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Full Length Research Paper

Myrothecium roridum's effectiveness in eradicating water hyacinth and species identification based on molecular information

Orawan Piyaboon[1], Arm Unartngam[1] and Jintana Unartngam[2]*

[1]Bioproducts Science, Department of Science, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand. *Corresponding author. E-mail: agrjne@ku.ac.th. Tel: 66-84-1582586.
[2]Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand.

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Myrothecium roridum **isolates were evaluated for their effectiveness in controlling the aggressive water hyacinth and molecular identification was conducted using internal transcribed spacer (ITS) rDNA region and amplified fragment length polymorphism (AFLP) markers.** ***M. roridum*** **isolates were collected from nine provinces of Thailand. Seventy isolates were included in the primary screening, using colonial growth rate and spore number measurement. The results indicate that five isolates were the most aggressive and so were selected to evaluate their effectiveness in controlling the water hyacinth. The result showed that** ***M. roridum*** **isolate KKFC 408 had significantly highest disease severity (P<0.05) and the highest reduction for the fresh weight of water hyacinth. These five isolates DNA were amplified and sequenced using ITS1 and ITS4 universal primer for species identification. The data analyzed showed that five sequences of** ***M. roridum*** **were in the same group agreeing with other sequences of** ***M. roridum*** **recorded in the GenBank database, which correlated to morphological observation results. The AFLP result indicated that fifteen isolates of** ***M. roridum*** **were divided into four subgroups which were not correlated to the geographical area.**

Key words: Amplified fragment length polymorphism (AFLP), internal transcribed spacer (ITS) of rDNA region, mycoherbicide.

INTRODUCTION

Water hyacinth (*Eichhorniacrassipes* (Mart.) Solms) is a water plant belonging to the family Pontederiaceae. This aquatic weed is counted as a major problem due to its rapid productivity and the difficulty of eradication. Water hyacinth affects irrigation, water flow, water use and navigation. The problems caused by water hyacinth include obstructing waterways, impeding drainage, destroying wildlife resources, reducing outdoor recreation opportunities, lowering dissolved oxygen levels resulting in reduced available oxygen for animals and other plants and a health risk by enabling the breeding of mosquitoes, bilharzias and other human parasites (Okunowo et al.,

2013). Each year, the government spends several millions baht removing this species from water resources. Control and removal can be by either mechanical or chemical methods. However, both methods have demonstrated that they do not remove this aquatic weed effectively. Consequently, a biological controlling method is a plausible alternative; this could reduce expense and would not be toxic to the environment. However, there is the disadvantage of biological method that takes time for working and controlling. In Thailand, biological control of the water hyacinth is still limited, although there is a wide range of research at the international level, especially in using asexual Ascomycete fungi to control the water hyacinth or other aquatic weeds. A pathogenic fungi may have many advantages as: (i) most pathogens of plants are fungi (ii) they are destructive (iii) they are widely prevalent (iv) most of them can be easily mass cultured, and, (v) they can be integrated into organized pest management systems (Lancar and Krake, 2002). Many species of fungi have been reported for the control of water hyacinth, such as *Acremonium zonatum* (Sawatum) Gams. (Martyn and Freeman, 1978) *Alternaria eichhorniae* (Shabana et al. 1995) *Cercospora rodmanii* (Conway and Freeman, 1977; Charudattan, 1986), *Rhizoctonia solani* (Shahjahan et al., 1987) and *Myrothecium roridum* (Liyanage and Gunasekera, 1989). In these fungal genera, several species of *Myrothecium* have been developed as a mycoherbicide for controlling the water hyacinth and others weeds such as *M. roridum* (Liyanage and Gunasekera, 1989), *Myrothecium verrucaria* (Hoagland et al., 2007; Okunowo et al., 2010). There are numerous enzymes that *M. roridum* can produce such as the cellulases enzyme, which hydrolyzes the β-1,4-D-glycosidic bonds within the cellulose molecules of the plant (Akiba et al., 1995; Baer and Gudmestad,1995; Zaldivar et al., 2001; Moreira et al., 2005; Okunowo et al., 2010). Mycotoxins are the series of macrocyclic trichothecenes such as epiroridin E, epi-isororidin E, roridin E, roridin H, trichoverrin A, trichoverrin B, verrucarin A and verrucarin J (Jarvis et al., 1985; Abbas et al., 2002).

Molecular data have been employed to identify and study the diversity of this fungus. Molecular-based identification techniques, including internal transcribed spacer (ITS) rDNA regions are effective tools for species identification. Due to the fact that ITS sequences are variable regions and rich in informative sites; hence, their usefulness for relationship of genus and species of fungi. Okunowo et al. (2013) successfully used ITS regions for identification of *M. roridum* isolated from water hyacinth in Nigeria. Moreover, several types of molecular marker such as amplified fragment length polymorphism (AFLP) is successful for study of the variability within and among fungal populations (Majer et al. 1996; Sibounnavong et al. 2012). The AFLP fingerprints are highly reproducible and used by unknown samples circumventing cumber-some multi-locus sequencing. Sibounnavong et al. (2012) reported that AFLP markers could divided *Fusarium oxysporum* f. sp. *lycopersici* isolated from tomatoes in Thailand into 3 subgroups as low, moderate and high virulence.

The objectives of this study were to determine the tendency of using *M. roridum* to control the water hyacinth in Thailand. Moreover, the molecular based methods were performed to identify the fungal species and to evaluate the genetic diversity.

MATERIALS AND METHODS

Fungal isolation and preliminary identification

Water hyacinth leaf blight disease was observed, then 32 samples were collected from different localities of provinces in the central region such as Nakhon Pathom (6 samples), Pathum Thani (5 samples), Phra Nakhon Si Ayutthaya (2 samples), Suphan Buri (2 samples), Sumut Songkhram (2 samples), Samut Sakhon (3 samples), Ratchaburi (5 samples) and in the western region such as, Phetchaburi (4 samples) and Kanchanaburi (3 samples). Fungal pathogen was isolated from the leaves using the tissue transplanting method. The diseased leaf samples were cut into 0.5×0.5 cm samples and then the surface was disinfected with a 10% sodium hypochlorite solution for 5 min and then washed with sterilized distilled water before being moved to a potato dextrose agar (PDA). The cultures were incubated at 25°C under diurnal light. Single spore isolation was carried out for each of the pure cultures and maintained on PDA slants and deposited at the Fungal Collection, Department of Plant Pathology, Faculty of Agriculture at Kamphaengsaen, Kasetsart University, Kamphaeng Sean Campus (KKFC), Thailand. Each fungal isolate was identified on the basis of morphological characters such as colony color, color and shape of conidia and conidiophores (Barnett and Hunter, 1987).

Pathogenecity test for controlling the water hyacinth

The most aggressive strains were primarily screened by colonial growth rate and spore number reproduction. Each isolate was grown on PDA and incubated at 28°C under diurnal light. The colonial dimension of *M. roridum* was measured every day for 9 days. The spore numbers were counted using a hemacytometer and the data were calculated for statistical analysis. The differences of mean value using ANOVA, was followed by Duncan's multiple range test (DMRT).

The most effective isolates were deposited on the water hyacinth leaves using the spraying method, with a spore suspension concentration of 10^8 spores/mL. The water hyacinths were planted until they reached the third generation, the size of leaves was 25-100 cm^2. The experiment was conducted using a completely randomized design (CRD) with 10 replications in each treatment. The experiment group (treatment), consisted of 5 treatments and control treatment (N = 10 water hyacinth plants, each).

The healthy plant was inoculated with KKFC 390, KKFC 400, KKFC 402, KKFC 403 and KKFC 408 using spraying method with 10 mL of spore suspension. While, control treatment was performed by spraying with 10 mL distilled water. The plants were then placed in a growth chamber with 100% relative humidity for 24 h and then moved to their natural conditions. The disease severity was observed at seven days after inoculation using rating scale as follow as; 0 = no disease, 1 = 1-25% of leaf blight, 2 = 26-50% of leaf blight, 3 = 51-75% of leaf blight, 4 = 76-100% of leaf blight (Sultana and Ghaffar, 2009). The disease severities were analyzed for the mean value and standard deviation. The differences of mean value was shown using ANOVA,

followed by DMRT. Statistical analysis was performed using SPSS statistics software (version 15.0, Window). A $P<0.05$ was considered to be statistically significant. Moreover, the fresh weight of each water hyacinth was recorded before and after spraying, then the data were analyzed for the mean value and standard deviation.

DNA extraction

All fungal isolates were cultured in potato dextrose broth (PDB) in a shaker for two to three days; the mycelia were then harvested on filter paper (Whatman No.1). Fungal mycelia were freeze-dried for 6-8 h using Lyophilizer, then stored at -20°C for later use. Freeze-dried mycelium was ground in a mortar using liquid nitrogen, then 50 mg of the ground mycelium was suspended in 500 µL of extraction buffer (200 mM Tris-HCl, pH 8.0; 250 mM NaCl; 25 mM EDTA and 0.5% SDS) and incubated at 65°C for 30 min. 500 µL of phenol: chloroform : isoamyl alcohol (25:24:1) was then added. After centrifugation at 13,000 rpm for 10 min, the upper aqueous phase was deproteinized by the addition of 1 volume of chloroform : isoamyl alcohol (24:1) and again centrifuged at 13,000 rpm for 10 min. The upper aqueous phase was transferred to a 1.5 mL microtube containing 2 µL of 10 mg/mL RNAase and incubated at 37°C for 30 min. After that 500 µL of chloroform : isoamyl alcohol (24:1) was added and placed in a centrifuge at 13,000 rpm for 10 min, two volumes of absolute ethanol were added to the upper aqueous phase and the solution was stored at -20°C for 1 h. The solution was again centrifuged at 13,000 rpm for 10 min. The precipitated DNA was washed with 200 µL of 70% ethanol and was centrifuged at 13,000 rpm by 2 times, each having duration of 10 min.Afterdrying,theDNAwasdissolvedwithTEbuffer(10mM Tris-HCl, pH 8.0; 1 mM EDTA) by a modified method of Zimand et al. (1994).

PCR amplification of ITS rDNA region

DNA samples of *M. roridum* were amplified in the ITS regions of rDNA using PCR amplification. Amplification of the ITS regions was done using 40 µL PCR reaction each containing 5 µL genomic DNA (50 ng), PCR buffer (1x), dNTP (0.2 mM), 5 pmole of each primer, $MgCl_2$ (2.5 mM) and *Taq* polymerase (1 unit). The PCR amplification of the ITS regions was amplified using primer: ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'TCCTCCGCTTATTGATATGC-3') (White et al., 1990). Amplification of the ITS regions was carried out using T professional Standard Gradient (Biometra) under the following condition: 95°C for 30 min, 35 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min, and finally at 72°C for 10 min. After amplification, 5 µl of the PCR product was detected on 1% (W/V) agarose gels and then added to 0.1 µL/ml GelStar (Nucleic acid Gel stain, 1,000 x concentrate in DMSO) in a TAE buffer (40 mM Tris, 20 mM sodium acetate,1 mM EDTA, pH 8.0). The PCR products were purified using Illustra^{TM} MicroSpin S-400 HR columns (GE Health care UK Limited). The purified PCR products were sequenced at 1st BASE DNA Sequencing Services, Malaysia.

Multiple sequences alignment and data analyses

Sequences generated from the ITS region of rDNA were aligned with other sequences retrieved from the GenBank database including *M. rodidum* (JF724157 and EU927366), *Myrothecium gramineum* (FJ235084), *M. verrucaria* (AB778924) and *Fusarium oxysporum* (KC292853) using MEGA 5 (Tamura et al., 2011). Phylogenetic tree were calculated from the dataset using neighbour-joining (NJ) (Saitou and Nei, 1987) method in the same program. Node support was evaluated by bootstrap analysis (Felsenstein, 1985) using 1,000 replications in the NJ analysis.

DNA fingerprinting analysis using AFLP marker

The AFLP reactions were used as described by Vos and Kuiper, (1997) with the following modifications: Genomic DNA (500 ng) was digested with two combinations of restriction endonuclease *Eco*RI (50 Units) and *Mse*I (10 uints) in a mix of 10x digestive buffer, 0.5 M NaCl and BSA. The digested DNA fragments were ligated with their respective adapter pair (5 pmol *Eco*RI adapter and 50 pmol *Mse*I adapter) of both enzymes in a reaction of T4 DNA ligase (1 unit), T4 DNA ligase buffer (1x) and 1 mM ATP. After the incubation at 37°C for 3 h, the restriction-ligation products were diluted 10 fold with distilled water.

The pre-selective amplifications were carried out with 1 selective nucleotide at 3' end of each primer in volume of 25 µL of PCR buffer, containing PCR buffer (1x), dNTP (0.2 mM), each primer; *Eco* RI primer (5'–GACTGCGTACCAATTC–3') with additional one base (E+A, E+C and E+G), *Mse*I primer (5'-GATGAGTCCTGTAGTA- 3') and additional one base (M+C, M+G, M+A and M+G) 5 pmole, $MgCl_2$ (2.5 mM), *Taq* polymerase (0.5 unit). The pre-selective amplification was carried out using a thermal cycler for 20 cycles of 30 s duration at 94°C; 60 s at 56°C; 60 s at 72°C and held at 16°C for 15 min.

The selective amplifications were performed using selected combinations of primers with two or three selective nucleotides (Blears et al., 1998) as shown in Table 1. All combination primers were screened to investigate the most suitable primers. These were carried out in 20 µL of PCR buffer containing 5 µL diluted (1:10) product of pre-selective amplified DNA, PCR buffer (1x), dNTP (0.2 mM), 5 pmole of each primer, $MgCl_2$ (2.5 mM) and *Taq* polymerase (1unit). The PCR amplifications were performed with an initial denaturation at 94°C for 30 s followed by 12 cycles at 94°C for 30 s, annealing at 65°C; this was then reduced by 1°C for 30 s followed by an extension step at 72°C for 60 s. For each of the following 10 cycles, the annealing temperature was reduced by a further 1°C. The next 30 PCR cycles continued at 94°C for 30 s, 56°C for 30 s and 72°C for 60 s.

For gel analysis, the PCR products were mixed with 10 µL of formamide dye (98% formamide, 10 mM EDTA pH 8.0, 0.3% bromophenol blue and 0.3% xylene cyanol), then heated at 95°C for 3 min and quickly cooled on ice. Each sample (2 µL) was examined on a 5% polyacrylamide gel plus 7 M urea on a Model S2 sequencing gel electrophoresis apparatus. Electrophoresis was performed at a constant power of 50 W for 2.5 h. After electrophoresis, the gel plate was removed, fixed in 10% acetic acid for 30 min, and washed three times in distilled water for 2 min each time. The gel plate was then stained for 30 min in a silver solution (1 g of silver nitrate and 1.5 mL of 37% formaldehyde per liter) and then rinsed with distilled water. After staining, the gels were developed in a developer solution (30 g of sodium carbonate, 1.5 ml of 37% formaldehyde and 0.01 g of sodium thiosulfate) until the bands appeared. The staining was stopped by adding 10% acetic acid (fixing solution) for 1-2 min, rinsed with distilled water for 2 min and dried under a fume hood.

Data analysis

Polymorphic bands were scored as binary data by 1 (present) or 0 (absent). The binary data were analyzed with the computer program NTSYS pc version 2.02 (Rohlf, 1993). An unweighted pair group method with arithmetic mean (UPGMA) cluster analysis was performed using the Jaccard's similarity coefficient. A dendrogram was generated with the tree option (TREE) and a cophenetic value was calculated with the COPH program in NTSYSpc. Bootstrap values were calculated with 1000 replications by the Winboot program (Yap and Nelson, 1996).

Table 1. Primer combinations used for screening in selective amplification.

*Eco*RI	*Mse*I
5' –GACTGCGTACCAATTC**C**- 3'	5'-GATGAGTCCTGTAGTA**C**- 3'
5' –GACTGCGTACCAATTC**A**- 3'	5'-GATGAGTCCTGTAGTA**G**- 3'
5' –GACTGCGTACCAATTC**G**- 3'	5'-GATGAGTCCTGTAGTA**AC**- 3'
5' –GACTGCGTACCAATTC**GT**- 3'	5'-GATGAGTCCTGTAGTA**AA**- 3'
5' –GACTGCGTACCAATTC**GC**- 3'	5'- GATGAGTCCTGTAGTA**ACG**- 3'
5' –GACTGCGTACCAATTC**AGC**- 3'	5'-GATGAGTCCTGTAGTA**CTA**-3'
5' –GACTGCGTACCAATTC**ACG**- 3'	5'-GATGAGTCCTGTAGTA**AAC**-3'
5'–GACTGCGTACCAATTC**ATC**- 3'	5'-GATGAGTCCTGTAGTA**CGC**- 3'
5' –GACTGCGTACCAATTC**ACT**- 3'	
5' –GACTGCGTACCAATTC**CGC**- 3'	

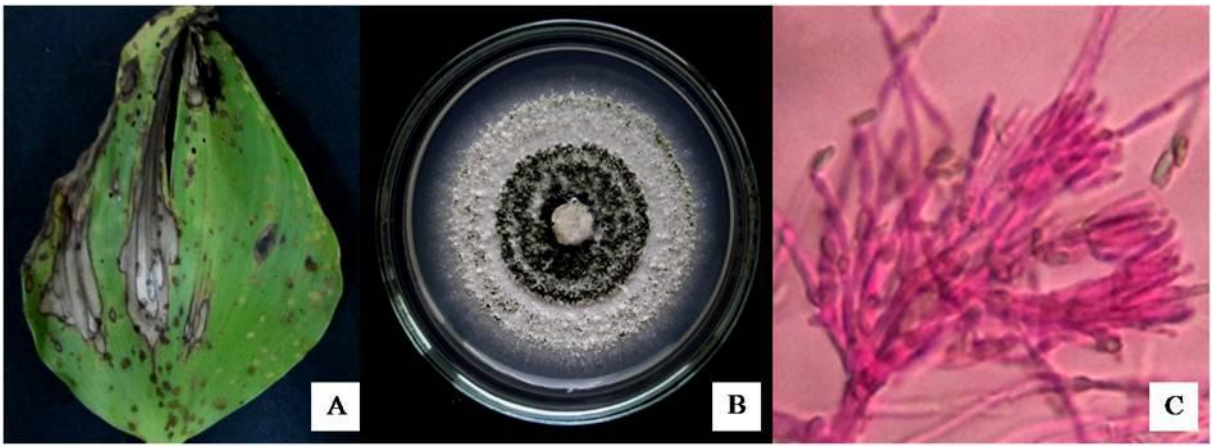

Figure 1. Blight symptoms causing by *Myrothecium roridum* on water hyacinth leaf with fungal mass (A) colony of *M. roridum* on PDA (B) and conidia and conidiophore of *M. roridum* under microscope (400 X) (C).

RESULTS

Fungal isolation and preliminary identification

The disease symptoms were teardrop-shaped leaf spot, rounded on the side facing the petiole and tapering to a narrow point in the direction of the leaf margin. Subsequently, the leaf spots turned to necrotic with dark brown margins with orange-white conidial masses (Figure 1A). Seventy isolates of *Myrothecium* sp. were identified using colony, conidia and conidiophore characteristics. Culture characteristics produced hyaline sporodochia and white margins. Spore masses were initially green, later a wet and shiny black. Sporulation often in concentric zones diffused in sporodochia (Figure 1B). The codiniophores were repeatedly branched and bearing phialides (Figure 1C). Conidia were rod shaped with rounded ends and colorless to pale olive green to black in mass. The conidial size was 3.36-4.88 x 0.73-2.10 µm. All characteristics were consistent with description of *M. roridum* (Tulloch, 1972). Moreover, the colonial growth observations indicated that there were five isolates including KKFC 390, KKFC 400, KKFC 402, KKFC 403 and KKFC 408; KKFC 408 had the highest colonial growth rate and spore production. Therefore, these isolates were selected for pathogenicity testing on the water hyacinth in greenhouse condition.

Pathogenicity test for controlling the water hyacinth

Five isolates of *M. roridum* were sprayed on water hyacinth leave and incubated in greenhouse condition. The results showed that there were variations among these isolates in disease virulence. In the preliminary stage of symptom, water hyacinth leaf was necrosis and developed into the form of a spot. After that the symptom became leaf blight with orange-white conidial masses on the water hyacinth leave (Figure 2C). The average disease severity of *M. roridum* isolates KKFC 390, KKFC 400, KKFC 402, KKFC 403 and KKFC 408 were 1.8, 1.0, 1.8, 1.5 and 3.5, respectively, as shown in Table 2. There

Figure 2. Disease symptoms on water hyacinth recorded at 7 days after inoculation. Leaf blight symptoms on inoculated plant (A) control treatment (B) leaf blight symptoms with conidial mass (C).

Table 2. The disease severity on water hyacinth infected by *Myrothecium roridum* isolates.

Myrothecium roridum	Disease severity*
KKFC 390	1.8 ± 0.4^{b}
KKFC 400	1.0 ± 0.0^{c}
KKFC 402	1.8 ± 0.8^{b}
KKFC 403	1.5 ± 0.8^{bc}
KKFC 408	3.5 ± 0.7^{a}
Control	0.0 ± 0.0^{d}

*Means followed by a common letter were significantly different by DMRT (P<0.05).

were statistical differences on disease severity among five isolates. The isolate KKFC 408 showed the highest disease severity on water hyacinth (P<0.05). The results were correlated to the fresh weight observed at 7 days after inoculation (Figure 3). The fresh weight of water hyacinth was reduced when compared with the control treatment**.** The results indicate that *M. roridum* isolate KKFC 408 had a potential for reducing the amount of water hyacinth in the water resource.

Molecular identification based on ITS region

Sequencing data of *M. roridum* isolates KKFC 390 (AB823651), KKFC 400 (AB823652), KKFC 402 (AB823653), KKFC 403 (AB823654) and KKFC 408 (AB823655) were 580-588 bp in length when aligned together with other sequences from the database. The similarity coefficient among five sequences of *M. roridum* was 99.99% when compared with the sequence of *M. roridum* obtained from the database (Figure 4). Moreover, the NJ clustering showed that five sequences of *M. roridum* were in same group with other sequences of *M. roridum* recorded in the database, this was supported by 99% bootstrap value and separated from other species such as *M. gramineum*, *M. verrucaria* and *F. oxysporum* (Figure 5). The result indicated that the five isolates could be identified as *M. roridum* based on ITS sequence which correlated to the morphological based identification.

DNA fingerprint analysis using AFLP markers

Twelve primer combinations were screened with five isolates of *M. roridum* for investigating a suitable primer combinations to be used in this study. The generated fingerprints were evaluated for overall clearness of the banding pattern and the number of polymorphic bands was recorded. The result showed that three of twelve primer recombinations such as *Eco*RI+AGC/*Mse*I+C (19/51), *Eco*RI+C/*Mse*I+ACG (24/ 57) and *Eco*RI+A/*Mse*I+CTA (21/ 26) gave a high number of polymorphic bands. Therefore, these three primer combinations were used to observe the genetic variation among *M. roridum* isolates. The results showed that a total of 166 bands were generated with 116 polymorphic bands among the fifteen isolates of *M. roridum* and one isolate of *Fusarium* sp. (Figure 5). All isolates of *M. roridum* were separated from out group (*Fusarium* sp.) with support by 100% boostrap value and cophenetic values (r) = 0.97. Cluster analysis divided all isolates of *M. roridum* into 4 subgroups at Jaccard's coefficients similarity ≥0.5. The subgroup 1 consisted of isolates KKFC384, KKFC390, KKFC401, KKFC388 and KKFC398; the subgroup 2 included isolates KKFC407, KKFC409 and KKFC410; the subgroup 3 included isolate KKFC403; subgroup 4 consisted of isolates KKFC385, KKFC387, KKFC411, KKFC406 and KKFC389 (Figure 6).

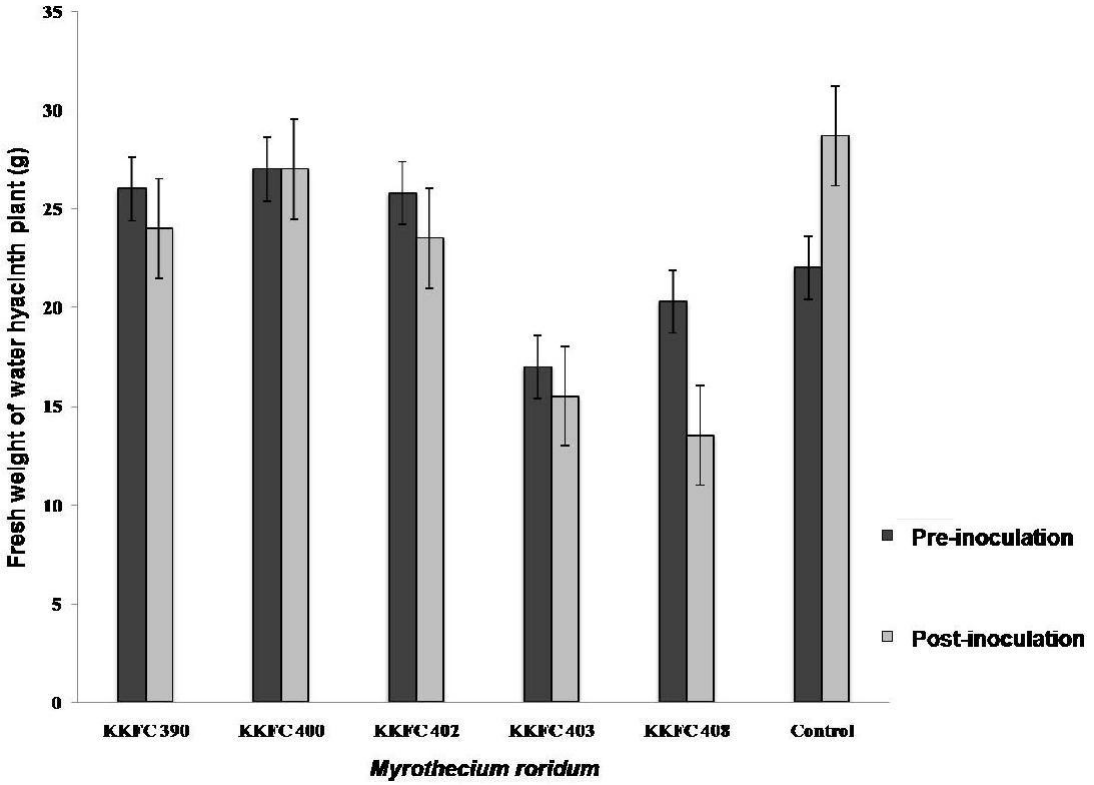

Figure 3. The fresh weight of water hyacinth plants measured before inoculation (black) and 7 days after spraying inoculation (gray).

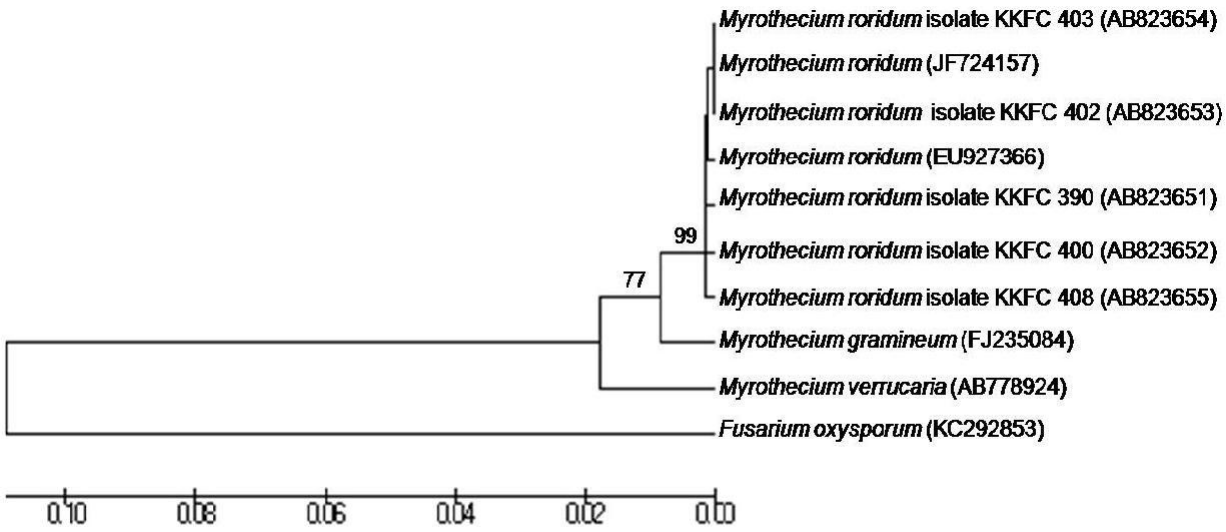

Figure 4. Neighbor-joining phenogram showing the phylogenetic relationship within the species of *M. roridum* and among species of *Myrothecium* based on ITS rDNA sequences. Bootstrap values are indicated on the branches (1000 replication).

DISCUSSION

Myrothecium roridum was specific pathogen to water hyacinth and could cause leaf blight disease. In addition, pathogen produced enzymes and phytotoxic metabolites. Dagno et al. (2012) report showed that *M. roridum* could

Figure 5. AFLP band patterns of *Myrothecium roridum* isolates obtained from three primer recombinations including *Eco*RI+AGC/*Mse*I+C, *Eco*RI+C/*Mse*I +ACG and *Eco*RI+A/*Mse*I+CTA. Lane M = 100 bp Plus DNA Leader; the polymorphic bands are indicated with arrows.

be used for controlling water hyacinth population growth because *M. roridum* produced and released several metabolites such as enzymes, antibiotics and mycotoxins.

There are numerous enzymes that *M. roridum* can produce and release onto the water hyacinth leaf such as cellulases enzymes which hydrolyze the β-1,4-D-glycosidic bonds within the cellulose molecules of plant (Akiba et al., 1995; Baer and Gudmestad, 1995; Zaldivar et al., 2001; Moreira et al., 2005; Okunowo et al., 2010), the series of macrocyclic trichothecenes such as epiroridin E, epi-isororidin E, roridin E, roridin H, trichoverrin A, trichoverrin B, verrucarin A and verrucarin J (Jarvis et al., 1985; Abbas et al., 2002). Wang et al. (2007) confirmed that *M. roridum* produced mycotoxin in group trichothecenes namely Roridin and Isororidin. While, Okunowo et al. (2010) reported that *M. roridum* is capable of producing cellulase and xylanase in submerged cultures containing different carbon sources. In this study, seventy isolates of *M. roridum* were isolated from the water hyacinth leaf blight disease from different geographical area in Thailand.

Five of them were found to be more aggressiveness strains; these strains had pathogenicity when tested on the water hyacinth. The results indicated that *M. roridum* isolate KKFC 408 had the highest disease severity when compared with the other isolates and the control treatment. In these fungal genera, several species of *Myrothecium* have been developed as a mycoherbicide for controlling the water hyacinth and other water weeds such as *M. roridum* (Liyanage and Gunasekera, 1989) and *M. verrucaria (*Hoagland et al., 2007; Okunowo et al., 2010).

In the previous study, Jonniaux et al. (2004) reported on phylogeny obtained from ITS sequences data of *Myrothecium* species including *Myrothecium leucotrichum*, *Myrothecium cinctum*, *M. roridum*, *Myrothecium verrucaria*, *Myrothecium atroviride*, *Myrothecium gramineu* and *Myrothecium inundatum*. The information indicated that the ITS region genes could be used for identification and differentiation among these species of *Myrothecium*. The present study agrees with Jonniaux et al. (2004) and Okunowo et al. (2013), five isolates of *Myrothecium* were consistent with the sequences of *M. roridum* obtained from the GenBank database and are separate from other species of *Myrothecium*.

According to the AFLP analysis, the results showed that the AFLP groups were not correlated to the geographical area. Within the group, there were the fungal isolates collected from different area. The results showed that there was movement of conidia among geographical areas or provinces.

This process is probably relative to genotype flow in which many genes move together as a block in asexual spores. Genotype flow occurs only for organisms that have asexual reproduction in their life cycle (McDonald, 2004). As an example, *F. oxysporum* has only asexual cycle, the genotype of *F. oxyspoum* f.sp. *melonis* (wilt on melon) moves from North America to Israel on the muddy boots of an agricultural scientist. Thus, the entire set of alleles in the clone is introduced into a new population. If this clone has high degree of fitness, it can become established in the new location (McDonald, 2004).

In the previous report, Mohmed et al. (2003) used AFLP fingerprinting to study genetic relationships within and between natural populations of five *Fusarium* species, that is, *F. oxysporum*, *Fusarium solani*, *Fusarium moniliforme* and *Fusarium semitectum* isolated from Egypt. The results of the AFLP analysis enabled the classification of *Fusarium* isolates into f major clusters.

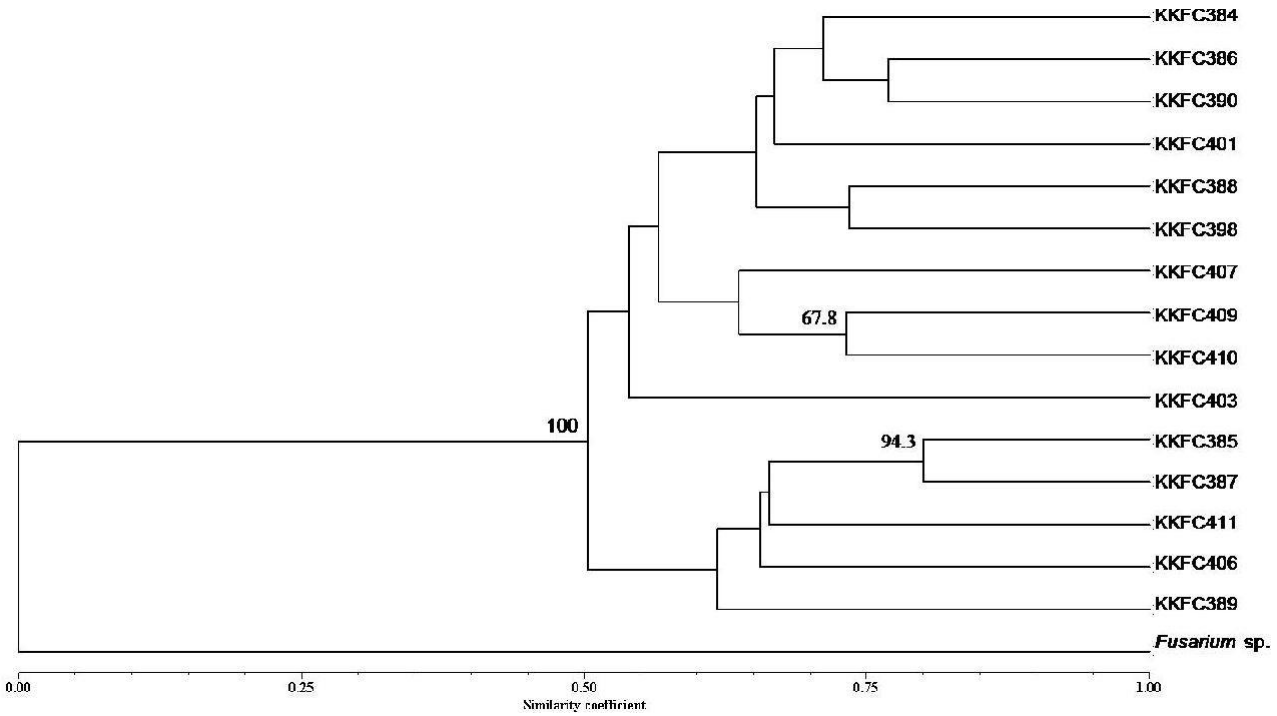

Figure 6. Dendrogram of *Myrothecium roridum* isolates based on the binary matrix of polymorphic bands, using the Jaccard's similarity coefficient and UPGMA algorithm. Bootstrap values above 50% from 1,000 replicates are indicated for the corresponding branch.

There was no correlation between AFLP data and geographic origin, host genotype.

Conclusion

Myrothecium is an effective fungal pathogen to control the water hyacinth in Thailand. The molecular based identification confirmed the species of *Myrothecium* as *M. roridum*. The AFLP based evaluation indicated that the conidial movement occurs among the geographical area, and then they can establish and survive in the new locality.

Conflict of interests

The author(s) have not declared any conflict of interests.

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