SHORT COMMUNICATION

# Potential of Endophytic Fungi of Barnyard Grass Weed for Biological Control of the Rice Sheath Blight Pathogen, *Rhizoctonia solani* Kühn

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**Abstract**—Endophytic fungi are group of microorganisms that colonize internal tissues of plants but do not cause any harm to their hosts. Although it has been reported that endophytic fungi from weeds have the potential as biological control agents against disease-causing pathogens of crops, the efficacy of those from barnyard grass weeds against sheath blight disease of rice caused by *Rhizoctonia solani* Kühn has not yet been studied. This study determined the potential of endophytic fungi of barnyard grass weed (*Echinochloa glabrescens* Munro ex Hook. f.) as biological control agents of *R. solani* and identified the most effective of these endophytic fungi.

Of 577 endophytic fungal isolates studied, rapid mycelial growth over *R. solani* was shown by sterile mycelia (SM EF-ds61-73, SM EF-ds375-97), EF-ds68-129, *Geotrichum* sp. EF-ds104-16 and *Mucor* sp. EF-ds158-2 in *in vitro* pre-screening tests. In dual culture tests, however, EF-ds68-129, *Geotrichum* sp. EF-ds104-16 and SM EF-ds375-97 exhibited mycelial growth increments of 17.0, 13.3 and 14.2 mm, respectively, resulting in 24% growth inhibition of *R. solani*. Closer observation revealed that only *Geotrichum* sp. EF-ds104-16 showed hyperparasitism through coiling and disruption of *R. solani* hyphae at 6 and 8 days after initial contact.

**Keywords**—Endophytic fungi, barnyard grass, *Echinochloa glabrescens*, sheath blight, *Rhizoctonia solani*, weed, rice, biological control, hyperparasitism, dual culture

## INTRODUCTION

Rice, *Oryza sativa* L., is the staple food of 90% of the Filipino population and carries the largest weight of a single commodity in the consumer price index. It is used as a barometer for inflation in the whole country (PhilRice 2003). Attaining higher yield in rice is sometimes not met due to occurrence of several factors like insect pests, weeds and diseases. Sheath blight of rice, caused by *Rhizoctonia solani* Kühn, is one of the major fungal rice diseases in tropical, sub-tropical and temperate regions of Asia, Africa and Americas (Ou 1985). The disease was first reported in Japan in 1909 and thereafter in Taiwan in 1912, Philippines in 1918 and India in 1920. In terms of severity and economic importance, sheath blight is considered second in rank after rice blast (Donayre et al. 2013; Endino et al. 2013; Banniza and Holderness 2001). Depending on the severity of infection, yield losses due to sheath blight range from 5 to 50% (Agres 2012; Raymundo 2006; Xuan and Alcala 2008). The durability of recently developed rice varieties with resistance to *R. solani* is uncertain due to the pathogen's wide host range and variability in terms of interaction with its host and environmental conditions (Perialde et al. 2013; Rillon and Duca 2013; Eizenga et al. 2002; Shahjahan and Mew 1992). Consequently, the use of fungicides is the main method of control despite the potential health risks and development of fungicidal resistance by the pathogen (Groth 2005; Slaton et al. 2003; Manila 1980).

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Biological control is considered an innovative approach to management of plant pathogens. This is of interest in sustainable agriculture due to increasing regulation and restriction of pesticides as well as the unsuccessful attempts to control diseases by other means (Maloy 1993). In plant pathology, this approach emphasizes the manipulation of microorganisms to control plant pathogens.

Weeds have been proven as good sources of beneficial organisms that can potentially be utilized to protect plants from infection. These beneficial microorganisms, called endophytes, include bacteria and fungi that colonize internal plant tissues (Petrini and Carroll 1981). Weed species like *Centella asiatica* (L.) Urb., *Parthenium hysterophorus* L., *Imperata cylindrica* (L.) Beauv. and *Cenchrus echinatus* L., have been reported to harbor several genera of endophytic fungi like *Acremonium*, *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Cylindrocarpon*, *Fusarium*, *Glomerella*, *Guignardia*, *Myriogenospora*, *Nigrospora*, *Penicillium*, *Periconia* and *Thialophora* (Rakotoniriana et al. 2008; Romero et al. 2001; Rachdawong 2002). In the course of studying endophytic fungi on rice weeds, Donayre et al. (2014) found that barnyard grass, *Echinochloa glabrescens* Munro ex Hook. f., is a host of different species of endophytic fungi. None of the many endophytic fungal isolates recovered from 7,680 tissue segments of barnyard grass, however, has been evaluated for biological control potential against *R. solani*. This study, therefore, was conducted to (a) determine the potential of endophytic fungi of barnyard grass weed as biological control against *R. solani* and (b) identify the most effective endophytic fungi against *R. solani*.

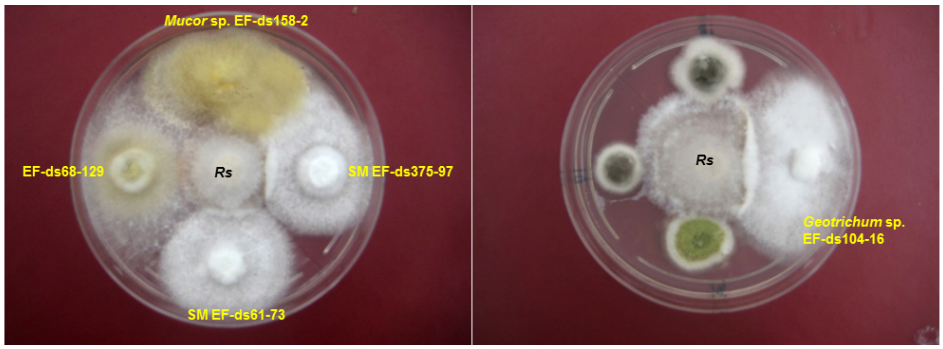


**Figure 1.** Rapid mycelial expansion of endophytic fungal isolates over *Rhizoctonia solani.*

## MATERIALS AND METHODS

### *Collection of Isolates and Preparation of Media*

Methodologies on collection of barnyard grass and isolation of endophytic fungi are discussed in Donayre et al. (2014). Two different culture media, namely potato dextrose agar (PDA) and malt extract agar (MEA) prepared by mixing 39 g and 33 g of their powder preparations, respectively, each in a liter of distilled water, were used to grow the endophytic fungi (EF). Each mixture of culture medium was transferred separately to storage bottles of 500 ml capacity, mixed and dissolved by heating in a microwave for 15 min, and sterilized in an autoclave for 15 min at 121ºC.

### *Isolation of Rhizoctonia solani*

Sheath blight infected rice leaves were collected at the nursery of the Crop Protection Division, Philippine Rice Research Institute, Muñoz, Nueva Ecija for the isolation of *R. solani* (*Rs*). Five cut pieces of infected tissues each measuring 25 $mm^2$ were surface sterilized with 10% NaOCl, rinsed thrice in sterile water, and then transferred to sterile petri plates filled with congealed PDA. The plates were incubated for 5 days at room temperature. To obtain pure cultures of the pathogen, a portion of its mycelial growth was transferred to PDA slants. Slant cultures with the pathogen were then kept under laboratory conditions.

### *Preliminary Bio-efficacy In Vitro Screening*

A total of 577 EF isolates from barnyard grass samples during the dry season were used in this part of the study (Donayre et al. 2014). Four EF isolates were seeded against *R. solani* in a petri plate. Seven-day old mycelial growth of each EF and *Rs* was excised with sterilized 10 mm cork-borer. A disc of EF was placed equidistantly on four sides with one mycelial disc of *Rs* at the center of the plated PDA. The interaction between the endophytic fungi and the pathogen was visually rated. EF isolates that developed fast and overgrew the pathogen's hyphae within three days were selected and further evaluated using the dual culture test.

### *Dual Culture Test (DCT)*

Each disc of EF and *Rs* were equidistantly placed on the plated PDA. EF and *Rs*, seeded in separate plates, served as controls. The interaction between the EF and *Rs* was evaluated using the rating scale of Bell et al. (1982) as follows: 1 = the antagonist completely overgrew the pathogen and covered the entire medium surface, 2 = antagonist overgrew the pathogen at least two-thirds of the medium surface, 3 = both the antagonist and the pathogen colonized one-half of the medium surface, 4 = pathogen overgrew the antagonist at least two-thirds of the medium surface, and 5 = pathogen completely overgrew the antagonist and occupied the entire medium surface. An endophytic fungus was considered antagonistic to the pathogen if the mean score for a given comparison when rounded to the nearest whole stage is less than or equal to two. High antagonism was not considered if the number was greater than or equal to three (Rebuta 2008). The growth of EF and *Rs* on DCT was compared to the growth of *Rs* or EF alone. The plates were incubated under room temperature for three days. Increment of growth (IG) was computed as IG (day 1 to 3) = [growth(day 3) – growth(day1)]/ 2 while inhibition of the pathogen's growth was determined by measuring the percentage reduction in colony diameter (CD) using the equation below:

$$\text{Growth inhibition (\%)} = \frac{\text{CD(pathogen alone)} - \text{CD(pathogen in DCT)}}{\text{CD(pathogen alone)}} \times 100$$

The experiment was arranged in a Completely Randomized Design (CRD) with five replications and five Petri plates per replication. All data gathered were subjected to statistical analysis using analysis of variance (ANOVA) while computed means were compared using FLSD values at 5% level of significance. The computer package SAS 9.1.3 was utilized in the analysis.

### *Test for Hyperparasitism*

Agar blocks (10$mm^2$), cut from plated PDA with a sharp and flame-sterilized scalpel were placed on opposite ends of a sterile glass slides. Mycelia of *Rs* and those EF that showed potential as biocontrol agents were placed on each of these agar blocks. A sterile cover slip was placed on top of each agar block. These agar blocks inside sterile petri plates were then incubated for 5 days with moistened sterile tissue paper. When visible growth of both the pathogen and the potential biocontrol agents overlapped, cover slips were removed and placed on separate glass slides with a drop of lactophenol cotton blue and sealed for examination using a microscope with an attached digital camera (Olympus DP72-BSW).

## RESULTS AND DISCUSSION

### *Preliminary Bio-efficacy In Vitro Screening*

Of 577 EF isolates that were tested, only five, coded as sterile mycelia (SM EF-ds61-73, SM EF-ds375-97), EF-ds68-129, *Geotrichum* sp. EF-ds104-16 and *Mucor* sp. EF-ds158-2, showed rapid mycelial growth over *R. solani* after 3 days of incubation in PDA culture medium (Figure 1).

### *Dual Culture Test*

In dual culture tests (DCT), all four of the five promising EF grew faster than the test pathogen with *Mucor* sp. EF-ds158-2 as the exception (Table 1). SM EF-ds61-73, EF-ds68-129, *Geotrichum* sp. EF-ds104-16 and SM EF-ds375-97 had mycelial growth increments of 11.1, 17.0, 13.3 and 14.2 mm, respectively. In terms of growth inhibition, only EF-ds68-129, *Geotrichum* sp. EF-ds104-16 and SM EF-ds375-97 were antagonistic by inhibiting 24% of the pathogen's growth (Figure 2). Based on Bell's rating, however, the three EF isolates only overgrew two-thirds of the mycelial growth of the pathogen in PDA medium. Nevertheless, the three EF isolates showed inhibitory effect on the pathogen by limiting its growth on the substrate. In studying endophytic fungi as biocontrol agents against *R. solani*, Lanceta (2010) reported that *Penicillium* and *Aspergillus* isolates effectively reduced the radial growth of the pathogen in the form of hyperparasitism and antibiosis. Sadoral (2010) also had similar findings on the effect of two endophytic fungi against pathogenic *Colletotrichum* sp. causing leaf spot on *Jatropha curcas* L. Both studies found out that *Geotrichum* and *Aspergillus* isolates effectively inhibited the growth of the pathogen by 46.74 and 44.74%, respectively, in dual culture tests. Naik et al. (2009) also reported that *Chaetomium globosum*, an EF isolated from rice, inhibited the mycelial growth of *Nigrospora oryzae* (64.29% inhibition), *R. solani* (62.13%), *Altenaria alternata* (62.15%), and *Phoma sorghina* (61.11%) in dual culture tests. They added that *Penicillium chrysogenum* greatly decreased mycelial growth of *Macrophomina phaseoli* by 79.81%. Moreover, Tian et al. (2004) also reported that endophytic fungal isolates from four rice varieties [Qilisimiao (Q-1), Huajingxian (H-1). Huaza35 (H-35), Jinfengzhan (J-1)], namely *Fusarium*, *Penicillium* and *Aspergillus* showed 58.8, 20.0 and 18.5% antagonism against *Magnaporthe grisea*, *Rhizoctonia solani*, *Xanthomonas oryzae* pv. *oryzae* and *Fusarium moniliforme*, respectively.

**Table 1.** Antagonistic activity of potential endophytic fungal isolates in dual culture test.

| EF ISOLATES | GROWTH INCREMENT (mm)[1/] | GROWTH INHIBITION (%)[2/] | BELL'S RATING[3/] |
|---|---|---|---|
| SM EF-ds61-73 | 11.1 $^{c}$ | 6.4 $^{b}$ | 3 $^{b}$ |
| EF-ds68-129 | 17.0 $^{a}$ | 24.2 $^{a}$ | 2 $^{a}$ |
| *Geotrichum* sp. EF-ds104-16 | 13.3 $^{b}$ | 24.2 $^{a}$ | 2 $^{a}$ |
| *Mucor* sp. EF-ds158-2 | 6.1 $^{e}$ | 1.6 $^{c}$ | 3 $^{b}$ |
| SM EF-ds375-97 | 14.2 $^{b}$ | 24.8 $^{a}$ | 2 $^{a}$ |
| *Rhizoctonia solani* | 9.4 $^{d}$ | - | - |
| FLSD | 0.89 | 4.35 | 0.00 |

Means with the same letters are not significantly different at 5% level of significance using FLSD.

1/ - Growth increment (%) (day 1 to 3) = {[growth(day 3) – growth(day1)] /2} x 100

2/ - Growth inhibition (%) = $\frac{\text{colony diameter (pathogen alone) – colony diameter (pathogen in DCT)}}{\text{Colony diameter (pathogen alone)}} \times 100$

3/ - Bell's rating: 1 = the antagonist completely overgrew the pathogen and covered the entire medium surface, 2 = antagonist overgrew the pathogen at least two-thirds of the medium surface, 3 = both the antagonist and the pathogen colonized one-half of the medium surface, 4 = pathogen overgrew the antagonist at least two-thirds of the medium surface, and 5 = pathogen completely overgrew the antagonist and occupied the entire medium surface

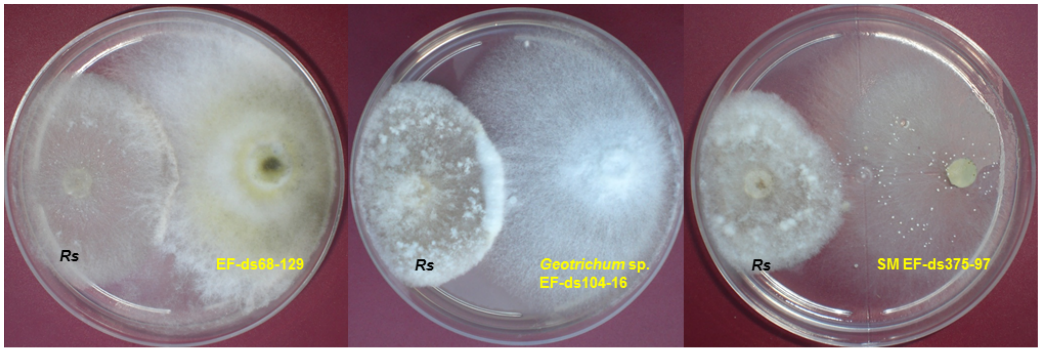


**Figure 2.** Antagonistic activity of three potential endophytic fungal isolates against *Rhizoctonia solani* in a dual culture test.

### *Hyperparasitism*

Of the three potential EF isolates, only *Geotrichum* sp. EF-ds104-16 showed hyperparasitism against *R. solani*. Initially observed as hyphal contact with the pathogen's hyphae as early as 2 days after inoculation (DAI), this was followed by penetration, coiling and disruption of *R. solani*'s hyphae at 4, 6 and 8 DAI, respectively (Figure 3). As there is no previous report on the effect of *Geotrichum* against any pathogen, it can be inferred that the degradation of hyphae might be the result of various enzymatic hydrolysis and cellular activities by chitinases such as 1,4-β-acetylglucosaminidases (GlcNAcases), endochitinases and exochitinases much like the secretions by *Trichoderma* (Kubicek et al. 2001 and Howell 2003). Gao et al. (2005) also found similar manner of mycoparasitism when they co-cultivated *Chaetomium spirale* Zoft ND35, a dominant endophyte c fungal strain isolated from *Populus tomentosa* Carr., with *R. solani* using the agar-block culture technique. Their observations under the light microscope and transmission electron microscope revealed that *C. spirale* Zoft ND35 densely coiled around the hypha of *R. solani* with intracellular growth coming from its penetration peg. At a later stage of the antagonism process, they found several hyphae of *R. solani* that were strongly degraded, emptied and abnormally shaped. When they incubated ultrathin sections from individual colonies of either *C. spirale* Zoft ND35 or *R. solani* with gold-labelled wheat germ agglutinin (WGA) for localizing N-acetylglucosamine residues (chitin), they concluded that chitinases might be involved in the cell-wall degradation of *R. solani*.

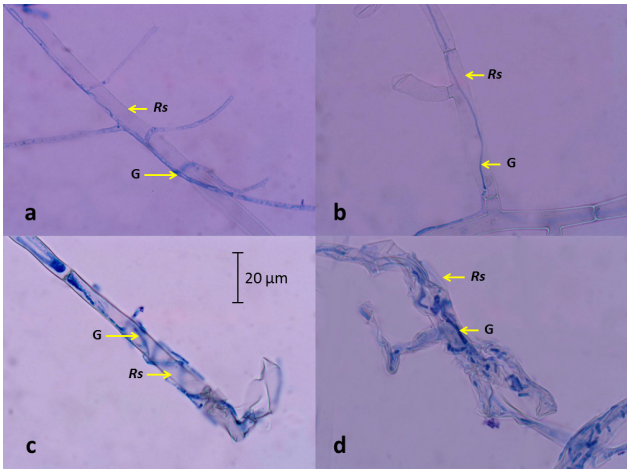


**Figure 3.** Hyperparasitism of *Geotrichum* sp. EF-ds104-16 (G) on *Rhizoctonia solani* (*Rs*) under the light microscope at 1000x magnification: (a) hyphal contact at 2 days after inoculation (DAI), (b) penetration inside the hyphae at 4 DAI, (c) coiling around the hyphae at 6 DAI, and (d) disruption of the pathogen's hyphae at 8 DAI.

## CONCLUSION

This study confirmed that endophytic fungi of barnyard grass weed have the potential as biological control agents against *R. solani*. More specifically, endophytic fungal isolates EF-ds68-129, *Geotrichum* sp. EF-ds104-16, and SM EF-ds375-97 were found effective against the pathogen. It is recommended that additional tests, including experiments under field conditions, be undertaken to confirm their effectiveness. Likewise, a bio-assay to determine their effect on other major disease-causing rice pathogens, such as *Pyricularia grisea* Sacc., *Xanthomonas oryzae* pv. *oryzae ex* Ishiyama, and *Bipolaris oryzae* (Breda De Haan) Shoemaker, must be conducted.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

## CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Both authors contributed in the conceptualization of the study. DKM Donayre was responsible for the implementation of the study, gathering and analysis of data, and writing of the paper while TU Dalisay for the editing of the paper.

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