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Some endophytes of *Juncus trifidus* from Tatra Mts. in Poland

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This is a first part of work devoted highland rush endophytes: *Penicillium expansum*, *Cladosporium oxysporum*, *Arthrinium* state of *Apiospora montagnei* and *Aureobasidium pullulans*. The basidiomycete strain, possibly *Lagarobasidium detriticum* was also isolated.

Key words: highland rush, basidiomycete endophyte, *Arthrinium*, *Penicillium*, *Cladosporium*, *Aureobasidium*

INTRODUCTION

Asymptomal fungal infections within tissues of healthy plants is a basic character of endophytic fungi. These fungi can be dormant saprotrophs, latent pathogen as well as mutualistic symbionts (Stone et al. 2000). Sieber et al. (1991) noted that the latent endophyte phase is inversely related to the virulence of pathogen. Fungal endophytes belong to different taxonomical groups of fungi such as Ascomycetes and their anamorphs, mitosporic fungi and very rare basidiomycetous fungi. There are some tentatively using groups of endophytes as xylariaceous endophytes, DSE (dark septate endophytes), aquatic mitosporic fungi, clavicipitaceous endophytes and others. Of them fungi from the order Clavicipitales are well known and evolutionary advanced endophytes (Bacon, Hill 1996). According to Clay (1989) and White (1987) clavicipitaceous endophytes are widely distributed in the grass family Poaceae and sporadically distributed in *Cyperaceae* and *Juncaceae*. Other non-clavicipitaceous endophytes were noted in grasses (see Larran et al. 2002) and rarely in rushes. According to Powell (1975) rushes and sedges are non-mycotrophic, avoid of mycorrhiza. Some endpohytes in rushes leaves of annual *J. bufonius*, perennial *J. effusus*, *J. patens* and *J. bolanderi* were reported by Cabral et al. (1993). Perennial rushes were inhabited by limited diversity of fungal species, mostly anamorphs and

teleomorphs of dothidealen fungi, but only one or two species were recorded in high frequency. Whereas annual rush was inhabited by distinctly diverse assemblage of mitosporic fungi including aquatic fungi with low isolation frequencies. According to Cabral et al. (1993) rush endophytes were restricted to single epidermal host cell - *Stagonospora innumerosa* (Desm.) Sacc., substomatal cavity - *Drechslera* sp., *Cladosporium cladosporoides*, *Alternaria alternata*, and intercellular area of mesophyll tissue - *Phaeosphaeria juncicola* and *Alternaria alternata*. Menéndez et al. (1997) noted *Platysporoides* aff. *togwotiensis* (Wehm.) Shoemaker & C. E. Babc. as endophyte in substomatal chamber of *Juncus imbricatus* var. *chamissonis*.

There are some articles devoted microfungi inhabiting different rushes. Volkmann-Kohlmeyer and Kohlmeyer (1994) and Kohlmeyer and Volkmann-Kohlmeyer (2000) described some marine fungi from saltmarsh *Juncus roemerianus*. Adamska (2005) noted six microfungi on various *Juncus* species in lowland of Poland. 30 taxa of microfungi (Tab. I) were noted on highland rush by Scheuer (1988, 1996, 1999); Chlebicki (1990, 2002); Scheuer & Chlebicki (1997); Suková (2004) and Suková & Chlebicki (2004) in the Alps, Carpathians, Sudetes and Ural Mts.

Table 1
List of fungal species noted on plant organs of *J. trifidus*

Arthrinium cuspidatum	*Lophodermium juncinum*
Arthrinium state of *Apiospora montagnei*	*Mycosphaerella perexigua*
Ascochyta junci	*Naeviella paradoxa*
Brikookea sepalorum	*Niptera eriophori*
Botrytis cinerea	*Periconia atra*
Brunnipila calycioides	*Phaeosphaeria juncicola*
Cladosporium herbarum	*Phaeosphaeria vagans*
Coronellaria caricinella	*Phialocephala* sp.
Cistella fugiens	*Pseudoseptoria* sp.
Dinemasporium strigosum	*Pycnothyrium junci*
Diplonaevia emergens	*Septoria* spp.
Epicoccum nigrum	*Septoria chanousiana*
Hysteronaevia minutissima	*Stagonospora* sp.
Hysteropezizella diminuens	*Stagonospora junciseda*
Lachnum dinminutum	*Unguicularia* sp.
Lachnum roseum	

Only some endophytes were noted on specimens of *Juncus* spp.: *Pleospora togwotiensis*, *Stagonospora* sp. on *Juncus imbricatus* – (Menéndez et al. 1997); *Stagonospora innumerosa*, *Alternaria alternata*, *Phaeosphaeria juncicola*, *Drechslera* sp., *Cladosporium cladosporioides, Coniothyrium* sp., *Exophiala salmonis*, *Gyoerffyella craginiformis*, *Spermospora* –like sp., *Varicosporium elodeae*, *Helicodendron* spp., *Gliomastix* sp., *Lambdosporium*, *Phoma*-like sp., *Titaea* sp., *Acremonium* (*Neotyphodium*) sp., on *J. bufonius*, *J. effuses*, *J. patens*, *J. bolanderi* – (Cabral et al. 1993). All these species are ascomycete fungi and its anamorphs, or mitosporic fungi.

Host plant. *Juncus trifidus* L. (highland rush) belongs to the section *Steirochloa* which is a sister group of *Luzula* (Drábkova et al. 2003). *J. trifidus* subsp. *trifidus* is the amphi-atlantic perennial plants of Boreal-arctic-alpine distribution. The species is divided into three subspecies. Of them *J. trifidus* subsp. *carolinianus* occurs in North America, *J. trifidus* subsp. *monanthos* is restricted to calcareous area of Alps and Appenines. The typical taxon *J. trifidus* subsp. *trifidus* occurs in silicate

mountains of Europe, Greenland and Northern Asia. The geographical distribution of the plant indicates its European origin (Böcher 1972). Plants from North America resembling those from Greenland and Scandinavia and differ in many characters from strains from Pyrenees and Tatra Mts. (Böcher 1972). During the Pleistocene it spread in the Arctic, reaching central Siberia in the East (Kulczyński 1924). In Poland it occurs in some localities such as the Karkonosze Mts. (the Sudetes), Mt. Babia Góra Range and Tatra Mts. (the Carpathians). The locality in Mt. Śnieżnik in the Central Sudetes, reported by Ciaciura (1988), is situated on the Czech side of this mountain. Highland rush is perennial caespitose rush forming dense tufts. Aerial stems are stiff and erected, leaves alternate, simple with acuminate apices. Inflorescence cymose or head-like. Number of chromosomes of Polish populations 2n=30 (Skalińska et al.1957). It is pioneering alpine species with effective strategy of colonization of disturbed sites (Marchand, Roach 1980).

METHODS

Material was collected on NE slope of Mt. Małołączniak in the Tatra Mts. (49° 55'N, 19° 14'E). 19 specimens of *Juncus trifidus* were screened for the presence of fungal endophytes. Small part of tuft including culms with rots were removed from the soil into sterile unclosed zip-lock plastic bags and putted in the refrigerator. After 3 days they were moving from the bags and processed according to isolation procedures (Bacon 1990). Three green culms of each sample were selected, central parts of each culm were cut into two segment ca 3 cm long. Six segments from each sample were used (totally 114 segments). All segments were dipped for 1 min in 96% ethanol, then surface sterilized for 3 min in 3% chlorox (NaOCl), rinsed in ethanol for 1 min and transferred to 90 mm Petri plates with Ferency medium and then were incubated in room temperature in daylight. Obtained isolates of different fungi were then removed on PDA and MAA media and incubated in room temperature. Taxa of fungi were identified on the basis of cultural characteristics and the morphology of hyphae and conidia.

RESULTS

Totally 114 segments were used. 11 strains of different fungi were isolated. Of them five taxa are presented in this article.

Arthrinium state of ***Apiospora montagnei*** Sacc., Nuovo Giorn. Bot. Ital. 7: 306, 1875.

Colonies superficial, widely effused, produced mycelial mats, mycelium colourless to pale olivaceous brown, composed of network of septate hyphae anastomosing and branched, smooth ca 3 μm diam, conidiophores colourless, smooth 1.5-2 μm diam., conidia brown 5-9 × 4-5 μm with a hyaline band at the junction of the two

sides. The fungus started growth after 5 months from two segments and overgrow every mycelia in the dishes.

COMMENTS. The anamorph of this species was earlier noted by the author on leaves and stems of *J. trifidus* from Ural Mts. The colonies of this Ural collection growing on stems, where circular, black with conidia 4.5-5(6) × 3.6-5 μm. It is cosmopolitan species, very common on bamboos and other dead plants (Ellis 1971), with long hosts list (Domsch, Gams and Anderson 1980). The fungi from the genus *Arthrinium* were isolated from leaves of *J. imbricatus* var. *chamissonis* (Menendez et al. 1995), bark of *Eucalyptus globulus* (Bettucci et al. 1999) and accidentally noted in tomato leaves (Larran et al. 2001) and lichen talli of *Cladonia*, *Stereocaulon* (Petrini et al. 1990) and *Xanthoparmelia taratica* (as *Parmelia taratica* Girlanda et al. 1997).

Aureobasidium pullulans (De Bary) G. Arnaud, Annals d'École National d'Agric. de Montpellier, N.S. 16: 39, 1918, var. ***pullulans.*** Figs 1A1, A2

Anamorph of *Discosphaerina* Höhn.

After 10 days at the ends of rush segments appeared two pale colonies (Fig. 1A1) which quickly joined and darken after 30 days (Fig. 1A2). Such black mycelium has been removed to the new plate with PDA. Colony black, convex, slightly pulvinate, surface covered by dense hyphae with conidia, margin entire to undulate, 21 mm diam. after 7 days. Hyphae hyaline to pale brown, 4-5 μm diam., septate and branched, conidiogenous cells intercalary, conidia very variable in shape, (4)7-10(19) × (2)3-4 μm.

COMMENTS. This black yeast-like fungus (meristematic fungus) is ubiquitous saprophyte of phyllosphere and other aerial plant parts (Domsch, Gams and Anderson 1980). It is frequently noted as endophyte of different plants, among them in bark and xylem of *Eucalyptus* (Bettucci et al. 1999), leaf fern *Pteridium aquilinum* (Fisher 1996), twigs, leaves and buds of *Acer pseudoplatanus* and other trees (Pugh, Buckley 1971). *A. pullulans* can be antagonistic against a number of phytopathogenic fungi (Andrews et al. 1983). Schena et al. (2002) noted high protection levels of *A. pullulans* against *Penicillium digitatum*, *Botrytis cinerea* and *Monilia laxa*. *A. pullulans* is also noted on painted wood, stone and glass (Yurlova et al. 1999).

Cladosporium herbarum (Pers.) Link, Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 7: 37, 1816.

Anamorph of *Davidiella tassiana* (De Not.) Crous & U. Braun, Mycol. Progr. 2: 8, 2003.

Conidiophores straith to flexuose, distinctly nodose (were present on three stem segments in a single plate), conidia cylindrical, limoniform, pale olivaceous, nearly smooth or very slightly verrucose 19-16 × 5-6 (7) μm.

COMMENTS. Conidia of the species complex of *C. herbarum* have minutely verruculose to echinulate or spiny conidia (Schubert et al. 2007). Conidia of similar species - *C. oxysporum* - are generally smooth. It is known that species of *Cladosporium* may inhabit substomatal chambers. Shorter superficial sterilization times may lead to higher percentage of *Cladosporium*. *C. oxysporum* was noted in bark and xylem of *Eucalyptus grandis* (Bettucci et al. 1999). *Cladosporium cladosporoides* was noted as endophyte in branches of *Fraxinus excelsior* and *Pinus sylvestris* (Kowalski, Kehr 1996) and *Juncus* spp. (Cabral et al. 1993). *C. herbarum* was isolated as endophyte

in many different plants. Suková (2004) noted this fungus on stems of *J. trifidus* in Czech Republik.

Penicillium expansum Link, Obs. Mycol. 1: 16, 1809. Figs 1B1, 1B2

Anamorph of *Eupenicillium* F. Ludw.

At the beginning colonies white (Fig. 1B1), after becoming grey to olivaceous-grey on Ferency medium (Fig. 1B2). Conidia pale yellow, smooth walled ellipsoidal 3-4 × 2,4-3 μm.

COMMENTS. Common fungus on fruits, meat, paper, various rotting substrates and soil (Domsch et al. 1980), known as *Sorbus* endophyte (Samson, Frisvad 2004). Also Batista et al. (2003) and Vega & Posada (2006) informed that fungi from the genera *Penicillium* and *Aspergillus* where isolated as endophytes. *P. expansum* strongly suppressed growth of black yeast strain growing on nearest segment.

Basidiomycete endophyte, possibly *Lagarobasidium detriticum* (Bourdot & Galzin) Jülich, Persoonia 10(3): 334, 1979. Syn.: *Hypochnicium detriticum* (Bourd. & Galzin) J. Erikss. & Ryvarden, Corticiaceae of North Europe (Oslo) 4: 701, 1976.

The white mycelium was growing on five segments of culm. Mycelium transferring on MAA medium has 54 mm diam. after five days and 80 mm after seven days. Mycelium white, velvety, pale yellow underside. At the beginning aerial mycelium cover all dish. Hyphae 3-5 μm diam., with clamps, spores smooth, ellipsoid 6-6.5 × 5-5.5 μm diam. with a small apiculus and big oil drop. Crystals not seen. Cystidia not present, however similar structures were seen.

COMMENTS. Size of spores resemble *L. detriticum*. Eriksson and Ryvarden (1976) noted *L. detriticum* on leaves of *Juncus*, *Carex*, *Eqisetum*, ferns, on rotten woods of *Juniperus*, *Alnus*, *Betula*, *Populus* and *Sorbus*. Basidiomycete endophytes are not often isolated from plants. Some species from the genus *Peniophora* were isolated from xylem and bark of *Eucalyptus globulus* and they probably initiated the decomposition process (Bettucci et al. 1999).

DISCUSSION

Totally 11 strains of different fungi were isolated. Of them a black yeast, basidiomycete fungus, DSE and non-sporulating colonies will be subject of the next article. In this article are presented first result of presence of endophytes in the highland rush stems. Four isolated species of fungi have worldwide general distribution and were earlier noted as endophytes of various plants. The precise determination of the most interesting basidiomycete strain need sequencing of ITS region (Nilson 2003). Also comparison of frequency of various ecological groups of endophytes needs further investigation. So far any endophytes were isolated from stems of highland rush.

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Kilka endofitycznych grzybów wyizolowanych z pędów situ skuciny (*Juncus trifidus*) z polskiej części Tatr

Streszczenie

Do badań wybrano populację situ skuciny rosnącą w masywie Czerwonych Wierchów w polskiej części Tatr. Pobrano materiały 19 prób pędów, wszczepiono 114 fragmentów pędów. Uzyskano 11 szczepów, z czego 5 opisano w niniejszym artykule: *Arthrinium*, anamorfa *Apiospora montagnei*, *Cladosporium herbarum*, *Pennicillium expansum*, *Aureobasidium pullulans* i szczep należący do grzybów podstawkowych, prawdopodobnie *Lagarobasidium detriticum*.

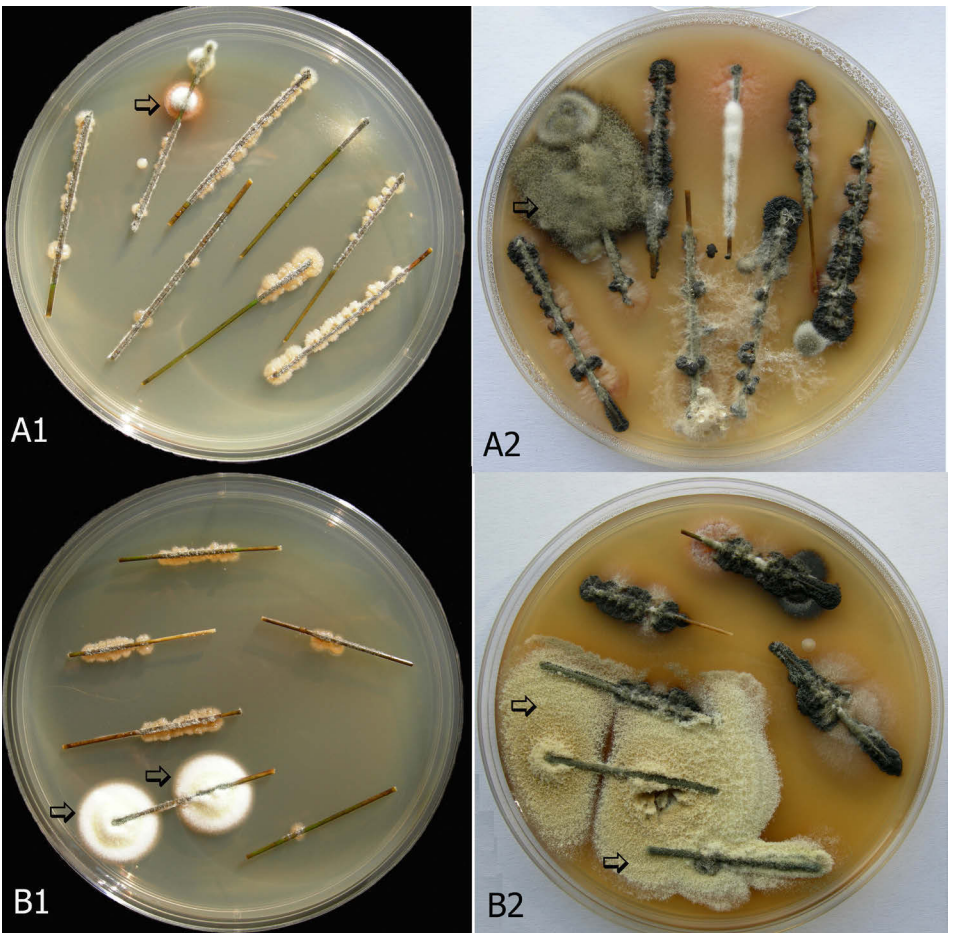

Fig. 1. *Aureobasidium pullulans*: A 1 – after 10 days; A 2 – after 30 days; *Penicillium expansum*: B1 – after 10 days; B2 – after 30 days. Both strains were cultivated on Ferency medium.