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Suppression of *Nigrospora oryzae* (Berk. & Broome) Petch by an aggressive mycoparasite and competitor, *Penicillium oxalicum* Currie & Thom

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Abstract

The objective of this research was to study by means of different techniques, the interaction between *Penicillium oxalicum* and *Nigrospora oryzae* under different temperatures (15 and 25 °C), water activities (0.95, 0.98, and 0.995) and culture media (rice and rice extract agar). In dual culture, *P. oxalicum* was dominant over *N. oryzae* in spite of presenting in the majority of cases, lower growth rates. The microscopic study revealed that *P. oxalicum* is a powerful mycoparasite, which attacks the conidiophores and the spores of *N. oryzae*, not only surrounding them, but also penetrating, deforming, destroying and developing reproductive structures inside them. The antagonist did not change its way of performance in the different tested conditions. Water activity and temperature showed a significant effect on fungus growth.

Keywords: Competition; Index of Dominance; Mycoparasitism; *Nigrospora oryzae*; Rice; *Penicillium oxalicum*; Water activity

1. Introduction

An alternative and effective method to control phytopathogenic fungi is the use of biocontrol agents. Among these, there are fungic species which have already been formulated and which have been applied to control diseases with varying levels of efficacy.

Fungal biological control agents have several mechanisms of action that allow them to control pathogens, including mycoparasitism, production of antibiotics or enzymes, competition for nutrients and the induction of plant host defences (Brimner and Bolam, 2003). Understanding the mode of action of the antagonist is important in order to develop more reliable procedures for the effective application of the antagonist and to provide a rationale for selecting more efficient antagonists (Golam and Ilag, 1999).

Mycoparasitism, the direct attack of one fungus on another is a very complex process that involves sequential events, including recognition, attack and subsequent penetration and killing of the host (Benítez et al., 2004). Mycoparasites utilize fungal cell-wall-degrading enzymes such as chitinases and glucanases to dissolve their fungal hosts' cell walls and penetrate the cells (Elad, 1995). The phenomenon of mycoparasitism is very widespread in nature (Paul, 1999).

When the strain secretes volatile or diffusible antibiotics or metabolites that disable the growth of the antagonized microorganism preventing it from colonizing from the substratum, we speak of antibiosis.

Competition occurs when one organism uses a resource and consequently reduces the availability of this resource to another organism. Fungal competition can be divided into primary resource capture (obtaining uncolonized resources) and secondary resource capture (struggle to obtain resources already colonized by other fungi) (Boddy, 2000). To ensure that competition exists it is essential that there is a shortage of an element, since if there is excess, there is no competition. Competition can also result from space, oxygen, etc.

Biological control agents may also induce changes in the plant that increase disease resistance similar to the phenomena of induced and systemic acquired resistance (Handelsman and Stabb, 1996; Harman, 2000).

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When opposed to a pathogen, each antagonist will be able to express one or several mechanisms of simultaneous form. Moreover, the success of these agents depends on many factors, such as the fungal species used – antagonist – pathogen-, environmental conditions – notably temperature and water activity –, pH, availability of nutrients, growth substrate, etc.

Penicillium oxalicum Currie & Thom is one of the more ubiquitous species with a greater distribution inside the genus *Penicillium* and it is considered to be a normal representative of the mycobiota of the soil (Pitt and Hocking, 1999).

Recent studies of biocontrol carried out on this filamentous fungus, describe that it is capable of controlling certain pathogenic fungal species by means of induction of resistance in the plants and by means of its metabolites. In this way, Larena et al. (2003) observed a reduction in vascular wilts caused by *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *lycopersici* under glasshouse and field conditions by inducing resistance in the tomato plant. On the other hand García et al. (2001) detected a high fungicidal activity of the metabolites secreted by this strain opposite to the filamentous fungi *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *gladioli* and slightly less opposite to *F. oxysporum* f. sp. *niveum* and *Botritis aclada*. In this assay, activity was not registered opposite to strains of the genera *Penicillium*, *Alternaria*, *Cladosporium*, *Colletrotrichum* and *Trichoderma*. The fungus has also been reported to be useful as a biocontrol agent for other crop diseases (Kommedahl and Windels 1978; Gintis and Benson 1987; Trapero-Casas et al., 1990).

Nigrospora oryzae (teleomorph: *khushia oryzae* Huds.) is a seedborne fungus which produces minute leaf and grain spot in rice crop (Mew and Gonzales, 2002). This saprophyte species has been reported of affecting glumes, culms, leaves, or other parts of rice plants that are weakened because of nutritional or climatic conditions, or suffering from disease or insect attack. Although its presence does not cause important illnesses, it can affect seed quality, germination, viability, plant strength and root or coleoptile growth (Sempere and Santamarina, 2006a).

Biological control experiments were conducted with the fungus *P. oxalicum* against the plant pathogen *N. oryzae* under different conditions of temperature, water activity and culture media. Initially a macroscopic study was realized and was completed with two microscopic techniques: light microscopy and cryo-scanning electron microscopy.

2. Materials and methods

2.1. Isolates

Nigrospora oryzae DAE 7406 was isolated at the laboratory of Agroforest Ecosystems of The School of Rural Environments and Enology from samples of rice grain collected from different rice fields and cooperatives of the main rice producing areas in Valencia.

Penicillium oxalicum Currie & Thom was isolated from national corn grain samples currently forming part of the fungal collection of the Department of Agroforest Ecosystems in the School of the Rural Environment and Enology (Avda. Blasco Ibañez, 21. 46010-Valencia. Spain).

All fungal strains were kept in Potato Dextrose Agar (PDA).

2.2. Culture medium

The basic medium used was Rice Extract Agar (REA) that was made by boiling 60 g of dry ground rice in l l of distilled water for 45 min. The resulting mixture was filtered through a double layer of muslin and the volume was made up to 1 l. The water activity of this basal medium was 0.995, and this a_w was modified by the addition of different amounts of glycerol −0.98, 0.95, 0.90 and 0.85: 47.5, 105, 182.5 and 240 g glycerol l^{-1} of medium, respectively- (Sempere and Santamarina, 2006b). Media were sterilized by autoclaving for 20 min at 121 °C and cooled to 45–50 °C before pouring into 90 and 150 mm Petri plates. Final a_w values were checked with a water activity meter (AquaLab, Pullman, WA, USA).

Plates with the same water activities (a_w) were placed in water impermeable plastic containers together with two 100 ml beakers containing a glycerol water solution with an equilibrium relative humidity value identical to the a_w of the plates (Sempere and Santamarina, 2007). In this way, equilibration to the target a_w levels was achieved within 24 h, maintaining a constant relative humidity inside the Petri dishes and also controlling the a_w of the substrate.

The water activity values studied were 0.85, 0.90, 0.95, 0.98 and 0.995, and the experiments were carried out at 15 and 25 °C.

2.3. Measurement of growth

Each Petri plate was inoculated with two disks (8 mm diameter) of each fungus species, 45 mm apart, obtained from the growing margins of the fungus colonies grown in PDA at 25 °C for 5 days.

The growth was registered for 5 days at intervals of 24 h according to the method described by Sempere et al. (2007). To calculate the growth rate (mm·day $^{-1}$) a linear regression of the radius (mm) as opposed to the time (days) was carried out. The computer software used was Microsoft Excel 2003.

2.4. Assessment of Index of Dominance (I_D)

The I_D was developed to measure the ability of a species to dominate under a particular set of environmental conditions (Magan and Lacey, 1984). Petri dishes containing rice extract agar modified with glycerol to 0.95, 0.98 and 0.995 water activities were incubated in polyethylene bags for 60 days at 25 and 15 °C. The interaction of each dual culture at different water activities was examined macroscopically, the type of interaction was determined, and numerical scores were assigned to obtain an Index of Dominance. Mutual intermingling (1); mutual antagonism on contact or with free space between fungus colonies <2 mm (2); mutual antagonism at a distance (3); dominance on contact (4 for the dominant species, 0 for the inhibited species); dominance at a distance (5 for the dominant species, 0 for the inhibited species).