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Suada, I Ketut
Study Program of Agroecotechnology, Faculty of Agriculture, Udayana University

Suhartini, Desak Made Weda Yati
Study Program of Agroecotechnology, Faculty of Agriculture, Udayana University

Sunariasih, Ni Putu Linda
Study Program of Agroecotechnology, Faculty of Agriculture, Udayana University

Wirawan, I Gede Putu
Study Program of Agroecotechnology, Faculty of Agriculture, Udayana University

他

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Ability of Endophytic Fungi Isolated from Rice to Inhibit *Pyricularia oryzae*–Induced Rice Blast in Indonesia

I Ketut SUADA[1], Desak Made Weda Yati SUHARTINI[1], Ni Putu Linda SUNARIASIH[1], I Gede Putu WIRAWAN[1], Kun Woo CHUN[2], Joo Young CHA[3]* and Shoji OHGA

Laboratory of Forest Recources Management, Division of Forest Environmental Sciences,
Department of Ago–environmental Sciences, Faculty of Agriculture,
Kyushu University, Fukuoka, 811–2415, Japan
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The aim of this research was to measure the ability of endophytic fungi isolated from rice to inhibit the growth of *Pyricularia oryzae*, the pathogen that causes rice blast. *Phialemonium curvatum* had the greatest inhibitory effect against *P. oryzae* (66.6%), followed by *Phaeosphaeriopsis musae* (63.3%), making these species good canditates for biocontrol agents against *P. oryzae*.

Key words: Endophytic Fungi, inhibition ability, *Pyricularia oryzae*, rice

INTRODUCTION

Indonesia, with its tropical climate, is habitat to an enormous number of fungal species including endophytes harbored within the tissues of various plants. Rice (*Oryza sativa* L.) is, on a global scale, one of the most important food crops and performance improvements are continuously being sought in order to increase rice yields.

Endophytic fungi colonize their hosts without causing any external disease symptoms (Carroll, 1998), except under stressful conditions. Studies of the microorganisms associated with tropical plant species are becoming more frequent, especially studies describing biological control and the ability of these microorganisms to biosynthesize pharmacologically important compounds (Azevedo *et al.*, 2000). Endophytic fungi have frequently been reported to be associated with crop plants including wheat (*Triticum aestivum*), wild barley (*Hordeum brevisubulatum* and *Hordeum bogdanii*), soybean (*Glycine max*), and maize (*Zea mays*) (Dingle and McGee, 2003; Istifadah and McGee, 2006; Larran *et al.*, 2010). Some of the endophytic fungi associated with these crops confer upon them resistance to insect or fungal pathogens (Sieber *et al.*, 1988).

The association of arbuscular mycorrhizal fungi and endophytic bacteria with rice has been well documented (Glassop, 2007; Mano and Morisaki, 2008; Mattos *et al.*, 2008). Less, however, is known about rice's fungal endophytes. Fungal endophytes have been detected in cultivated rice (Naik *et al.*, 2009; Yuan *et al.*, 2007), and antagonistic or plant growth–stimulating properties have been claimed for some of them. For example, endophytic *Fusarium* spp. from cultivated rice roots proved to be an effective biocontrol against a root–knot nematode (Le *et al.*, 2009). The occurrence of mycorrhizal and endophytic fungi in a variety of rice cultivars has also recently been reported (Vallino *et al.*, 2008).

Endophytic fungi, in symbiotic associations with rice plants, provide advantages to the plants such as increased growth and resistance against pathogens such as *Pyricularia oryzae*. The use of endophytic fungi to increase plant resistance to pathogens would be a step toward decreasing the use of fungicides and other synthetic chemicals in rice agriculture. In the present study, we screened endophytic fungi isolated from rice plants *in vitro* for possible biocontrol activity against the rice blast pathogen, *P. oryzae*.

MATERIALS AND METHODS

Isolates of *P. oryzae* and endophytic fungi

For the isolation of *P. oryzae*, diseased rice leaf parts were sterilized with 90% alcohol for 1 min and 70% alcohol for 2 min, and then rinsed with sterile water before being cultured in 2% MA medium (20 g malt extract and 18 g agar in 1000 ml distilled water). After incubation for 5 days, the emerging colony was identified according to its conidia. The 11 endophytic fungi used in this study were isolated from rice seeds and seedlings in Bali (Cha *et al.*, unpublished data).

Test for endophytic fungal species inhibition of *P. oryzae*

Each endophytic fungal isolate was cultured, together with *P. oryzae*, in MA medium to determine its inhibitory activity against the pathogen. Agar plugs, 5 mm in diameter, taken from 7–day–old colonies of the endophyte and pathogen pure cultures were inoculated 3 cm apart on MA medium in 9–cm diameter Petri

[1] Study Program of Agroecotechnology, Faculty of Agriculture, Udayana University, Bali, 80361, Indonesia
[2] Department of Forest Sciences, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon, 200–701, Korea
[3] Field Science Center for Northern Biosphere, Hokkaido University, Nayoro, 096–0071, Japan
* Corresponding author (E–mail: jycha@fsc.hokudai.ac.jp)

dishes. These were incubated for 1 week at 28°C. The pathogen inoculated onto the same media without the endophyte and incubated for 6 weeks at 28°C served as a positive control. The diameters of *P. oryzae* colonies grown with each endophyte isolate were used to calculate the amount of pathogen control, referred to as the percentage of inhibition, as follows:

% inhibition = (A–B)/A

where A is the diameter of the control pathogen colony and B is the diameter of the pathogen colony grown with the endophyte (Nuangmek *et al.*, 2008).

Data analysis

The colonization frequency (CF) of a single endophyte species was calculated as the number of segments colonized by an endophyte species divided by the total number of segments examined × 100%. The percentage contribution to an endophyte assemblage by the dominant endophyte (DE) was calculated as the CF of the dominant endophyte divided by the sum of the CF of all the endophytes in an assemblage × 100% (Xing *et al.*, 2010).

RESULTS AND DISCUSSION

Most of the endophytic fungi isolated from rice seeds and seedlings, with the exception of *Cladosporium* sp. and *Nodulisporium* sp., showed greater than 50% inhibition of *P. oryzae* in *in vitro* testing. The endophyte that showed the greatest inhibition of the pathogen (66.6%) was *Phialemonium curvatum* W. Gams & W. B. Cooke, which had been isolated from seeds. The next most effective endophyte was *Phaeosphaeriopsis musae* M. Arzanlou & Crous, which inhibited the pathogen by 63.3% (Table 1). Some species of endophyte may be beneficial to the plant in other ways (e.g., nutrient and water uptake), but would provide less benefit as the plant produces fewer defensive chemicals in response to increasing damage (Ahmad *et al.*, 1985; Cheplick and Clay, 1988). The effect of a single fungal strain on the plant can change when a combination of fungal strains infects the same individual (Lewis and Clements, 1986). These endophytic microorganisms are ubiquitous, increasing plant growth (Varma *et al.*, 1999) and fitness through their ability to improve plant tolerance to heavy metals and drought, and by reducing herbivory and/or phytopathogen settling through the induction of plant self–defense mechanisms (Azevedo *et al.*, 2000). Self–defense may also occur via chemicals produced by endophytic fungi and/or space competition within the plant tissue (Azevedo *et al.*, 2000).

In the future, endophytic microorganisms will be used to increase rice growth through mechanisms such as plant defense against herbivores. Studying these microbes may lead to methods to enhance their ability to improve rice productivity and/or their secondary metabolite production. Many secondary metabolites from endophyte–plant interactions have been isolated and used in raw or derived forms as drugs for the treatment of many conditions (Shennan, 2008). Since plants use the antibiotic qualities of endophytic fungi for defense against pathogens, this mostly chemically–based defense mechanism makes endophytic fungi a perfect group for novel antibiotic research, in the same way that other fungi have yielded such useful drugs as penicillin and streptomycin (Schade, 2007). The endophytic fungi studied here have shown high pathogen inhibition ability and should be considered good candidates for biological control of rice blast caused by *P. oryzae*.

Table 1. Percentage of inhibition ability of endophytic fungal species to *P. oryzae*

Fungal species	Inhibition ability (Value±SE) (%)
Cladosporium sp.	46.6±0.1
Nigrospora oryzae	51.1±2.1
Nigrospora sp.	51.1±0.5
Nodulisporium sp.	45.6±1.4
Penicillium citrinum	60.0±0.6
Penicillium pinophilum	52.2±0.5
Penicillium radicum	55.6±2.2
Phaeosphaeriopsis musae	63.3±0.1
Phialemonium curvatum	66.6±0.1
Sarocladium oryzae	61.1±0.6
Sordariomycetes sp.	58.8±0.7

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