# Quantitative composition and taxonomic structure of endophytes of agricultural crops

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**Abstract.** Various vegetative organs of the studied crops yielded 320 isolates of cultivated forms of micromycetes, with a predominance of filamentous fungi (249 strains). The level of colonization by fungi varied widely, ranging from 6 to 40%. This indicator was lower for yeast, ranging from 2 to 12%. The isolation ratio of fungal strains ranged from 0.08 to 0.54, while yeast strains ranged from 0.02 to 0.2. The taxonomic composition of endophytic micromycetes complexes in the studied agrocultures included 7 genera of filamentous fungi (Penicillium, Aspergillus, Fusarium, Trichoderma, Beauveria, Metarhizium, and Cladosporium) and 4 genera of yeasts and yeast-like fungi (Rhodotorula, Aureobasidium, Metschnikowia, and Cryptococcus). The greatest taxonomic diversity was observed in soybean and sweet clover. Barley and sainfoin also exhibited considerable diversity. The quantitative composition and taxonomic structure of endophytes are largely influenced by species, physiological state and stage of development of the host plant, type of plant tissues and organs, and environmental conditions.

## 1 Introduction

Endophytic filamentous fungi and yeasts play an important role in plant development and influence their physiological and biochemical processes. Due to the production of phytohormones, volatile organic substances, vitamins, antimicrobial metabolites, alkaloids and other compounds by endophytic micromycetes, growth and development of plants are improved, their resistance to infections and unfavourable conditions is increased, and protection of crops from insect pests and, to a certain extent, from mammalian herbivores is ensured [1, 2]. A number of authors have demonstrated the effectiveness of endophytic micromycetes in case of such abiotic stresses as drought, soil salinity, extreme temperatures, as well as soil contamination with toxic organic pollutants and heavy metals (HM). In addition, some endophytes are capable of converting hard-to-access potassium and phosphorus compounds into forms easily digestible for plants [3, 4].

Endophytic filamentous fungi and yeasts can colonise vegetative and generative organs of plants: leaves, stems, roots, seeds, fruits, buds, and flowers. Endophytes are present both within the intercellular space, where they feed on apoplastic substances, and within the cells of plant tissues, as well as in the vascular system [1].

Micromycetes colonise the endosphere *de novo* from the environment (horizontal spread) or are transmitted vertically to subsequent generations from seed to seed. The mode

of colonisation is influenced by ecological and evolutionary relationship factors. The vast majority of endophytic filamentous fungi and yeasts penetrate into plants from the soil through the root system. Another part of endophytes, which infest aboveground plant organs, penetrates mainly through stomata, lenticels, hydatodes, microdamage of cuticle and epidermis. Some representatives of micromycetes secrete lipolytic and pectolytic enzymes, with the help of which they locally hydrolyse the cuticle and thus penetrate into the internal tissues of plants [5]. Endophytic filamentous fungi can enter the plant by means of appressoria and haustoria and subsequently spread through the xylem vessels or through the intercellular space. Unlike filamentous fungi, yeasts are unable to spread by apical movement through the intercellular space [5].

The highest abundance of endophytic micromycetes is observed in roots, and there is a tendency for their number to decrease from the underground part of the plant to leaves and other aboveground organs [6, 7]. The taxonomic diversity of micromycete endophytes depends not only on the species but also on the variety of agricultural crop, and the density of infestation depends on the type of plant tissue, phenological phase of development and physiological state of the agroculture, and environmental factors. In agrocenoses, the application of mineral and organic fertilisers is an important form of influence on the colonisation of crops by endophytic micromycetes. It has been shown that long-term application of nitrogen fertilisers reduces the concentration of sucrose used by endophytic filamentous fungi and yeasts, due to which their penetration into plants is impaired [8].

A number of authors have shown that in dicotyledonous plants phytohormones and hormone-like compounds (salicylic acid, ethylene, jasmonic acid) limit colonisation by endophytic micromycetes. Antimicrobial peptides synthesised by some agricultural plants, such as rice and maize, reduce the degree of endophytic colonisation of these plants [9, 10].

The aim of this work was to study the composition of fungal community structure in an agrocultural system.

## 2 Materials and methods

The material of the study was microbial strains isolated from 7 agricultural crops: Soybean (*Glycine max*), Barley (*Hordeum vulgare*), Alfalfa (*Medicago sativa*), Rapeseed (*Brassica napus*), Safflower (*Carthamus tinctorius*), Sweet clover (*Melilotus officinalis*), Sainfoin (*Onobrychis viciifolia*).

Place of collection of plants and soil samples: Almaty region, private agro-industrial firm "Turgen" (43°27′N 77°34′E, 817 m above sea level). According to the international classification all investigated soil samples are classified as light loamy dark chestnut soils. The isolation of endophytic micromycetes from plant organs (roots, stems and leaves) was carried out by fragment seeding [11] using potato dextrose agar (PDA). Twenty mature leaves, five mature stems and five main roots were obtained from each plant species except barley from which ten leaves were collected. The collected plant organs were washed under running tap water and then cut into 0.5 cm fragments (50 pieces per plant organ of each species). The surface of these fragments was sterilised by immersion in 70% ethanol for 1 min and 3% sodium hypochlorite for 2 min, followed by washing in sterile distilled water for 2 min to remove epiphytic microorganisms. Sterilised fragments were placed in Petri dishes containing PDA supplemented with 50 mg/L streptomycin and 50 mg/L tetracycline to avoid contamination by endophytic bacteria. Cultures were incubated at 25 °C for 14 days and checked daily. To check the surface sterilisation of the plant fragments, 100 µl of water used for the final wash was also seeded on PDA.

Quantification of endophytic micromycetes was performed according to the following indicators: number of isolates, colonisation level and isolation rate. Colonisation rate was defined as the number of fragments of plant samples colonised by one or more endophytic

micromycetes divided by the total number of incubated fragments, expressed as a percentage. The endophyte isolation rate was calculated as the number of isolates obtained from plant fragments divided by the total number of fragments [11].

The taxonomic structure of micromycete communities was assessed by the relative abundance of representatives of different genera. The relative abundance of a genus was defined as the ratio of colonies of a given genus to the total number of colonies in a given variant, expressed in per cent [12].

## 3 Results

From various vegetative organs of the studied crops 320 isolates of cultivated forms of micromycetes with predominance of filamentous fungi (249 strains) were isolated. The level of colonisation by fungi varied in a wide range from 6 to 40%. This indicator for yeast was much lower (2 to 12 %). The isolation ratio of fungal strains ranged from 0.08 to 0.54, yeast strains from 0.02 to 0.2 (Table 1).

The highest colonisation by endophytic mycoflora was characteristic of sainfoin, soybean and alfalfa, the lowest - for sweet clover (Table 1). This may be due to the specific for each plant species composition of nutrient resources required for the development of endophytic microorganisms.

**Table 1.** Distribution of endophytic micromycetes in different organs of crops.

| Agroculture | Filamentous fungi | | | Yeasts | | |
|---|---|---|---|---|---|---|
| | Number of isolates | Level of colonisation, % | Isolation rate | Number of isolates | Level of colonisation, % | Isolation rate |
| Soybean | | | | | | |
| root | 23 | 40 | 0.46 | 10 | 12 | 0.2 |
| stem | 11 | 16 | 0.22 | 5 | 8 | 0.1 |
| leaves | 8 | 10 | 0.16 | 4 | 6 | 0.08 |
| Barley | | | | | | |
| root | 19 | 24 | 0.38 | 7 | 8 | 0.14 |
| stem | 7 | 10 | 0.14 | 3 | 4 | 0.06 |
| leaves | 6 | 6 | 0.12 | 1 | 2 | 0.02 |
| Alfalfa | | | | | | |
| root | 21 | 36 | 0.42 | 7 | 10 | 0.14 |
| stem | 10 | 16 | 0.2 | 4 | 6 | 0.08 |
| leaves | 5 | 6 | 0.1 | 1 | 2 | 0.02 |
| Rapeseed | | | | | | |
| root | 20 | 30 | 0.4 | 3 | 4 | 0.06 |
| stem | 5 | 8 | 0.1 | 1 | 2 | 0.02 |
| leaves | 7 | 12 | 0.14 | 1 | 2 | 0.02 |
| Safflower | | | | | | |
| root | 17 | 28 | 0.34 | 4 | 6 | 0.08 |
| stem | 8 | 10 | 0.16 | 2 | 4 | 0.04 |
| leaves | 8 | 14 | 0.16 | 2 | 4 | 0.04 |
| Sweet clover | | | | | | |
| root | 13 | 12 | 0.26 | 4 | 6 | 0.08 |
| stem | 6 | 8 | 0.12 | 1 | 2 | 0.02 |
| leaves | 4 | 6 | 0.08 | 1 | 2 | 0.02 |
| Sainfoin | | | | | | |
| root | 27 | 38 | 0.54 | 7 | 12 | 0.14 |
| stem | 14 | 14 | 0.28 | 1 | 2 | 0.02 |

| | | | | | | |
|---|---|---|---|---|---|---|
| leaves | 10 | 16 | 0.2 | 2 | 4 | 0.04 |

The taxonomic composition of endophytic complexes of micromycetes of the studied agrocultures was represented by 7 genera of filamentous fungi (*Penicillium, Aspergillus, Fusarium, Trichoderma, Beauveria, Metarhizium* and *Cladosporium*) and 4 genera of yeasts and yeast-like fungi (*Rhodotorula, Aureobasidium, Metschnikowia* and *Cryptococcus*) (Figure 1).

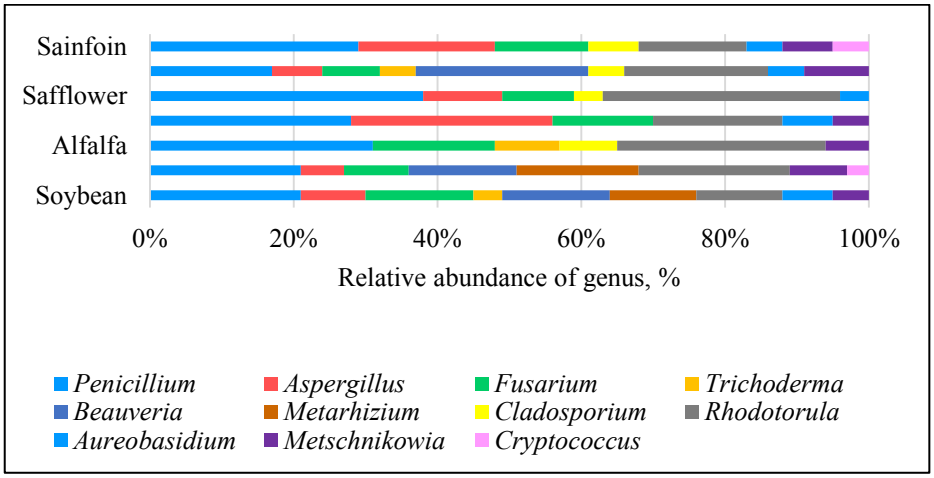


**Fig. 1.** Taxonomic structure of endophytic micrormycete communities (at the genus level)

The greatest taxonomic diversity was characteristic of soybean and sweet clover. Barley and sainfoin were also characterised by considerable diversity. In these plants the number of genera reached 8-9, while in other plants the number of identified genera was lower and did not exceed 6 (Figure 1).

The main component of microfungal communities was fungi of the genus *Penicillium,* the content of which varied from 17 to 38% depending on the plant species. The abundance of *Aspergillus* and *Fusarium* fungi was also observed, accounting for 6 to 28% and 8 to 17%, respectively. The largest share among yeasts was occupied by various species of the genus *Rhodotorula* (12-33%). Representatives of the genera *Trichoderma, Cladosporium* and *Cryptococcus* occupied the smallest share in the structure of endophytic micromycete communities, their relative abundance did not exceed 9% (Figure 1).

## 4 Discussion

Currently, the composition of endophytic micromycete communities is well enough studied only in some species of agricultural plants: alfalfa, soybean, barley, and rape [13, 14]. However, most attention in these studies is paid to filamentous fungi, while yeasts remain a poorly studied group of endophytic microflora. There are also few studies concerning the peculiarities of their distribution depending on the plant organ. In addition, there is no information on the taxonomic structure of endophytic micromycetes of safflower, sweet clover and sainfoin. All this emphasises the high significance and determines the novelty of the conducted research.

The quantitative composition and taxonomic structure of endophytes are largely influenced by such factors as species, physiological state and stage of development of the host plant, type of plant tissues and organs, and environmental conditions [8].

The number of isolates in the underground part of plants was found to be significantly higher than in the aboveground part [15]. The distribution of micromycetes in plant organs can be expressed by the following sequence: roots (182 isolates) > stems (78) > leaves (60). It should be noted that this feature is characteristic of both filamentous fungi and yeasts (Table 1). Similar results were obtained by a number of authors in the study of endophytic fungi of herbaceous plants [16]. Such peculiarities in the distribution of endophytic micromycetes in different plant organs may be due to several reasons. The main source of readily available substrate is roots and their exudates, so the root system can be considered as a relatively stable habitat suitable for many fungal species. In addition, unfavourable factors such as desiccation, UV radiation and nutrient deficiencies affect primarily the aboveground organs of plants, which may be the reason for less frequent infestation of leaves and stems compared to roots [5]. Another reason is that the amount of airborne fungi is much lower compared to soil fungi, which may be responsible for less colonisation of aboveground organs [17].

It is known that plants have an individual unique microbiome due to the selective admission of certain microorganisms into their endosphere [9]. In the present work, it is shown that representatives of the genera *Penicillium*, *Fusarium* and *Rhodotorula* were found in all the studied plants. Various species of fungi of the genus Aspergillus were isolated from all crops except alfalfa, endophytic yeasts of the genus *Metschnikowia* were not detected only in safflower. Representatives of the genera *Metarhizium* and *Cryptococcus* were most rarely detected (Figure 1).

## 5 Conclusion

Thus, the study of the quantitative composition and taxonomic structure of endophytic micromycete communities of agrocenoses revealed a number of features and regularities of their distribution:

- uneven distribution of micromycetes in plant organs was revealed, which can be expressed by the following sequence: roots > stems > leaves;
- the highest colonisation by endophytes was characteristic of sainfoin, soybean and alfalfa, the lowest - for sweet clover;
- representatives of the genera *Penicillium, Aspergillus* and *Fusarium* occupied the largest share in the structure of endophytic micromycete communities among filamentous fungi, and species of the genus *Rhodotorula* among yeasts.

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