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Morphological and Physiological Development of *Pyricularia oryzae* Isolates from North-western Region of Sarawak on Different Media under Laboratory Conditions

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ABSTRACT

Rice blast (causal agent: *Pyricularia oryzae)* is an important disease of rice in Sarawak. Understanding the pathogen's morphological characteristics, genetic diversity and pathogenicity is important. Having a suitable medium for culturing and maintaining *P. oryzae* is important to ensure the availability of inoculum or materials under laboratory conditions. Oatmeal agar (OMA) and potato dextrose agar (PDA) are common media used for growing *P. oryzae*. OMA allows better mycelial growth and better sporulation as compared to PDA. There are also other alternatives such as fresh rice leaf agar and rice straw agar. Although OMA seems to be the best medium, unfortunately the opaqueness of the medium causes difficulty in observing the morphology and growth of mycelia. In addition, it is known that different isolates of *P. oryzae* will respond differently to different media. This study aims to identify the best media for culturing and maintaining *P. oryzae* isolates from Sarawak. A total of 14 *P. oryzae* isolates were characterised for their morphological characteristics, growth rate and sporulation rate using seven growing media. These 14 isolates included seven newly identified isolates in this study and seven isolates from a previous study, which were verified using internal transcribed spacer DNA sequence. The colony surface of the 14 *P. oryzae* isolates varied on different growing media. The pigmentation of colony surface varied from different shades of grey, translucent light brown, white and colourless. *Pyricularia oryzae* isolates grew better on OMA and PDA, while OMA was the best for sporulation. These two media can be recommended for culturing and maintaining different *P. oryzae* isolates under laboratory conditions.

Keywords: Growth media, *Pyricularia oryzae*, rice blast, Sarawak, sporulation

INTRODUCTION

Rice feeds 50% of the world population (Zhou, 2016). Rice production can be at stake due to diseases and pest infestations, which may lead towards the economic losses and threat to food security. Rice blast is one of the most important rice diseases, caused by ascomycetes fungus *Pyricularia oryzae* (teleomorph: *Magnaporthe oryzae*). The fungus can attack at different growth stages and on almost every part of paddy plant such as leaf, neck, sheath, nodes, panicle, and pedicels. Rice blast infection typically appeared as diamond-shaped lesions with greyish centre and brown margin on leaves. The lesions may grow rapidly under favourable conditions and tend to merge leading to plant death (Wang *et al.*, 2014).

Rice blast has caused 10 – 30% global yield loss annually (Skamnioti & Gurr, 2009; Zhou, 2016) and up to 70% yield loss in Malaysia (Gianessi, 2014). A survey conducted by Malaysian Agricultural Research and Development Institute (MARDI) in 2015 showed 43% of rice field in Peninsular Malaysia were infected by rice blast (Harun, 2015). In 2017, rice blast disease had infected 10% of rice field area under Muda Agricultural Development Authority (MADA) and approximately 10,000 tons of rice grains were lost, causing severe economic loss up to 50% of production cost (Suzalina, 2017). In Sarawak, 58% of the surveyed rice fields (128 farms in total) between the year 2009 to 2012 were infected by *P. oryzae* with disease severity ranging from moderate (6 – 25% affected leaf area) to high (>50% affected leaf area) (Lai & Eng, 2011, 2013; Lai, 2016).

Studies on the morphological characterisation, genetic diversity, and pathogenicity of *P. oryzae* are important towards controlling rice blast disease. These studies require the fungus itself to be isolated from the field, cultured, and maintained using suitable medium before performing *in vitro* or *in vivo* experiments (Pandey, 2014). Experiments may require either mycelia or spores, so *P. oryzae* needs to be grown on a suitable medium that supports its growth and induces sporulation of the fungus (Gad *et al.*, 2011).

Various factors such as type of medium, pH, temperature, incubation period, light exposure, and moisture level affect the growth of *P. oryzae* (Gad *et al.*, 2011; Pandey, 2014). Oatmeal agar (OMA) and potato dextrose agar (PDA) are common media used for growing *P. oryzae*. OMA allows better mycelial growth (Kulmitra *et al.*, 2017; Manjunatha & Krishnappa, 2019) and better sporulation (Hussin *et al*., 2020) as compared to PDA. Previous study reported that fresh rice leaf agar, OMA, and rice straw agar are the best media for sporulation and mycelial growth of *P. oryzae* (Gohel & Chauhan 2015). Czapek-Dox medium was reported as inappropriate medium for *P. oryzae* growth because it suppresses mycelial growth and do not support sporulation of the fungus (Gad *et al.*, 2011; Pandey, 2014; Gohel & Chauhan, 2015). The most favourable pH level for maximum mycelial growth of *P. oryzae* on medium is between 5 and 7, while at pH 4, no sporulation is recorded with least mycelial growth of *P. oryzae* (Pandey, 2014; Gohel & Chauhan, 2015). The optimum temperature for culturing *P. oryzae* with maximum mycelial growth ranges from 25 °C (±0.5 °C) (Gohel & Chauhan, 2015) to 27 °C (Rajput *et al.*, 2017). A combination of 16/8 hours of light/dark interval for culturing *P. oryzae* was reported to be most suitable condition for mycelial growth and spore induction (Hosseini-Moghaddam & Soltani, 2013). Relative humidity (RH) of ≥ 96% was reported as the optimum RH to induce sporulation for *P. oryzae* (Li *et al.*, 2014).

For *P. oryzae* isolates from Sarawak, Hussin *et al.* (2020) reported OMA supported growth and sporulation of their seven isolates. OMA, however, is opaque which hamper the observation of morphology and mycelial growth of *P. oryzae*. It is also known that different isolates of *P. oryzae* would respond differently to different media. Therefore, this study was to test additional media and select the best medium for culturing and maintaining *P. oryzae* isolates from Sarawak.

MATERIALS AND METHODS

Sample Collection

Rice blast infected leaf samples from lowland and upland paddy landraces were collected from different paddy fields (smallholder) in four different divisions of Sarawak: Serian, Sri Aman, Kuching, and Miri. For this study, lowland paddy is those planted in rainfed or irrigated (flooded) ecosystems. It is also known as wet paddy. Upland paddy is those planted in rain-fed dryland (IRRI, n.d). It is commonly known as 'padi bukit' by the locals (Yusop *et al.*, 2021).

Media Preparation

A total of eight media were used in this study: water agar (WA), OMA, PDA, malt extract agar (MEA), rice agar (RA), cornmeal agar (CMA), barley agar (BA), and glucose agar (GA). WA was used for fungal isolation, and the other media were used to study the morphological variation and sporulation rate. Ingredients used for media preparation are shown in Table 1. The pH of the media (except WA) was adjusted to 6.5 using 1.0 M NaOH and 1.0 M HCl. The media were sterilised at 121 °C at 15 psi for 15 minutes.

Table 1. List of ingredients used in preparing water agar (WA), oatmeal agar (OMA), potato dextrose agar (PDA), malt extract agar (MEA), rice agar (RA), cornmeal agar (CMA), barley agar (BA), and glucose agar (GA) (modified from Kumar *et al.* (2005))

Media	Ingredients (for 500 ml)
WA	• 10 g of agar stick
OMA	• 15 g of instant oatmeal • 7.5 g agar stick
PDA	• Commercial brand: Lab M®
MEA	• Commercial brand: Merck
CMA	• 10 g cornmeal • 10 g agar stick
BA	• 10 g barley grain • 10 g agar stick
RA	• 10 g rice grain • 10 g agar stick
GA	• 15 g glucose • 7.5 g agar stick

Fungal Isolation

Spore-drop isolation method was according to Hussin *et al.* (2020). Each rice blast lesion on an infected leaf was cut in half with each half of the lesion having a section of healthy part at one end. The leaf segments were surface sterilised with one percent sodium hypochlorite for 1 minute and rinsed three times with sterilised distilled water (each lesion was treated separately). Each leaf segment from a lesion was then attached onto the lid of a Petri dish containing WA with adaxial surface facing towards the medium. Subsequently, the plates were incubated in a humidity box at room temperature and observed daily for single spore colony of *P. oryzae* under a light microscope (ECLIPSE E100LED MV R).

Single spore colony was then picked and transferred onto OMA. The plates were incubated under dark condition for 5 days and under fluorescent light condition for the subsequent days, at room temperature maintained at ca. 28 ^{o}C. Alternatively, if spores were observed on leaf segment but no single spore colony on WA, the spores were dislodged with 250 µl of sterilised distilled water and spread on a new plate of WA. The plate was then incubated under light condition at room temperature. Single spore colony of *P. oryzae* was picked and cultured as described above.

Molecular Identification

Universal primer pair Internal Transcribed Spacer (ITS), ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') was used for colony PCR (White *et al.,* 1990). In 25 µL, the PCR reaction comprised of sterilised distilled water, 1X PCR Buffer with Mg^{2+} (EasyTaq), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM ITS-1 and ITS-4, 1.5 units *Taq* DNA Polymerase (Easy*Taq*) and a pinch (using forceps) of young fungal mycelium (culture age ranged from 5 to 10 days) was used as DNA material. PCR amplification was performed using T100™ Thermal Cycler (Bio-Rad Laboratories, USA) with the following profile: initial denaturation at 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, extension at 72 °C for 30 seconds and final extension at 72 °C for 5 minutes.

The PCR amplicons were visualised on 1% agarose gel and purified by using QIAquick Gel Extraction Kit. Purified PCR amplicons were then sent for sequencing to Apical Scientific Sdn. Bhd. The sequences obtained were BLAST using BLASTn from National Centre for Biotechnology Information (NCBI) website (Altschul *et al.*, 1990; Morgulis *et al.*, 2008). The species verification was determined through percentage of identity and expectation value (E-value).

Morphological Characterisation on Different Growing Media

Morphological characterisation method described by Mohammadpourlima *et al.* (2017) was adapted. There were seven types of media used for morphological characterisation: OMA, PDA, MEA, RA, CMA, BA, and GA (Table 1). Ten isolates from lowland paddy; three from current study and seven from Hussin *et al.* (2020), were cultured on six different media: OMA, PDA, MEA, BA, CMA and RA, while the other four isolates from upland paddy were tested on five different media: OMA, PDA, MEA, RA, and GA (Table 2).

Ten days old cultures were used as the inoculum source. An inoculum plug (5 mm Ø) was cut from the edge of actively growing mycelia and transferred (mycelium-side down) onto the centre of a Petri dish containing 20 ml media. For each medium, there were 14 replicates per isolate. The Petri dishes containing fungal plugs were then incubated for 5 days in dark condition and subsequent days under light condition, at room temperature maintained at ~28 ^{o}C. Morphological characteristics of the colonies (form, elevation, margin, colour, and surface) were described on the 10th day after inoculation.

The colony radial growth was measured along the perpendicular lines drawn on Petri dish (Figure 1). Measurement was done daily until the 10th day. Average radial growth was calculated by using Eq. (1) followed by growth rate using Eq. (2). Then, spores were collected from three randomly selected plates on the 20th day. Approximately 1 ml of 0.05% Tween 20 solution was added onto the surface of fungal mycelia. A sterilised spatula was used to scrap the mycelia off the agar, and the spore suspension was filtered by using sterilised

Table 2. List of *Pyricularia oryzae* isolates from different regions of Sarawak

	Isolate	Location	Division	GPS	Paddy Landrace	Lowland/ Upland
From this study	POS14	Kg. Tema Penggal	Serian	N1°00'5.6", E110°52'50.3"	Unknown	Upland
	POS17	Jalan Patung, Kg. Riih Mawang	Serian	N1°08'14.1", E110°26'11.2"	Unknown	Upland
	POS18	Jalan Patung, Kg. Riih Mawang	Serian	N1°08'14.1", E110°26'11.2"	Unknown	Upland
	POS19	Jalan Patung, Kg. Riih Mawang	Serian	N1°08'14.1", E110°26'11.2"	Unknown	Upland
	POK1	Senibong, Lundu	Kuching	N01°35'48.0", E109°53'22.2"	Unknown	Lowland
	POS4	Paon Gahat	Serian	N0°56'41.5", E110°39'16.3"	Wangi	Lowland
	POMI1	Bario	Miri	N03°45'14.6", E115°26'55.3"	Adan	Lowland
Hussin *et al.* (2020)	POS2	Kg. Remun	Serian	N1°08'19.9", E110°39'02.9"	Bajong	Lowland
	POK6	Kg. Stunggang, Lundu	Kuching	N1°38'58.9", E109°51'15.9"	Unknown	Lowland
	POK3	Pueh, Sematan	Kuching	N01°35'48.0", E109°53'22.2"	Unknown	Lowland
	POSA1	Stumbin	Sri Aman	N1°18'06.8", E111°22'46.6"	Unknown	Lowland
	POK4	Kg. Siru Melayu, Sematan	Kuching	N01°49'51.9", E109°43'38.7"	Unknown	Lowland
	POSA2	Tanjung Bijat	Sri Aman	N1°20'58.8", E111°23'00.9"	Unknown	Lowland
	POMI2	Bario	Miri	N03°45'14.6", E115°26'55.3"	Adan	Lowland

muslin cloth. A volume of 10 µl of spore suspension was added into the loading chamber of haemocytometer. Sporulation rate was estimated based on spore count on the haemocytometer.

$$Average\ radial\ growth\ (r) = \frac{a+b+c+d}{4} \quad \text{Eq. (1)}$$

$$Growth\ rate = \frac{(r2-r1)+(r3-r2)+\cdots}{N-1} \quad \text{Eq. (2)}$$

where a, b, c, d – Reading on axis A, B, C and D (respectively) of Petri dish; r1, r2, r3 – average radial growth on day 1, 2, 3…; N - total days of incubation period.

Statistical analyses were carried out using SPSS software (Version 20). Analysis of variance (ANOVA) followed by Tukey HSD post hoc test was used to compare the mean growth rate and sporulation rate of isolates between media at 0.05 significance level except for CMA, BA, and GA due to missing data. Pearson correlation test was also performed to observe any correlation between mycelial growth rate and sporulation rate of isolates on each medium at 0.05 significance level except for CMA, BA, and GA due to missing data.

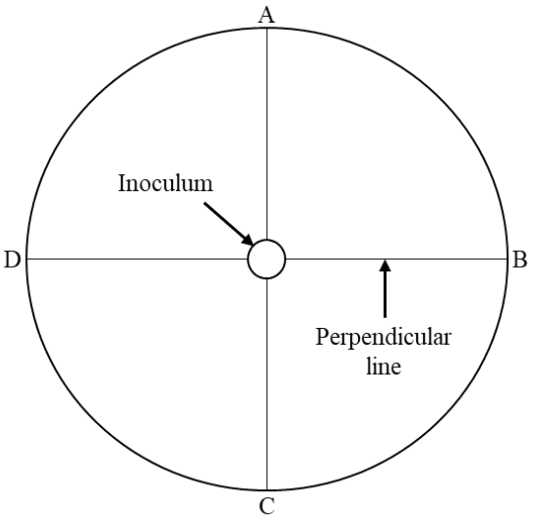

Figure 1. Illustration of *Pyricularia oryzae* inoculum position and perpendicular lines drew on Petri dish used in experiment

RESULTS AND DISCUSSION

Isolation and Identification

Infected leaf samples collected had typical rice blast disease symptom (Figure 2a), diamond shape with greyish centre and brown margin (Wang *et al.*, 2014; Hussin *et al.*, 2020). The spores were borne along the conidiophore (Figure 2b) with the basal of the spore attached at the tip of branches of conidiophore. In average, one conidiophore holds more than 10 spores (number of plates observed = 3). The morphology of the spores obtained showed pear-shaped with narrowed apex and broad basal, hyaline in colour, two septa and three celled (Figure 2c).

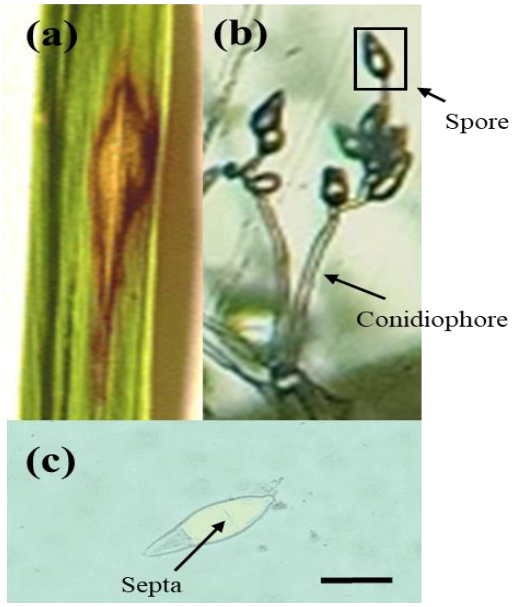

Figure 2. (a) Rice blast symptom on paddy leaf (b) Structure of conidia and conidiophore at 10x magnification (c) Spore of *Pyricularia oryzae* and septa structure at 40x magnification. Scale bar = 10µm

Table 2 shows seven isolates, isolated from different regions of Sarawak. The seven isolates were preliminarily identified based on the spore morphology (Figure 3). The spore for the isolates obtained matched the spore descriptions from previous studies of *P. oryzae* (Hussin *et al.*, 2020; Ou, 1985; TeBeest *et al.*, 2007). ITS amplification was successful for the seven isolates (Table 3). The sequences obtained were between 290 and 500 bp. Based on the percentage of identity and E-value from BLASTn, the isolates were verified as *P. oryzae*.

The isolates were 99 – 100% similarity to the target sequence of *M. oryzae* in the database with E-value near to or equal to zero.

Morphological Characterisation

Table S1 summarises the morphological characteristics for all isolates. All 14 isolates were grouped based on the similarities of their morphology (form, elevation, margin, and surface), excluding the colony colour. There were eight groups of morphology characterisations for OMA (Figure S1), nine groups for PDA (Figure S2), six groups for MEA (Figure S3), four groups for RA (Figure S4) and BA (Figure S6), three groups for CMA (Figure S5) and two groups for GA (Figure S7).

Based on the observation, the colony surface of the different isolates varied from smooth and fluffy to rough and flattened mycelia; some had concentric rings, perforated, corrugated-like surface and some with aerial mycelia. The elevation (viewed from side way) was either raised or flat. All isolates grew on RA and GA showed flat elevation, while the other five types of media had either raised or flat elevation.

Table 3. Summary of BLASTn search results for ITS sequence of the *Pyricularia oryzae* isolates from this study. All isolates were identified as *Pyricularia oryzae*

Isolate	Accession number	Amplicon size (bp)	Percentage of identity (%)	E-value	NCBI accession number with highest similarity
POS17	OP874839	295	100	9e-152	MT023687
POS18	OP874840	450	99.33	0.0	MN338375
POS19	OP874841	496	99.8	0.0	MK880271
POS14	OP874838	249	100	1e- 125	MT023687
POS4	OP874837	490	100	0.0	MH715386
POK1	OP874835	490	100	0.0	MH715386
POMI1	OP874836	296	100	9e-152	MH715393

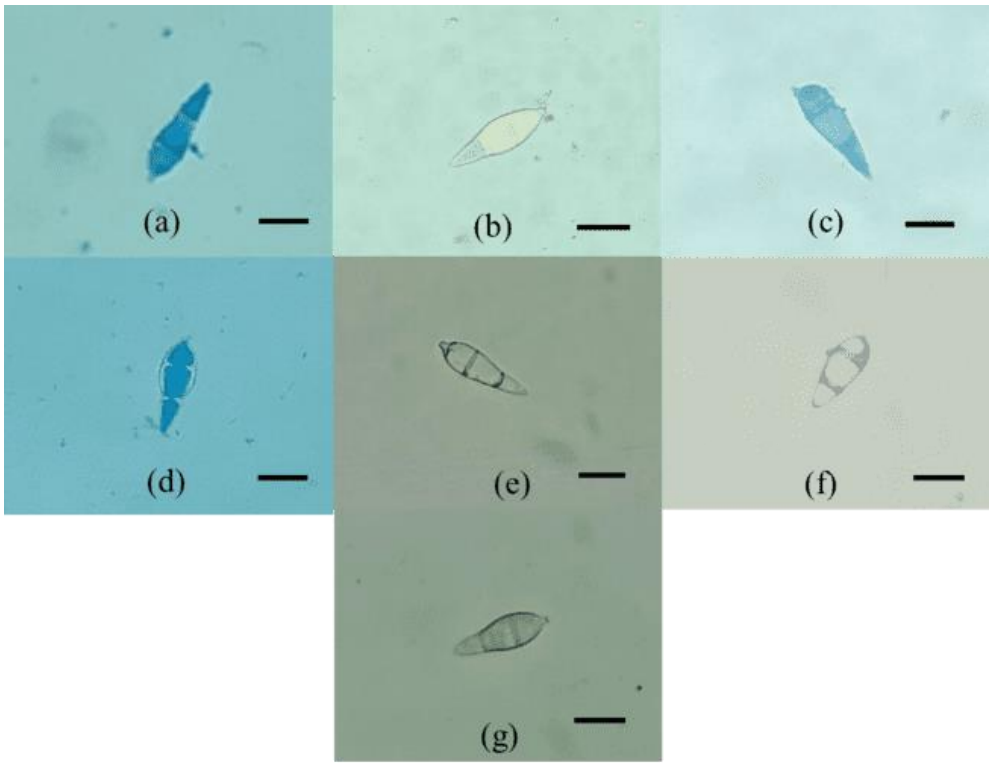

Figure 3. Spores of seven *Pyricularia oryzae* isolates under light microscope at 40x magnification (a) POS14 (b) POS17 (c) POS18 (d) POS19 (e) POK1 (f) POS4 (g) POMI1. Scale bar = 10 µm

Front pigmentation of the colony varied from dark grey to light grey, some with brownish colour, translucent grey or brown in colour. For reverse-view pigmentation, the colony varied from white to dark with different shades of black and brown. Most isolates had greyish colony pigmentation on the front view and brownish or greyish pigmentation for the reverse view. Despite of media used, all isolates had circular form and entire margin.

In summary, all 14 isolates isolated from four divisions showed morphological variations, which suggests that the isolates might be genetically different from each other. There is also possibility that the 14 isolates are having different pathogenicity capability based on the variation of dark pigmentation observed on the colony surface of the isolates. Dark pigmentation of colony surface has been reported to correlate with pathogenicity of isolate (Lujan *et al.*, 2016; Oh *et al.*, 2017). This could be due to the presence of melanin pigment which is important in formation of melanized appressorium for successful penetration into the host cell wall (Motoyama, 2020). It was also reported that melanin promotes sporulation of *P. oryzae* (Huang *et al.*, 2022). Studying the correlation of *P. oryzae* pathogenicity and sporulation to the intensity of *P. oryzae* colony surface pigmentation could be an interesting area of further research.

The isolates with the same morphology are not necessarily originated from the same location. For example, all *P. oryzae* isolates from Kuching were grouped differently based on their morphology (Figure 4). This finding is supported by Srivastava *et al.* (2014) who stated that there is no correlation between geographical distribution and morphological characteristics between *P. oryzae* isolates. However, this does not conclude that isolates from the same location are genetically unrelated. A study by Abed-ashtiani *et al.* (2016) reported that there is positive correlation between molecular data and geographical origin of *P. oryzae* isolates from Peninsular Malaysia, where isolates from the same location tend to cluster together based on two different markers.

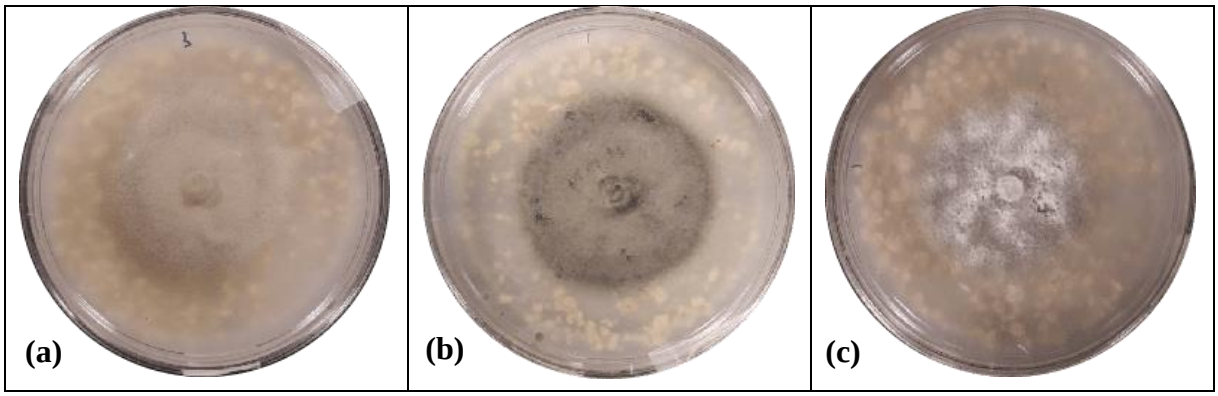

Figure 4. *Pyricularia oryzae* isolates from Kuching (a) POK6 (b) POK3 (c) POK1

Growth Rate and Sporulation Rate

Growth rate and sporulation rate are tabulated in Table 4 and Table 5. Data on growth rate of all 14 isolates on PDA and RA were complete, but there were some missing data for some isolates on either one (e.g., OMA, MEA), or more media (e.g. CMA, BA, and GA). Growth rate of an isolate varied on different media, and it was significantly different between isolates on different media (on all media $p<0.01$). A few isolates had higher growth rate on most of the media but slower growth rate on other media and vice versa. For example, isolate POK6 was among the isolates with fast, if not the fastest (Table 4), growth rate on PDA (0.37 cm/day ± 0.01) and RA (0.34 cm/day ± 0.01) but was slower compared to other isolates on other media. Isolate POK3 was among the slower growth isolate on PDA (0.29 cm/day ± 0.03), but was among the isolates with intermediate, if not fast growth rate on other media. These variations of growth rate on different media might suggest that different isolates might have different nutritional needs or preferences, or different metabolism rate (Mohsen *et al.*, 2016).

Table 4. Growth rate summary of *Pyricularia oryzae* on oatmeal agar (OMA), potato dextrose agar (PDA), malt extract agar (MEA), rice agar (RA), cornmeal agar (CMA), barley agar (BA) and glucose agar (GA)

Isolate	Growth rate (cm/day)													
	PDA	Std. error	OMA	Std. error	MEA	Std. error	RA	Std. error	CMA	Std. error	BA	Std. error	GA	Std. error
POK1	0.35^{fg}	0.0032	0.36^{f}	0.0044	0.31^{fg}	0.0038	0.34^{h}	0.0013	0.37^{e}	0.002	0.36^{e}	0.0022	-	-
POK3	0.29^{ab}	0.0067	0.32^{cd}	0.0017	0.30^{ef}	0.0026	0.32^{fg}	0.0046	0.34^{bc}	0.0032	0.34^{de}	0.0038	-	-
POK4	0.32^{de}	0.0034	0.32^{bc}	0.0045	0.29^{de}	0.0031	0.29^{bcd}	0.0057	0.31^{a}	0.0058	0.31^{ab}	0.0062	-	-
POK6	0.37^{g}	0.0027	0.34^{de}	0.0019	0.33^{g}	0.0024	0.34^{h}	0.0028	0.34^{cd}	0.0022	0.35^{e}	0.0015	-	-
POMI2	0.32^{de}	0.0043	0.32^{cd}	0.0033	0.25^{a}	0.0025	0.30^{cde}	0.0055	0.33^{b}	0.0027	0.32^{b}	0.0033	-	-
POS2	0.35^{f}	0.0017	0.33^{de}	0.0049	0.31^{fg}	0.0027	0.32^{ef}	0.0034	0.35^{cd}	0.0024	0.34^{de}	0.004	-	-
POS4	0.32^{de}	0.0041	0.32^{bcd}	0.0024	0.28^{cde}	0.003	0.33^{gh}	0.0017	0.34^{bc}	0.001	0.33^{cd}	0.0013	-	-
POSA1	0.30^{bc}	0.0009	0.32^{bcd}	0.0019	0.27^{bcd}	0.0031	0.31^{de}	0.0017	0.33^{b}	0.0017	0.32^{bc}	0.0021	-	-
POSA2	0.33^{de}	0.0037	0.35^{e}	0.0022	0.31^{g}	0.0049	0.34^{gh}	0.0041	0.35^{de}	0.0025	0.34^{de}	0.0037	-	-
POMI1	0.29^{ab}	0.0042	-	-	-	-	0.30^{bcd}	0.0015	0.30^{a}	0.0016	0.30^{a}	0.0023	-	-
POS14	0.27^{a}	0.0057	0.28^{a}	0.0045	0.27^{bcd}	0.0024	0.26^{a}	0.0046	-	-	-	-	0.18^{a}	0.0051
POS17	0.33^{e}	0.0025	0.32^{cd}	0.0038	0.27^{bcd}	0.0044	0.28^{b}	0.0033	-	-	-	-	0.26^{b}	0.0023
POS18	0.31^{cd}	0.0031	0.31^{b}	0.0049	0.26^{ab}	0.007	0.29^{bc}	0.002	-	-	-	-	0.25^{b}	0.0051
POS19	0.31^{cde}	0.0028	0.31^{bc}	0.0025	0.27^{bc}	0.0021	0.30^{bcd}	0.004	-	-	-	-	0.25^{b}	0.0058
Mean	0.32		0.32		0.29		0.31		0.34		0.33		0.23	

Note: ANOVA followed by Tukey HSD post hoc test was used to compare the difference in growth rate between isolates in each medium at 0.05 significance level. The same alphabet within column indicates the variances do not differ significantly.

Table 5. Sporulation rate summary of *Pyricularia oryzae* on oatmeal agar (OMA), potato dextrose agar (PDA), malt extract agar (MEA), rice agar (RA), cornmeal agar (CMA), barley agar (BA) and glucose agar (GA)

Isolate	Sporulation rate (10^5/5mL)													
	PDA	Std. error	OMA	Std. error	MEA	Std. error	RA	Std. error	CMA	Std. error	BA	Std. error	GA	Std. error
POK1	0.03[a]	0.0133	0.12[a]	0.025	0.04[a]	0.0147	0.03[a]	0.008	0.06[a]	0.0201	0.07[a]	0.0111	-	-
POK3	0.33[a]	0.1351	2.08[ab]	0.6906	0.08[a]	0.0273	0.50[ab]	0.0931	1.79[b]	0.3369	2.23[c]	0.3992	-	-
POK4	0.07[a]	0.0086	0.19[a]	0.0291	0.03[a]	0.0042	0.04[a]	0.0191	0.33[a]	0.1245	0.15[a]	0.0267	-	-
POK6	0.73[a]	0.1335	1.79[ab]	0.1817	0.09[a]	0.0287	0.04[a]	0.0111	0.38[a]	0.0789	0.26[a]	0.02	-	-
POMI2	0.06[a]	0.0167	0.16[a]	0.05	0.06[a]	0.0232	0.02[a]	0.0017	0.09[a]	0.0231	0.34[ab]	0.1246	-	-
POS2	5.39[b]	1.2594	1.87[ab]	0.6863	0.08[a]	0.0133	0.60[b]	0.2335	3.47[c]	0.4443	2.27[c]	0.3718	-	-
POS4	0.03[a]	0.0042	0.09[a]	0.0186	0.04[a]	0.011	0.03[a]	0.0111	0.02[a]	0.0051	0.04[a]	0.0133	-	-
POSA1	0.18[a]	0.0138	0.07[a]	0.0102	0.04[a]	0.0143	0.03[a]	0.0068	0.06[a]	0.0096	0.09[a]	0.0138	-	-
POSA2	0.06[a]	0.0182	3.67[bc]	0.5498	1.09[c]	0.1977	0.05[a]	0.016	0.08[a]	0.0152	0.07[a]	0.0088	-	-
POMI1	0.09[a]	0.0563	-	-	-	-	0.03[a]	0.0054	0.12[a]	0.0148	1.02[b]	0.0249	-	-
POS14	0.95[a]	0.4016	5.44[c]	1.2024	0.25[ab]	0.0133	-	-	-	-	-	-	0.07[a]	0.0133
POS17	1.03[a]	0.2871	2.53[ab]	0.8308	0.26[ab]	0.0143	-	-	-	-	-	-	-	-
POS18	0.75[a]	0.1739	1.52[ab]	0.3413	0.47[b]	0.0392	1.63[c]	0.0933	-	-	-	-	0.08[ab]	0
POS19	1.07[a]	0.1852	4.29[bc]	1.0149	0.15[ab]	0.0481	1.00[b]	0.3002	-	-	-	-	0.20[b]	0.04
Mean	0.79		1.83		0.21		0.33		0.64		0.65		0.12	

Note: ANOVA followed by Tukey HSD post hoc test was used to compare the difference in growth rate between isolates in each medium at 0.05 significance level. The same alphabet within column indicates the variances do not differ significantly.

Sporulation of nine *P. oryzae* isolates was observed on all seven media. Sporulation rate for another five isolates were not observed either in one or few media. There were significant differences on the sporulation rate observed on different media (on all media $p<0.01$ except GA, $p<0.05$). The sporulation rate of each isolate was also varied on different media (Table 5). For example, isolate POS2 was the isolate or among the isolates having the highest sporulation rate on PDA, CMA and BA. Sporulation rate of isolate POS2 was approximately 5.39 x 10^5/5 ml of spores on PDA, but only sporulated 0.08 x 10^5/5 ml of spores on MEA. Drastic change in sporulation rate on different media can also be observed for isolate POSA2, POS14 and POS19, to name a few.

Table 6 shows the comparison of mean growth rate and sporulation rate of 14 *P. oryzae* isolates on OMA, PDA, MEA, and RA. Based on the result, isolates grown on OMA (0.32 cm/day ± 0.02) and PDA (0.32 cm/day ± 0.03) had the fastest average growth rate as compared to their growth on MEA (0.29 cm/day ± 0.03) and RA (0.31 cm/day ± 0.03). The highest mean sporulation rate was recorded on OMA (1.83^5/5 mL ± 2.17). The sporulation performance of isolates in this study shows that PDA (0.79^5/5 mL ± 1.62) might not be a preferable medium for sporulation. Only one isolate, POS2 showed good sporulation rate on PDA (5.39^5/5 ml ± 3.09).

OMA was reported as the most favourable media for mycelial growth (Gad *et al*., 2011; Kulmitra *et al.,* 2017; Manjunatha & Krishnappa, 2019) and sporulation of *P. oryzae* (Gad *et al.,* 2011; Gohel & Chauhan, 2015; Hussin *et al.,* 2020). PDA also being reported as the best medium for *P. oryzae* mycelial growth (Pandey, 2014; Hussin *et al.*, 2020). The findings above were in accordance with our present result. Meanwhile, RA was reported as fair medium for sporulation (Kulkarni & Peshwe, 2019) which also agrees to our finding where *P. oryzae* isolates in this study sporulated less on RA compared to OMA but no significant difference from PDA. Interestingly, a study by Vanaraj *et al.* (2013) reported that *P. oryzae* was rapidly growing on MEA. This is contradicting to the result of this study, where MEA in general, does not support the mycelium growth as well as sporulation of the *P. oryzae* isolates (Table 6).

Table 7 shows the correlation between growth rate and sporulation of *P. oryzae* on each tested media. Blast isolates grown on PDA and MEA show weak positive significant correlation ($p<0.05$) while weak negative significant correlation was shown on OMA

($p<0.05$) and moderate negative significant correlation shown on RA ($p<0.01$). This result indicates that isolates having a high growth rate might not necessarily have high sporulation rate. For instance, isolate POK1 grown on OMA had the highest growth rate but was among the isolates that had the lowest sporulation rate.

The change of growth and sporulation rate pattern of each *P. oryzae* isolate on different media in this study shows that different media affect the physiology of isolates differently. Some isolates in this study can sporulate in wider range of media such as isolate POS2 that had good sporulation rate in PDA, CMA, and BA compared to other isolates. Isolates that can sporulate very well in a particular medium also not necessarily can grow well on the same medium. For example, isolate POS14 had the highest sporulation rate but the slowest growth on OMA. Hence, it is important to have the knowledge on the suitability of the medium as a good substrate especially when dealing with pathogens that possess varied physiological performance.

Table 6. Comparison of mean growth rate and sporulation of *Pyricularia oryzae* on oatmeal agar (OMA), potato dextrose agar (PDA), malt extract agar (MEA) and rice agar (RA)

Media	Growth rate (cm/day)	Std. error	Sporulation rate (10^5/5 ml)	Std. error
OMA	0.32^c	± 0.0016	1.83^b	± 0.2458
PDA	0.32^c	± 0.0021	0.79^a	± 0.1840
MEA	0.29^a	± 0.0020	0.21^a	± 0.0395
RA	0.31^b	± 0.0020	0.26^a	± 0.0596

The same alphabet within column indicates the variances do not differ significantly.

Table 7. Pearson correlation between growth rate and sporulation rate of *Pyricularia oryzae* on oatmeal agar (OMA), potato dextrose agar (PDA), malt extract agar (MEA), and rice agar (RA)

Media	Pearson correlation (r)
OMA	-0.28*
PDA	0.26*
MEA	0.24*
RA	-0.34**

*-correlation is significant at the 0.05 level (2-tailed).
**-correlation is significant at the 0.01 level (2-tailed).

CONCLUSION

In conclusion, the morphological variations of all 14 *P. oryzae* isolates in this study were not specific to location or district. The pigmentation of the isolates ranged from dark grey to colourless regardless type of media. Growth and sporulation of all 14 *P. oryzae* isolates demonstrated that OMA and PDA were the most suitable media for mycelial growth, while OMA was the best medium for spore production. However, there was no strong correlation between growth rate and sporulation rate of *P. oryzae* on each media tested. The information obtained from this study may contribute towards understanding of morphological and physiological development of *P. oryzae* isolates from Sarawak on different media especially under laboratory conditions.

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