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## MORPHO-PHYSIOLOGICAL CHARACTERISTICS AND INTERACTIONS OF ISOLATES OF *MYCOGONE PERNICIOSA* (MAGNUS) DELACR.

**ABSTRACT:** *Mycogone perniciosa* (Magnus) Delacr., which causes wet bubble disease of *Agaricus bisporus* Lange (Imb), results in a considerable crop loss on mushroom farms in Serbia. The isolation and identification of five isolates of *M. perniciosa* from diseased fruit bodies of white button mushroom from mushroom units in Serbia, Bosnia and Herzegovina and Holland were made. Morpho-physiological characteristics and inter-relationships of the obtained isolates were studied. Macroscopic and microscopic investigations of different zones between colonies of the isolates of *M. perniciosa* revealed the phenomenon of the hyphal interference between different isolates. The obtained results suggest that hyphal interference could serve as an additional parameter for a more reliable determination of fungal specificity.

**KEY WORDS:** *Mycogone perniciosa*, mycopathogen, hyphal interference

### INTRODUCTION

Wet Bubble Disease, caused by mycopathogen *Mycogone perniciosa* (Magnus) Delacr., is still considered as one of the most important diseases of the cultivated mushrooms *Agaricus bisporus* Lange (Imb), wherever white button mushroom are produced commercially (Sisto et al., 1997, Sharma and Kumar 2000, Bora and Özakta 2000, Nanagulyan and Yesayan 2002). Mushroom cultivation in Serbia is still less developed than in other countries, and *M. perniciosa* has a significant influence on quality and yield of mushrooms. *Mycogone perniciosa* produces small thin-walled phialoconidia on *Verticillium*-like conidiophores, together with much larger bicellular

conidia (aleuriospores) that develop on short, lateral hyphae, consisting of dark, spherical thick-walled, verrucose apical cell and thin-walled basal cell. Besides vegetative mycelium, both phialospores and aleuriospores of *M. pernicioso* are infectious (Holland and Cooke 1990). Studies of the *M. pernicioso* isolates directed toward the distinction of different isolates, is of great importance from both theoretical and practical point of view. This fungus is known by extremely high mycopathogenic potential and frequently attacks crops of white button mushroom, a common edible mushroom with major economic value and a cosmopolitan distribution (Kerrigan, 1995).

According to Webster (1970) hyphal interference can be of great significance if the hypotheses that it represents the most obvious and most clear form of interspecific competition, and that it could serve as an additional parameter for determination of different isolates of *M. pernicioso*, are true.

In this study, we examined the morpho-physiological characteristics and inter-relationships of the obtained *M. pernicioso* isolates.

## MATERIALS AND METHODS

Samples of diseased mushrooms were collected from mushroom farms in Serbia, Bosnia and Hercegovina and Holland. Pure culture of the *M. pernicioso* was isolated from *A. bisporus* in the Mycological Laboratory, Institute for Biological Research "Siniša Stanković". Fungal isolates used in this work were as follows: MPS from mushroom farms in Bosnia and Herzegovina (Sarajevo); 2 from mushroom farms in Serbia: MPPS (Padinska Skela) and MPR (Ripanj) and 2 (MPH1 and MPH2) from the Mushroom Experimental Station, Wageningen, Holland. The isolates were maintained on potato dextrose agar (PDA). The cultures were stored at 4°C and subcultured once a month (Booth 1971).

Morpho-physiological characteristics of the isolates were recorded on the colony, grown on Petri plates with PDA medium, during 10 days, at 25°C. Colony characteristics and growth measurements were made daily. Hyphae, phialoconidia and aleuriospores were placed on microscopic slides and stained with Lactophenol cotton blue (Johnson, 1940). Measurements (at least 30 of each spore type) and photographs were made on Reichert microscope with Canon power Shot S40.

Different combinations each consisting of three fungal isolates, were inoculated in Petri plates containing PDA medium. The three isolates were inoculated equi-distant from the others in all possible combinations in the same Petri plates. The cultures grown under laboratory conditions (day light, at 25°C) were examined after 5 and 10 days of age.

## RESULTS AND DISCUSSION

Colony characteristics of all isolates varied (Table 1). The colonies were very regular in growth with either dense aerial mycelium MPH2, MPPS and

MPR, or sparse MPH1. Only MPS isolate had colony, which grew in sectors compact and aerial, irregular in growth. Colony colour, which to some extent indicates the production of aleuriospores, varied from white MPS to dark brown MPPS (Table 1).

Tab. 1 — Some morphological characteristics of isolates of *Mycogone perniciosa*

Isolates	Colony tipe	Colony size (mm)	Hyphae max-min mostly ( $\mu\text{m}$ )	Phyalospores max-min mostly ( $\mu\text{m}$ )	Aleuriospores Upper cell (max-min, mostly) Lower cell (max-min mostly) ( $\mu\text{m}$ )
MPS	white, growth in sectors, compact and aerial mycelium, irregular growth	75x76x77	4—8 6	2x6—6x18 2x12	12x16—22x24 (18x20) 6x10—10x18 (10x14)
MPPS	dark brown, with white edge, aerial mycelium	68x69x68	3,75—5 3,75	3,75x12,50 — 5x18,75 3,75x12,50	15x17,50—21,25x23,75 (18,75x20) 7,50x11,25—13,75x15 (10x13,75)
MPR	light brown, with white edge, aerial mycelium	70x50x76	2,50—5 3,75	3,75x7,50 — 3,75x11,25 3,75x12,50	12,50x17,50—23,75x25 (18,75x21,25) 7,50x10—12,50x13,75 (12,50x12,50)
MPH1	white, supstrate mycelium	74x70x74	2,50—5 3,75	2,50x7,50 — 2,50x13,75 2,50x11,25	12,50x13,75—22,50x22,50 (20x20) 6,25x6,25—15x17,50 (10x10)
MPH2	amber brown, with white edge, aerial mycelium	80x80x79	2,50—5 3,75	2,50x7,50 — 3,75x14 3,75x12,50	15x17,50—22x22 (18,75x20) 6,25x8,75—12,5x17,50 (8,25x11,25)

Growth rates and sizes of the colonies of all isolates were similar (Table 1). All isolates produced both phialospores and aleuriospores. The most intensive sporulation was detected in MPPS and MPR, then in MPH2, and the lowest intensity was in MPS and MPH1. Phialospores size varied within the range 2x12  $\mu\text{m}$  (MPS) — 5x18,75  $\mu\text{m}$  (MPPS). Aleuriospores varied within the range 23,75 x 25  $\mu\text{m}$  (MPR) to 12,50 x 13,75  $\mu\text{m}$  (MPH1) for the upper cell, and 10 x 18  $\mu\text{m}$  (MPS) to 6,25 x 6,25  $\mu\text{m}$  (MPH1) for the lower cell (Table 1). Our isolates of *M. perniciosa* came from mushroom farms from Serbia, Bosnia and Herzegovina and Holland but differences between them were not so obvious when compared to the other literature data (Gray and Morgan-Jones, 1980; Umar et al., 2000; Sharma and Kumar, 2000 and Potočnik, 2006). In contrast to the data of Smith (1924), Atkins and La Touche (1948), Hsu and Han (1981) and Fletcher et al. (1995) which reported two-cell phialospores, we could not find this form in our isolates.

Albouy and Lapierre (1972) and Fletcher et al. (1995) found that some pathogenic strains of *M. perniciosa*, which were slow-growing on agar, were highly pigmented and produced numerous aleuriospores. Other

were weakly pathogenic, producing much vegetative growth and little pigmentation. The slow-growing forms were found to contain numerous virus like particles.

We observed interactions between all tested isolates after 10 days. There were several types of interaction: detaining of growth at the site of contact without visible changes, overgrowth of mycelium of one isolate and demarcation lines between the isolates (Table 2). According to the morpho-physiological characteristics and hyphal interferences of five *M. pernicioso* isolates, the isolates from Serbia were similar; the isolates from Holand showed mutually similar characteristics, but they were different from the isolates from Serbia. Isolate from Bosnia and Herzegovina was different from these two groups (Figure 1—4).

Tab. 2 — Interactions appearing between colonies of isolates of *Mycogone pernicioso*

Izolat	MPH2	MPH1	MPR	MPPS	MPS
MPS	detaining of growth at the site of contact	overgrowth of mycelium	demarcation line 4—5 mm	demarcation line	detaining of growth at the site of contact
MPPS	detaining of growth at the site of contact	detaining of growth at the site of contact	demarcation line	demarcation line	
MPR	detaining of growth at the site of contact	overgrowth of mycelium	detaining of growth at the site of contact		
MPH1	overgrowth of mycelium	overgrowth of mycelium			
MPH2	detaining of growth at the site of contact				

Earlier investigations of hyphal interference phenomenon on other fungal species showed different interspecies interaction. As demonstrated previously (Franić-Mihajlović et al., 1996), the isolates of *Diaporthe/Phomopsis* known for their extremely phytopathogenic potential, showed different reactions during the investigation of hyphal interference. Demarcation lines were formed between the isolates which originated from one plant. Formation of the lines between the colonies of the same morphological group of isolates was named as inter-species antagonism by Brayford (1990 a, b). Phenomenon of demarcation line formation results from the incompatibility between genetically different colonies. On the basis of comparison of interactions between the different isolates *M. pernicioso*, we can conclude that these belong to different groups. Genetical difference between these isolates based on molecular-genetic characteristics (Glamočlija, 2006), supports such a conclusion.

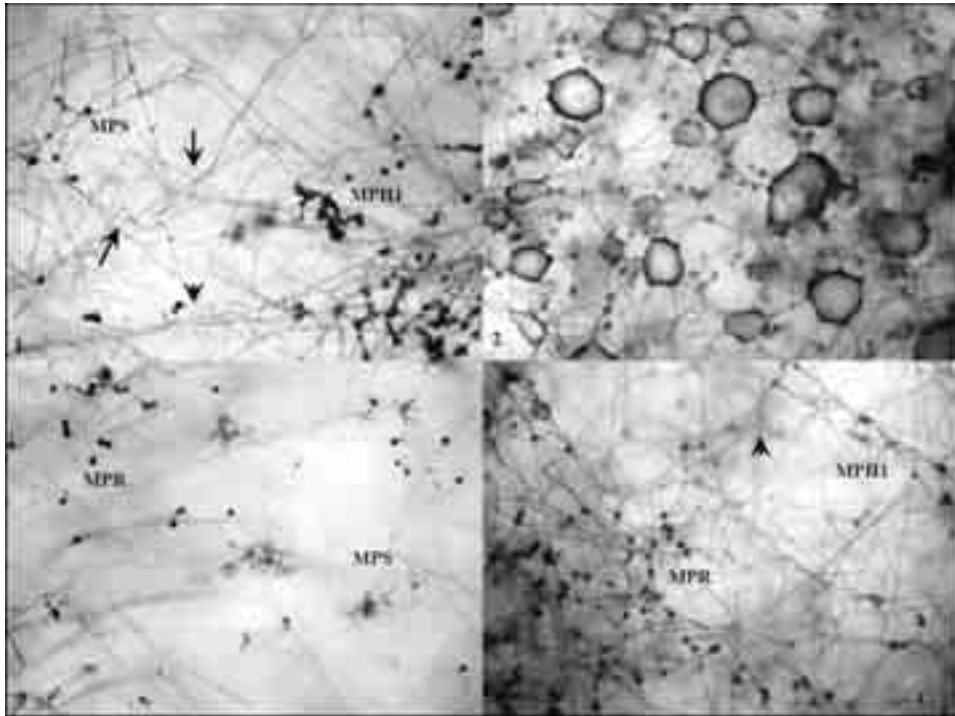


Fig. 1—4 — Types of interactions between isolates of *M. perniciosa*: 1. anastomosis (arrows) of hyphae MPH1 and MPS; 2. overgrowth of MPH1 and MPH2 with exudation; 3. demarcation line between MPR and MPS; 4. anastomosis of hyphae MPH1 and MPR

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МОРФО-ФИЗИОЛОШКЕ КАРАКТЕРИСТИКЕ И ИНТЕРАКЦИЈЕ  
ИЗОЛАТА *MYCOGONE PERNICIOSA* (MAGNUS) DELACR.

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Резиме

*Mycogone perniciosa* (Magnus) Delacr., изазивач обољења мокре трулежи, најчешћи је узрочник губитака у гајилиштима *Agaricus bisporus* Lange (Imb) у Србији. Извршена је изолација и идентификација 5 изолата *M. perniciosa* са оболе-них плодноносних тела шампињона из гајилишта у Србији, Босни и Херцеговини и Холандији. Испитиване су морфо-физиолошке карактеристике као и степен сродности проучаваних изолата на основу анализе међусобног деловања колонија, односно коришћењем феномена хифалне интерференције. Макроскопска и микроскопска истраживања односа изолата и добијени резултати указују да хифална интерференција може представљати додатни параметар у разликовању изолата *M. perniciosa*. Изолати добијени из гајилишта у Србији слични су међусобно, као и изолати из Холандије који су показали међусобну сличност али се разликују од претходних. Изолати из Босне и Херцеговине разликовали су се и од српских и од холандских.