synonym for *Aspergillus repens* (Corda) by Bary, but the last description belongs to K. Raper and Fennell Dorothy in 1965.

The colony grows with difficulty on Czapek medium at 20^0C reaching only 2-3cm in diameter two weeks after subculturing procedure. The aerian hyphae look fluffy and grow from colony up to the lid of Petri dishes. The colony is yellow-orange with the edges gray and its back is light yellow-orange. The sporophore bodies are radially arranged with sterigme proliferating branches. The conidiophores have smooth walls, 4-6µm in diameter or even wider. Occasionally, under the sporophore head we can notice bumps by 20µm. The sterigme by 8-10x3.5µm appear on a single line, often elongated. The conidias are globular or subglobular, dark colored, 5-7.5µm and echinulated.

The micromycete was isolated from psamic soil collected from Matca-Galati and solonchak from Vanatori-Iasi.

6. *Aspergillus terreus* var. *aureus*, Thom and Raper, A Manual of Aspergilli, p.198-200, fig. 57B (1945); Gilman, A Manual of Soil Fungi, p.225 (1957); Raper and Fennell, The Genus Aspergillus, p.572, Fig. 128-D (1965).

The fungal colonies hardly reach 3-4cm in diameter and they look flocculent. The colony reverse color is golden yellow. The conidiophores can reach 500µm in height; their sporophore heads are cream, with vesicles of 13-18µm supporting sterigme arranged in two series: the former 5-7x2µm and the latter of 5x1.5-2µm. The conidias have smooth walls and 1.8-2µm in diameter.

This micromycete was isolated from cambic chernozem from Ezareni-Iasi.

7. *Penicillium coryophilum* Dierckx, Essai de revision du genre Penicillium Link., Soc. Sci., Bruxelles, XXV, p.86 (1901); in Biourge, Monogr., La Cellule, XXXIII, fasc. 1, p.266-267, Pl. IX and XIV, fig. 83 (1923); Thom, The Penicillia, p.254-255 (1930); Gilman, A Manual of Soil Fungi, p.251 (1957); Raper, Thom and Fennell, A Manual of the Penicillia, p.341, figs. 90, 91 (1968).

The colonies developed on Czapek medium have 2.5-3cm in diameter; at first they are white, then white with bluish gray, soft, small, easily radiated with white edges. The colony reverse is colored in light brown. The conidiophores are 50-75x2.5µm; they have branches with metule in the whorl or on both sides, with smooth walls. The penicilii are typically biverticilate, asymmetric, with 2-3 metule of 12-20x2.5µm and fialides of 10x2µm. The conidias are subglobular, smooth with diameter of 2.2mm, arranged in short chains.

The micromycete was isolated from cambic chernozem from Ezareni, Iasi.

8. *Penicillium frequentans* Westling, Arkiv för Botanik, XI:58, p.133-134, figs. 39, 78 (1911); Biourge, Monograph., La Cellule, XXIII, fasc. 1,

p.292-293, fig. X et XVII (1923); Thom, The Penicillia, p.216-217 (1930); Gilman, A Manual of Soil Fungi, p.241 (1957); Raper and Thom, A Manual of the Penicillia, p.172, Pl. IX -D, fig. 49 (1968).

The fungal colonies grow rapidly on the medium, having about 5cm in diameter after 10-12 days of incubation at 20^0C. The colonies are gray, soft, with brown reverse at maturity. The conidiophores are 200x2.5µm and conidias of 2.5-3µm in diameter with a slightly warty surface are arising from the phialides of 10x2µm.

This micromycete was isolated from cambic chernozem from Ezareni, Iasi.

9. Stysanus medius Saccardo, Syll. ung. IV (1886); Gilman A manual of Soil Fungi, p.352 (1957).

On Czapek medium the fungus develops black colonies, that are in the begining and submerged on the surface it grows black coremii composed of hyphae of 3µm in diameter. Conidiophores have ovate spores, unicellular, of 5x3µm, arranged in divergent gray-black chains.

The micromycete was isolated from solonchak soil from Vanatori-Iasi.

10. *Verticillium cellulosae* Daszewska, Etude sur désegrégation de la cellulose dans la terra de bruyere et la tourbe, Bul. Soc. Geneva, II-4, pag.294 (1912); Gilman, A Manual of Soil fungi, p.303 (1957).

The micromycete colony developed on Czapek medium is white, but becomes dark green at maturity. Mycelium has 5µm in diameter. Three phialides in whorls apper on conidiophores and they measure 6x3µm and eliminate the conidias that are forming sporophore heads of 18-20µm in diameter. The dispersed or associated green conidias are slightly rough by 5x4µm.

The micromycete was isolated from cambic chernozem from Ezareni-Iasi.

CONCLUSIONS

1. From cambic chernozem, a subtype of soil that contains "calcium mull", the following species of micromycetes: *Aspergillus niger*, *Aspergillus okazaki*, *Aspergillus terreus* var. *aureus*, *Penicillium coryophilum*, *Penicillium frequentans* and *Verticillium cellulosae* were isolated and identified.

2. The solonchak soils have very low humus content with a neutral or low alkaline reaction and from this type of soil *Aspergillus candidus*, *Aspergillus proliferans* and *Stysanus medius* were isolated and identified.

3. Both species *Aspergillus candidus* and *Aspergillus proliferans* were isolated and identified from psamic soil. From gleyic chernozem the species *Aspergillus funiculosus* was identified.

4. The micromycetes presence in these soils is not necessarily an indicator of soil quality, although it is known that the cambic chernozem soil is very high in quality, but rather shows that the micromycetes tolerate variations in soil reaction.

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