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Isolation of Soil and Endophytic Fungi from Rice (*Oryza sativa* L.)

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The objective of study was to isolate, identify fungi from rhizosphere soil and endophytic fungi from rice (*Oryza sativa* L.). Fungi were isolated from rhizosphere soil and healthy leaves, shealths and roots by using the soil plate method and tissue transplanting methods. The results showed that rhizosphere soil fungi were encountered in soil Bangkok Series at 3 sites. The total rhizosphere soil fungi isolates found were 11 species as follows:- *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp., *Chaetomium* spp., *Curvularia* spp., *Fusarium* spp., *Gliocladium* sp., *Phytopthora* spp., *Rhizoctonia* spp., *Rhizopus* spp. *Xylaria* sp. Endophytic fungi were isolated from different parts of healthy plants from 2 rice var. Khao Jow Hawm Suphan Buri and Pathum Thani 80. Endophytic fungi isolates were identified into 9 species, *Aspergillus* spp., *Chaetomium* spp., *Colletotrichum* spp., *Curvularia* spp., *Fusarium* spp., *Penicillium* spp., *Phytophthora* spp., *Rhizopus* spp. and *Trichoderma* spp.

Keywords: Endophytic Fungi, Rhizosphere Soil, Rice

Introduction

Rice belongs to Gramineae, there are only two species of cultivated rice in the world, Asian rice (*Oryza sativa)* and African rice (*Oryza glaberrima*). *Oryza sativa* produces higher yield than African rice, and softer grain easier to mill. (Matsuo *et al*., 1995). It is one of the most important food stable in Thailand. The export was above one million tons, especially in Asia. It is revealed that the agricultural commodity with the third highest worldwide production. The problem in cultivation of rice found that the damage to the rice is often influenced by environmental factors, disease and insect pests which

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reduced in yield (John *et al.*, 2007). Application of chemical fertilizers and pesticides cause chemical residues in agricultural products, and the ecological environment imbalance (Naik *et al*., 2009).

Rhizosphere is a thin layer of soil immediately surrounding plant roots. It is an extremely important and active area for root activity and metabolism. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist (Saharan and Nehra, 2011). It is a source for high diversity of microorganisms which a major role in the enhancement of the plant. The plant and microbe interaction influences the plant growth. Endophytic fungi are found in every plant species. The relationship between the host plant and the fungi is mutualism and the plant does not show disease symptoms. Endophytic fungi have the potential to be used as fertilizer. The major benefit of these microorganisms also promote the growth of plants that live soil *around rice roots* such as nitrogen fixation in rice rhizosphere, growth hormone producer, root have a large surface area, which makes absorbing both water, minerals and nutrients more efficient using osmosis (Rodriguez *et al*., 2009; Strobel *et al*., 2004). 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).

Rhizosphere soil and endophytic fungi can enhance plant productivity and endophyte can survive in the host plant for long periods. Therefore, fungi have the potential for using as a part of integrated pest management or in organic farming systems for sustainable crop production. Further the micorganisms would be screened for production of growth promoting substances which can be used as natural biofertilizers in the enhancement of the yield from rice.

The objectives of this experiment was to isolate of rhizosphere soil and endophytic fungi from rice.

Materials and methods

Isolation of rhizosphere soil

Soil samples were collected from the rhizosphere soil in Bangkok Series. randomly sampling the area around the roots of rice 10 g. The surface soil to a depth of 0-15 cm. Soil samples were isolated fungi by soil plate method on water agar (WA), then incubated at a temperature 28-30 ℃ for 3-5 days, and observed colonies of fungi growing in culture, and a single colony was transferred to be a pure culture. The culture in petri dishes were incubated at 28-30 ℃ for 1-12 days for observation, identification based on morphological characteristics.

Isolation of Endophytic fungi

Sampling plants for isolation of endophytic fungi were isolated from rice by tissue transplanting method. Plant samples were collected from 2 varieties of rice, Khao Jow Hawm Suphan Buri and Pathum Thani 80. The healthy plants without disease symptoms or pest infestation were selected. The samples were processed within 24 hr. Leaves, branches and roots of rice plants were cleaned under running tap water and cut into 3x3 cm segments. Surface sterilization was done by washing with 70% ethanol for 2 min followed by two rinses in sterile distilled water. Leaf, branches and roots segments were placed on petri dishes containing WA for 2-3 days. Endophytic fungal colony growing out from the plant tissue were transferred into PDA plates (Schulz *et al*., 2002). Sterilized tissue segments were placed on to the surface of PDA and incubated in a light chamber at 28-30 ℃ for 1-12 days to yield pure culture**.**

Morphological Identification

All isolates of fungi were grew very quickly, usually covering the whole surface of the petri dish and the showed profuse sporulation on PDA medium. Endophytic fungi were identified on the basis of cultural morphological characteristics of fungi of each isolate, examined on PDA agar in petri dishes. After 1-12 days of incubation, different characteristics of colonies were recorded such as shape, size, elevation, surface, margin, color, pigmentation, fruiting bodies and spores structures on agar medium and observed under stereo and compound microscope, respectively by following the standard mycological manuals of Domsch *et al*. (1993) and Ellis (1971).

Results and discussion

Result showed that the isolates from rhizosphere soil from rice in Bangkok Series as seen in Table 1. It is morphological identified into 11 species as follows:- Aspergillus spp. such as *A. flavus*, *A. niger*, *A. terreus* , *Fusarium* spp., *F. oxysporum* and *F. solani*, *Trichoderma* spp., *T. harzianum*, *Chaetomium* sp., *Ch.cupreum*, *Curvularia* spp., *Gliocladium* sp., *Penicillium* spp., *Pythium* sp., *Rhizoctonia* sp. *Rhizopus* spp. and *Xylaria* sp. as seen in Fig.1.

Table 1. Isolates of rhizosphere soil fungi in Bangkok Series.

No.	Fungal species	Location
1	*Trichoderma harzianum*	Bangkok Series 1
2	*Rhizopus* spp.	Bangkok Series 1
3	*Rhizoctonia* spp.	Bangkok Series 1
4	*Aspergillus niger*	Bangkok Series 1
5	*Chaetomium* sp.	Bangkok Series 1
6	*Phytophthora* sp.	Bangkok Series 1
7	*Trichoderma* spp.	Bangkok Series 1
8	*Penicillium* spp.	Bangkok Series 2
9	*Fusarium oxysporum*	Bangkok Series 2
10	*Aspergillus niger*	Bangkok Series 2
11	*Trichoderma* spp.	Bangkok Series 2
12	*Chaetomium cupreum*	Bangkok Series 2
13	*Fusarium solani*	Bangkok Series 2
14	*Aspergillus flavus*	Bangkok Series 3
15	*Gliocladium* sp.	Bangkok Series 3
16	*Curvularia* spp.	Bangkok Series 3
17	*Xylaria sp.*	Bangkok Series 3
18	*Fusarium* spp.	Bangkok Series 3
19	*Aspergillus terreus*	Bangkok Series 3
20	*Fusarium oxysporum*	Bangkok Series 3

Table 2. Isolates of endophytic fungi from different parts of rice.

Rice varieties	Fungal species	Parts of plants
	Colletotrichum spp.	Leaf
Khao Jow Hawm	*Trichoderma* sp.	Leaf
Suphan Buri	*Aspergillus flavus*	Root
	Penicillium sp.	Leaf
	Chaetomium cupreum	Leaf
	Rhizopus spp.	Root
	Fusarium oxysporum	Stem
	Curvularia lunata	Leaf
	Curvularia lunata	Leaf
Pathum Thani 80	*Aspergillus fiavus*	Root
	Trichoderma harzianum	Stem
	Penicillium sp.	Stem
	Fusarium solani	Leaf
	Pythium spp.	Root
	Aspergillus niger	Root
	Chaetomium globosum	Stem
	Colletotrichum spp.	Leaf
	Chaetomium brasilense	Stem

Result showed that the dominant genera or endophytic fungi from different parts of rice is shown in Table 2. It is found species in this study similar to the findings of previously reports for isolated fungi from soil (Niharika *et al.*, 2012) and isolated endophytic fungi from different parts of rice. Plant samples were collected from rice 2 varieties of Suphan Buri and Pathum Thani 80. The total endophytic fungal isolates were identified as 9 species as seen in Fig. 2. Most of the endophytic fungi isolated from leaves were identified as *Chaetomium cupreum*, *Colletotrichum* spp., *Curvularia lunata*, *Fusarium solani*, *Penicillium* spp., *Trichoderma* sp., followed by endophytic fungi isolated from stems were *Ch. globosum*, *Ch. brasiliense*, *F. oxysporum*, *T. harzianum* and *Penicillium* sp.and *Aspergillus niger*, *A. fiavus*, *Pythium* spp., *Rhizopus* sp. which had been isolated from roots. Similar findings reported by Kaewchai (2009) stated that the endophytic fungi from leaves of Hom Kradung-Nga rices found *Chaetomium* sp., *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., *Fusarium* sp., *Colletotrichum* sp. and endophytic populations were isolated from roots and leaves were *Ch. globosum*, *Penicillium* sp. and *F. oxysporum* which dominant endophytes in this study (Naik *et al.*, 2009; Yuan *et al.*, 2007).

1. *Rhizopus* spp.

Colonies rapidly growing on PDA with fluffy, cotton-candy like growth in under 5 days were recorded. Growth is generally whitish in colour which can turn brown with age as a result of the maturation of the sporangiospores within the sporangium. Pigmented rhizoid and sporangiophore, apophyses and columella often collapse to form an umbrella-like structure, sporangiospores short-ellipsoidal.

Niharika *et al*. (2012) isolated this fungus from soil agricultural fields at Salur Mandal, a total of 15 species belonging to 6 genera of fungi were isolated by using soil dilution technique and soil plate technique on PDA. The most fungi common among them viz; *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. nidulans*, *A. terreus*, *Penicillium chrysogenum*, *P. frequentans*, *P. funiculosum*, *Trichoderma viride*, *T. harzianum*, *Fusarium oxysporum*, *F. solani*, *Curvularia clavata*, *C. lunata* and *Rhizopus stolanifer*.

2. *Penicillium* sp.

Colonies grow rapidly growth on PDA. The surface appearance is usually velvety to powdery. The colony colour varies with the species but is usually green, blue-green or grey-green, often with a white edge. *Penicillium* produces septate, hyaline hyphae. Conidiophores are simple or branched depending on the species, single celled conidia which extend as basipetal chains, conidia globose to subglobose.

Rodolfi *et al.* (2006) reported that endophytic colonisation of seeds by Penicillium, Fusarium and Aspergillus which were detected on more than one Italian rice cultivar. In addition to paddy, endophytic species of *Aspergillus* and *Penicillium* were also discovered from banana leaves and roots (Cao *et al.* 2002)

3. *Xylaria* spp.

The colonies grow quite slowly on PDA. Mycelia are white color, often growing in groups "finger-like or hand shaped" forms which emerge around stumps dark grey to brown, becoming dark black, upper branches appear powdered white, finally tipped black when mature, stalk black and hairy. Stromata are cylindrical, This isolate do not found to produce ascocarp.

Rogers *et al.* (1987) reported the morphological characters of this *Xylaria* spp. is identical with *Xylaria angulosa* found from soil, and endophytic fungi which isolated from living leaves of *Piper aduncum* (Piperaceae), an evergreen small tree used in traditional medicine (Silva *et al.* 2010).

4. *Aspergillus niger*

Colonies grow very rapidly on PDA with a white to yellowish look likes mycelia, quickly turning black as conidia develop the pigment during maturation. Reverse remains white to pale in colour.

5. *Aspergillus flavus*

Colonies grow very rapidly on PDA with surface growth is velvety, downy or powdery, showing various shades of green, most commonly and may have a white border. Texture is often floccose, especially near the center and overall can be velvety to woolly.

Muthomi *et al.* (2012) found *Aspergillus* spp. isolated from whole and unprocessed maize grain and soil from North Rift and Eastern regions as follwos:- *A. flavus, A. niger, A. fumigatus, A. versicolor, A.terreus, A. clavatus* and *A. ochraceus.* The most frequently isolates were *A. flavus* and *A. niger, while A. clavatus* was the least frequently isolate.

6. *Pythium* spp.

Colonies grow fast on PDA with white, fluffy short aerial mycelium that is started to submerge back to the medium surface, mostly colourless, hyphae is by branched. Not all of them produce zoospores but have very large sporangia which are globose to cylindrical, oval, and at times peanut shaped mostly catenulate and intercalary. These structures are densely granulated and the larger one with a clear hyaline central zone.

Owen-Going (2003) found isolate of *Pythium aphanidermatum* in root systems of sweet pepper and some species of *Pythium* are found in field soil, sand, pond and stream water and their sediments, and dead roots of previous crops. *P. irregulare* and *P. cryptoirregulare* are isolated from almost every type of greenhouse crop grown.

7. *Chaetomium globosum*

Colonies grow slowly with little superficial mycelium, ascomata dark brown or black, globose to subglobose; lateral hairs dark brown with paler tips, minutely roughened; terminal hairs dark olive brown with paler tips, wavy or loosely coiled and interwined. Ascospores are pale greenish to dark olive-brown, flattened lemon-shaped, smooth surfaced with apical papillae.

8. *Chaetomium cupreum*

Colonies grow slowly and usually red due to a red pigment. Hyaline septate hyphae. Ascomata are mature within 10-14 days, ascomatal hairs arcuate, apically circinate or coiled, asci are clavate in shape, and ascospores are reniform, with a single apical germ pore.

Kaewchai (2009) study on the endophytic fungi from leaves of Hom Kradung-Nga rice. Endophytic fungi from leaves of rice have frequently isolated as *Chaetomium* sp., *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., *Fusarium* sp., *Colletotrichum* sp.

9. *Curvularia lunata*

Colonies grow rapidly on PDA. Septate hyphae, generally brown in appearance, Conidiophores are brown, erect, simple or branched. geniculate, producing conidia in sympodial order. Conidia are ellipsoidal, often gently curved, brown in colour, usually with 3 to 4 septa.

Raviraja (2005) reported on the fungal endophytes in five medicinal plant species from India which isolated 18 species of endophytic fungi from bark, stem and leaf. The dominant species were *Curvularia clavata, C. lunata*, *C. pallescens and F. oxysporum*. Endophytic fungi was found in the leaf segments rather than the stem and bark segments.

10. *Fusarium oxysporum*

Colony is fast-growing on PDA. Aerial mycelium is sparsed to abundant and becoming felted, white with purple tinge. Conidiophores are shorted, formed singly and branched. Macroconidia shaped in fusiform, slightly curved, 3-5 septate, elliptical or cylindrical, chlamydospores terminal or intercalary in hyphae, smooth or roughen wall.

Tian *et al*. (2004) reported *Fusarium*, *Aspergillus* and *Penicillium* were the most common endophytic fungal genera discovered from healthy paddy plant in China. From healthy leaves and roots of paddy plants, *Penicillium chrysogenum* and *F. oxysporum* were frequently isolated.

11. *Fusarium solani*

Colonies grow rapidly to produce of white floccose (cottony) colonies with aerial mycelium white to cream, becoming bluish-brown. Macroconidia are short multiple branched conidiophores on sporodochia, 3-5 septate, fusiform, cylindrical, often moderately curved, with a short blunt apical cell. Microconidia are usually abundant, cylindrical to oval, 1-2 celled and formed from long lateral phialides. Chlamydospores are hyaline, globose, smooth to rough-walled, borne singly or in pairs on short lateral hyphal branches.

Endophytic fungus isolated from *Taxus baccata* bark. The fungus was identified as *Fusarium solani* (Tayung et al., 2011) and *F. solani* isolated from various crops such as potato, chickpea, wheat, rice, melon, olive and soil.

12. *Trichoderma* spp.

Colonies grow rapidly on PDA with fluffy white tufts, green tufts develop within the colony due to the production of conidia. The reverse is typically a light tan to yellow or pale orange. Septate and hyaline hyphae. Conidiophores are rather short, branching at wide angles, septate, and hyaline hyphae. Conidia are round to ellipsoidal, single celled conidia.

Thongrat *et al*. (2016) reported that isolates from oil palm rhizosphere soil in Trang, Pattani, Phangnga, Phuket and Songkhla provinces found 185 isolates which 16 genera in 26 species:- *Acremonium* spp., *Aspergillus* spp., *Chaetomium* spp., *Chrysosporium* spp., *Cladosporium* spp., *Curvularia* spp, *Eupenicillium* spp., *Fusarium* spp., *Glioladium* spp., *Mucor* spp., *Paecilomyces* spp., *Penicillium* spp., *Rhizopus* spp., *Scopulariopsis* spp., *Talaromyces* spp. and *Trichoderma* spp.

13. *Colletotrichum* spp.

The colony grow very quickly, usually covering the whole surface of the petri dish, and mycelia initially white-grey become dark brown. Conidia produce on conidiophore in acervulus with a single cell, mostly oval shape.

Lapmak *et al*. (2009) studied on the diversity of filamentous fungi associated with brown rice from Pattalung province and identified into 10 genera:- *Acremonium*, *Aspergillus*, *Bipolaris*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Fusarium*, *Geotrichum*, *Nigrospora*, and *Penicillium*. Among these, the most common genus was *Colletotrichum*.

Wongcharoen (2014) stated that rhizosphere soil and endophytic fungi from rice found to be a source of high fungal diversity which play a major role in the enhancement of plant. The plant and microbe interaction influences plant growth. The relationship between the host plant and the fungi is mutualism and the plant does not show disease symptom. The roles of the endophytic fungi in plant disease control. Moreover, endophytic fungi can enhance plant productivity and survive in the host plant for long periods and promote the growth of plants.

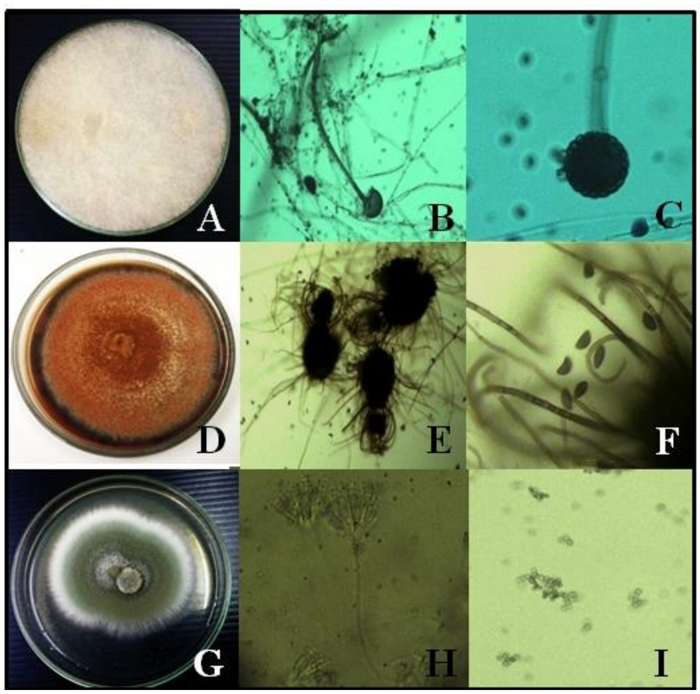

Fig 1. Isolate from rhizosphere soil:- *Rhizopus* spp. (A-C), *Chaetomium cupreum* (D-F), *Penicillium sp.* (G-I)

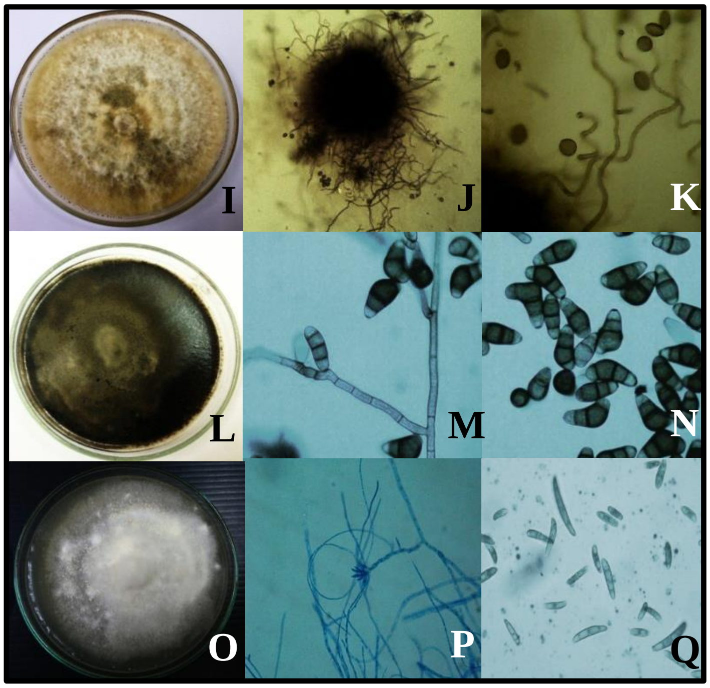

Fig 2. Endophytic fungi from different parts of two varieties of rice 2. *Chaetomium globosum* (J-L), *Curvularia spp.* (M-O) and *Fusarium spp.* (P-R

Isolation of soil and endophytic fungi from rice (*Oryza sativa* L.). Of which, 11 species of rhizosphere soil fungi fro rice in Bangkok Series. The most frequently isolates were *Aspergillius* spp., *Fusarium* spp. and *Trichoderma* spp. Endophytic fungi were isolated from different parts of two varities of rice. The endophytic fungal isolates were found 9 species. The most fungi frequently found were *Aspergillus* spp., *Chaetomium* spp., *Fusarium* spp., *Penicillium* spp., *Trichoderma* spp.

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