



## Isolation and characterization of *Pyricularia oryzae* isolated from lowland rice in Sarawak, Malaysian Borneo

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### ABSTRACT

**Aims:** Rice blast disease caused by *Pyricularia oryzae* is one of the major biotic diseases of rice in Sarawak, Malaysian Borneo. This study aims to isolate and characterize rice blast fungus obtained from infected leaf collected from four different divisions in Sarawak, viz, Miri, Serian, Sri Aman, and Kuching.

**Methodology and results:** Twelve succeeded isolates were pre-identified as *P. oryzae* by morphological characteristics of spores, followed by verification through (internal transcribed spacer) ITS sequencing. The isolates were evaluated for morphological characteristics, growth rate and sporulation rate, which were grown on two types of media, (filtered oatmeal agar) FOMA and (potato dextrose agar) PDA. Morphological characterization showed that the colony surface of the different isolates varied from smooth and fluffy to rough and flattened mycelia; some were with the present of concentric rings, and some with aerial mycelia. The growth rate and sporulation rate of each isolate varied based on types of media used. Most of the isolates grew faster on PDA than on FOMA but produced higher number of spores on FOMA as compared to PDA.

**Conclusion, significance and impact of study:** This preliminary study showed that there were variations observed based on morphological and physiological characterization for the different isolates collected in Sarawak, Malaysian Borneo. This study is the first step towards understanding variation in the population of *P. oryzae* from Sarawak.

**Keywords:** *Pyricularia oryzae*, Sarawak rice, morphological characteristics, growth rate, sporulation rate

### INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial food crop that is widely grown to feed half of the world's population. More than 90% of rice is cultivated and consumed in Asia (Khush, 2005; Talbot and Wilson, 2009; Global Rice Science Partnership, 2013). In Malaysia, the rice production caters approximately 65% of the population demands. As a result, Malaysia still depends on imported rice to meet the total demand. Rice production in Malaysia needs to be increased to reach the status of self-sufficiency and to meet the demand of the rapid growing population (Abdul Rahim *et al.*, 2017). Increasing the rice production is always challenged by rice diseases. One of the diseases is rice blast. Rice blast is recognized as one of the major biotic stresses that could lead up to 10% and 30% significant yield losses each year, globally (Skamnioti and Gurr, 2009; Zhou, 2016;). In Malaysia, rice yield loss due to rice blast can reach up to 50% (Gianessi, 2014; Elixon

*et al.*, 2017).

Rice blast disease is caused by filamentous ascomycete fungus, *Magnaporthe oryzae* (T.T. Hebert) M. E. Barr (anamorph *P. oryzae* Sacc.) (Silva *et al.*, 2009; Talbot and Wilson, 2009). This fungus can infect all stages of rice development and different parts of rice plants; leaves, stems, nodes and panicles (Talbot and Wilson, 2009). The lesion is typically a diamond shape with grayish center and brown margin. Under favorable conditions, the lesions can enlarge rapidly and tend to coalesce, leading to plant death (Wang *et al.*, 2014).

Breeding blast resistant varieties is a promising method in rice blast management (Ashkani *et al.*, 2015). However, the resistance might eventually be overcome by *P. oryzae* due to their genetic diversity and their ability to recombine (Scheuermann *et al.*, 2012). For example, rice blast resistant cultivar MR219 (Hussain *et al.*, 2012) in

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Peninsular Malaysia had its resistance breakdown which led to rice blast disease outbreak (Abed-Ashtiani *et al.*, 2016). It is a constant challenge for breeders in Peninsular Malaysia to breed for new resistant varieties. It is expected that the same phenomenon will be observed in Sarawak (Malaysian Borneo), which is a new 'rice bowl' state to increase rice production in Malaysia. Surveys from 2009 until 2012 in Sarawak showed that more than 50% of the surveyed rice field had moderate to high disease severity (Lai and Eng, 2011; Lai and Eng, 2013; Lai, 2016).

The knowledge on the genetic variations of *P. oryzae* could aid in managing rice blast disease. High genetic variations in a population will allow higher genetic recombination. Consequently, the breaking down of disease resistance will be rapid (Scheuermann *et al.*, 2012). In Peninsular Malaysia, there are already four reports on the variations of *P. oryzae* (Abdul Rahim *et al.*, 2013; Mat Muni and Nadarajah, 2014; Hasan *et al.*, 2016; Abed-Ashtiani *et al.*, 2016). Unfortunately, there is yet study on *P. oryzae* from Sarawak, neither their genetic variations nor pathogenicity. This paper provides a preliminary study on the variation of rice blast fungus isolated from selected rice fields in Sarawak based on their morphological characteristics.

## MATERIALS AND METHODS

### Samples collection

The rice blast infected leaf samples were collected from different rice fields (smallholders) in four different divisions in Sarawak: Miri, Kuching, Serian and Sri Aman during planting seasons of 2012 until 2016. Sampling points were decided based on the size of rice fields. In a one-hectare rice field, five sampling points were designated covering the field. If different rice landraces were planted by smallholders in one field, infected leaf samples were collected separately from each rice landrace.

### Fungal isolation

Spore drop method modified from a method described by Choi *et al.* (1999) was used. Each rice blast lesion on an infected leaf was cut in half with each half of the lesion having a section of healthy part on one end. The specimens were surface sterilized with 1% commercial bleach (Clorox®) containing 5.25% sodium hypochlorite for 1 minute and rinsed 3 times with sterilized distilled water (each lesion was treated separately). Each piece of a lesion was then attached onto the upper-lid of a Petri dish containing water agar [WA; 2% agar (agar stick) w/v] with adaxial part facing towards the medium. Then, 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). Each single spore colony was then picked and transferred onto oatmeal agar (OMA; 15 g of instant oatmeal and 7.5 g agar stick/500mL). The plates were

incubated under dark condition for 5 days and light condition for the subsequent days. Alternatively, if spores were observed on leaf segment but not on WA, the spores were dislodged with 250 µL of sterilized distilled water and spread on a new plate of WA. It was then incubated under light condition at room temperature. Each single spore colony of *P. oryzae* was picked and cultured as described above.

### Molecular identification

Universal primer pair Internal Transcribed Spacer (ITS)-1 (5'-TCCGTAGGTGAACCTGCGG-3') and -4 (5'-TCCTCCGCTTATTGATATGC-3') was used for colony PCR (White *et al.*, 1990). The PCR solution comprised of distilled water (ddH<sub>2</sub>O), 10x PCR Buffer with Mg<sup>2+</sup> (EasyTaq®), 25 mM MgCl<sub>2</sub>, 10 mM dNTPs, 10 µM ITS-1 and ITS-4, Taq DNA Polymerase (EasyTaq®), and pinch of young fungal mycelium (culture age ranged from 5 to 10 days). PCR amplification was performed using T100™ Thermal Cycler (Bio-Rad Laboratories, USA) with the following profile: initial denaturation at 94 °C for 2 min; followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 30 sec, and extension at 72 °C for 1 min; final extension at 72 °C for 5 min. The PCR products were visualized on 1% agarose gel and purified by using QIAquick® Gel Extraction Kit and sent for sequencing at Apical Scientific Sdn. Bhd. company. The sequences were used to blast (Blastn) against the sequences in gene bank (NCBI). The species verification was determined through percentage of identity and expectation value (E-value).

### Morphological characterization

Morphological characterization method described by Mohammadpourlima *et al.* (2017) was adapted and modified. There were two types of media used for morphological characterization, viz, filtered oatmeal agar (FOMA; 15 g instant oatmeal and 7.5 g agar stick/500 ml) and potato dextrose agar (PDA; brand MERCK). FOMA was prepared in a similar manner as for OMA except that the oatmeal flakes and clumps were filtered out (for ease of scoring). Both media were then sterilized in an autoclave at 120 psi for 15 min.

For all isolates, 10 days old cultures were used for subculture. An inoculum plug (5 mm Ø) was cut from the edge of actively growing mycelia and transferred (mycelium-side down) onto the center of a Petri dish containing 20 mL medium. For each medium, FOMA and PDA, there were ten replicates per isolate. The Petri dishes containing fungal plugs were then incubated in the dark for 5 days, followed with light condition for the subsequent days, at room temperature. Morphological characteristics of the colonies (form, elevation, margin, color and surface) were described (Microbiology, 2014.).

The colony growth was measured daily until the 10<sup>th</sup> day and their growth rate was calculated. Then, sporulation rate was recorded for each isolate based on three randomly selected plates. Statistical analyses were

carried out using SPSS software. ANOVA (analysis of variance) followed by Tukey HSD post hoc test was used to compare the difference in growth rate and sporulation rate between isolates in each medium at  $p < 0.05$  significance level. Mann-Whitney test was used to compare the growth rate and sporulation rate of each isolate on different media at  $p < 0.05$  significance level. This analysis excluded isolates POMI2, POSA3 and POS1 which did not produce spores on both media.

## RESULTS AND DISCUSSION

### Isolation and identification

In the rice fields of this study, the rice blast symptom (Figure 1a) observed on leaf was recognized as typical blast disease symptom, diamond shape with grayish center and brown margin (Wang *et al.*, 2014). In total, there were 12 isolates of *P. oryzae* successfully isolated from four divisions (Table 1). There was one isolate from Miri, three from Serian as well as Sri Aman, and five from Kuching.

Preliminary identification of the isolates was done based on the morphology of spores before they were verified through molecular based method. The spores (Figure 1b) obtained were pear-shaped with narrowed apex and broad basal, hyaline in color, two septa and three celled. The spores were borne along the conidiophore (Figure 1c) with the basal of the spore attached at the tip of branches of conidiophore. In average, one conidiophore can hold more than 10 spores ( $n=3$ ).

The characteristics of the observed spores for 12 isolates obtained (Figure 2) were in agreement with descriptions from previous studies (Ou, 1987; TeBeest *et al.*, 2007) and these characteristics allowed the pre-identification of the different isolates as *P. oryzae*.

ITS amplification was successful for eight isolates (Table 2). The ITS amplicon size (bp) for the six isolates was approximately 500 bp, while for POK3 and POK4 isolates with the amplicon size (bp) of approximately 300 bp. BLASTn search verified the eight isolates as *P. oryzae* based on the percentage of identity and expectation value (E-value). The results (Table 2) showed

99–100 % of the query sequence homolog to the target sequence of *M. oryzae* in the database with E-value less than or equal to zero.

### Morphological characterization

The morphological characteristics of all isolates are summarized in Table 3. The isolates were grouped based on morphological similarities (form, elevation, margin, and surface), excluding the criteria of color. There are 7 and 6 groups for FOMA and PDA, respectively.

The colony surface of the different isolates varied from smooth and fluffy to rough and flattened mycelia; some were with the presence of concentric rings, and some with aerial mycelia. All isolates showed raised elevation (viewed from side way) in both media except for three isolates (POMI2, POS1 and POSA3) which had raised elevation on one medium but flat elevation on the other. Despite of media used, all isolates had circular form and entire margin. The front colony was grey and light grey colored for most isolates on both media except for four isolates. While, the reverse colony was black colored for most isolates on both media except for three isolates (Table 3). The differences in morphology of the isolates in this study are similar to those reported by Srivastava *et al.* (2014) and Asfaha *et al.* (2015).

In short, morphological variations were observed among the 12 isolates from four different divisions. Such variations may suggest that the isolates were genetically different from each other. It might also be possible that the 12 isolates may have different pathogenicity capability. This assumption is based on the variations of dark pigmentation observed between the colony surface of the 12 isolates, and it has been reported that dark pigmentation correlates with pathogenicity (Lujan *et al.*, 2016; Oh *et al.*, 2017). The dark pigmentation of *P. oryzae* is crucial for the pathogen penetration into the host and to exhibit pathogenicity (Woloshuk *et al.*, 1980; Chida and Sisler, 1987; Wheeler and Greenblatt, 1988). The correlation of pathogenicity with pigmentation intensity of *P. oryzae* could be an interesting area for further research.

The morphological variations of isolates from different

**Table 1:** The isolates of *P. oryzae* that were successfully isolated from different regions of Sarawak.

| Isolate | Location             | Division | GPS                         | Rice Landrace |
|---------|----------------------|----------|-----------------------------|---------------|
| POMI2   | Bario                | Miri     | N03°45'14.6", E115°26'55.3" | Adan          |
| POS1    | Kg Remun, Tebedu     | Serian   | N01°08'19.9", E110°39'02.9" | Biris         |
| POS2    | Kg Remun, Tebedu     | Serian   | N01°08'19.9", E110°39'02.9" | Bajong        |
| POS3    | Paon Gahat           | Serian   | N0°56'41.5", E110°39'16.3"  | Wangi         |
| POSA3   | Stumbin              | Sri Aman | N1°18'06.8", E111°22'46.6"  | Wangi Halus   |
| POSA1   | Stumbin              | Sri Aman | N1°18'06.8", E111°22'46.6"  | Unknown       |
| POSA2   | Sri Aman, Tg Bijat   | Sri Aman | N1°20'58.8", E111°23'00.9"  | Unknown       |
| POK2    | Senibong, Lundu      | Kuching  | N01°35'48.0", E109°53'22.2" | Unknown       |
| POK3    | Pueh, Sematan        | Kuching  | N01°49'52.5", E109°43'30.5" | Unknown       |
| POK4    | Siru Melayu, Sematan | Kuching  | N01°49'51.9", E109°43'38.7" | Unknown       |
| POK5    | Siru Melayu, Sematan | Kuching  | N01°49'51.9", E109°43'38.7" | Unknown       |
| POK6    | Kg Stunggang, Lundu  | Kuching  | N1°38'58.9", E109°51'15.9"  | Unknown       |

**Table 2:** Summary of the BLASTn search results for ITS sequence of the *P. oryzae* isolates from this study.

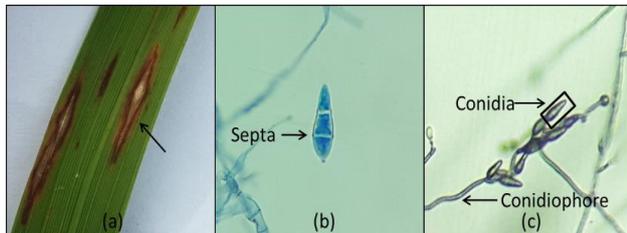
| Isolate           | Accession number | Amplicon size (bp) | Percentage of identity (%) | E-value  | Accession number (NCBI) |
|-------------------|------------------|--------------------|----------------------------|----------|-------------------------|
| POSA2             | MK629260         | 490                | 99.59                      | 0.0      | MH715386                |
| POK4 <sup>#</sup> | NA               | 314                | 100                        | 7e - 162 | MH715393                |
| POK3 <sup>#</sup> | NA               | 290                | 100                        | 1e - 148 | MH715373                |
| POSA1             | MK629259         | 489                | 99.59                      | 0.0      | MF583110                |
| POS2              | MK629262         | 490                | 99.59                      | 0.0      | MH715386                |
| POS1              | MK629264         | 491                | 99.59                      | 0.0      | JX469384                |
| POMI2             | MK629261         | 490                | 99.59                      | 0.0      | MH715386                |
| POK6              | MK629263         | 490                | 99.59                      | 0.0      | MH715386                |
| POK2*             | NA               | NA                 | NA                         | NA       | NA                      |
| POS3*             | NA               | NA                 | NA                         | NA       | NA                      |
| POSA3*            | NA               | NA                 | NA                         | NA       | NA                      |
| POK5*             | NA               | NA                 | NA                         | NA       | NA                      |

\* identified based on morphology of spores only

<sup>#</sup> isolate with partial sequence

NA - not available

locations were random. There was no particular variation which is specific to one location that is the isolates with similar morphology were grouped differently despite the origin. For instance, the morphology of isolate POMI2 from Miri (northeastern Sarawak) was similar to that of an isolate from Serian division (southwestern Sarawak) on OMA. On PDA, the morphology of the Miri isolate was similar to three isolates originated from three different divisions in southwestern Sarawak, respectively. This finding agrees with that by Srivastava *et al.* (2014). However, this does not conclude that isolates from the same location are genetically unrelated, because positive correlation between molecular data and geographical origin of different isolates of *P. oryzae* was reported in Peninsular Malaysia (Abed-ashtiani *et al.*, 2016).



**Figure 1:** (a) The arrow points out the rice blast symptom, diamond shape with grayish center and brown margin (b) conidia (spore) (c) conidiophore.

### Growth rate and sporulation rate

Growth rate and sporulation rate were tabulated in Table 4. Growth rate between isolates in each medium varied significantly. Isolate POS1 significantly had the fastest growth rate on FOMA with the mean value of 0.38 cm/day. On PDA, however, POS2 grew significantly faster than the other 11 isolates. Both isolates POS1 and POS2 were approximately 1½ days faster in growth as compared to isolate POS3 which was one of the slowest growing isolate on OMA and the slowest on PDA. Comparison of the growth rate of each isolate between

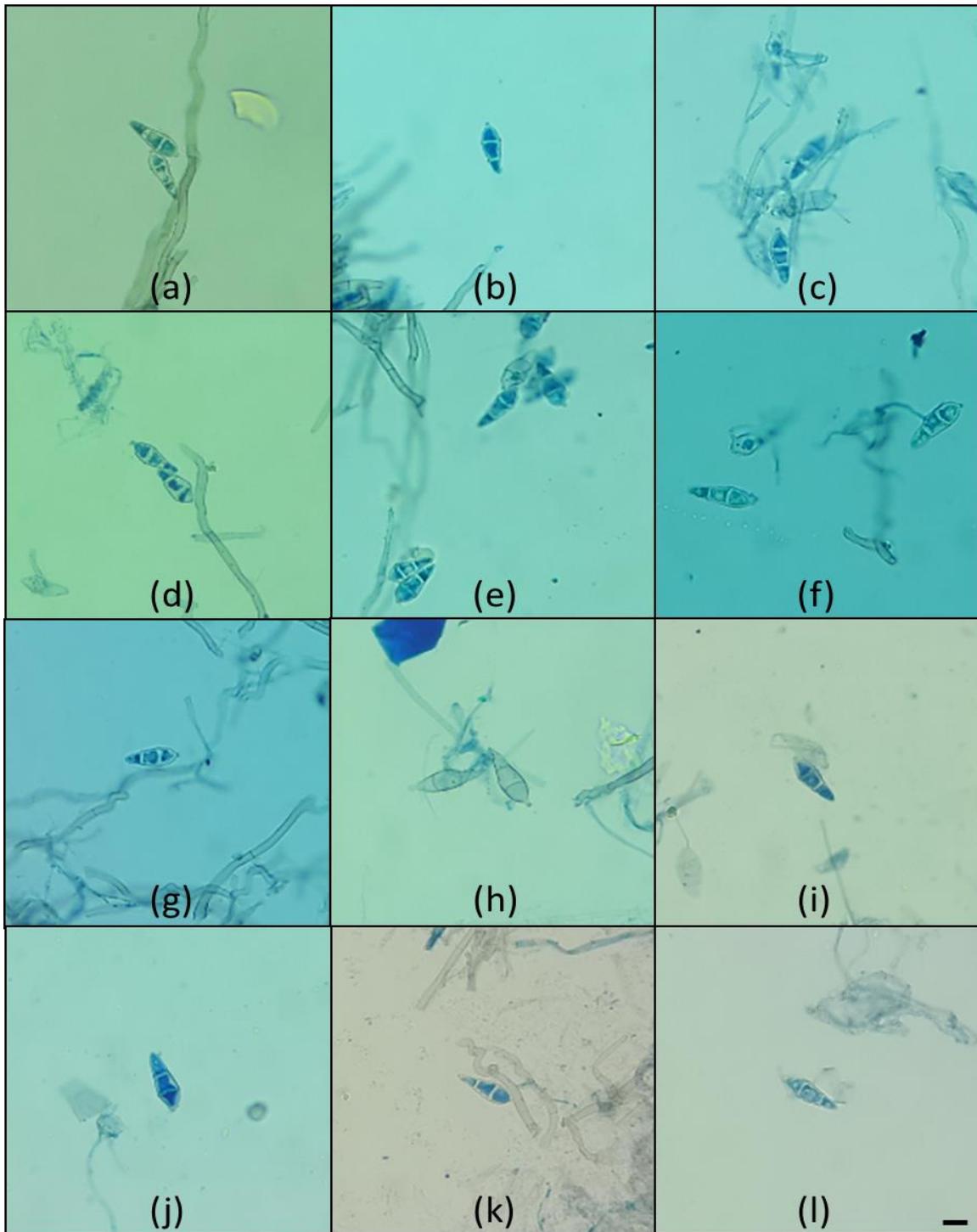
the two media showed that seven isolates rapidly grew on PDA, and one isolate on FOMA. The results in this study are in agreement with that of Vanaraj *et al.* (2013).

Means of sporulation rate revealed the significant differences in sporulation between isolates on each medium. Isolate POS2 produced the highest number of spores on FOMA, whereas isolate POK6 produced the highest number of spores on PDA. Isolate POS3 produced the lowest amount of spores on both media. In general, most isolates seemed to be able to produce higher number of spores on FOMA as compared to PDA. Unfortunately, there was insufficient number of replicates per isolate for each medium to give a reliable statistical analysis.

There is a change in the ranking of isolates based on growth rate as well as sporulation rate on the two different media. This suggests that the physiological performance of an isolate may have been affected by the different media used. It was also observed in this study that there were very weak and moderate positive correlation between the pathogen growth rate and sporulation rate,  $r = 0.03$  (FOMA) and  $r = 0.65$  (PDA) respectively.

The morphological and physiological variations observed in this study may have associated with the nutrients present in each medium. FOMA was made from oat (*Avena* sp.), a close relative to rice, and it has been reported that oat is a host to *P. grisea* (Marangoni *et al.*, 2013), a close relative to *P. oryzae*. The fact that isolates of *P. oryzae* exhibited different morphology, growth rate and sporulation rate on FOMA from PDA might suggest that oat contains comparable host material which is needed for *P. oryzae* for growth and sporulation.

The effect of the host material in the media can be seen in the induction of sporulation (Su *et al.*, 2012), where all isolates (with sporulation) produced at least two times higher the number of spores on FOMA in comparison to PDA. Two of the isolates even produced four times higher the spore number on FOMA (no statistical evidence). The effect of media on sporulation of *P. oryzae* will be studied in the future.

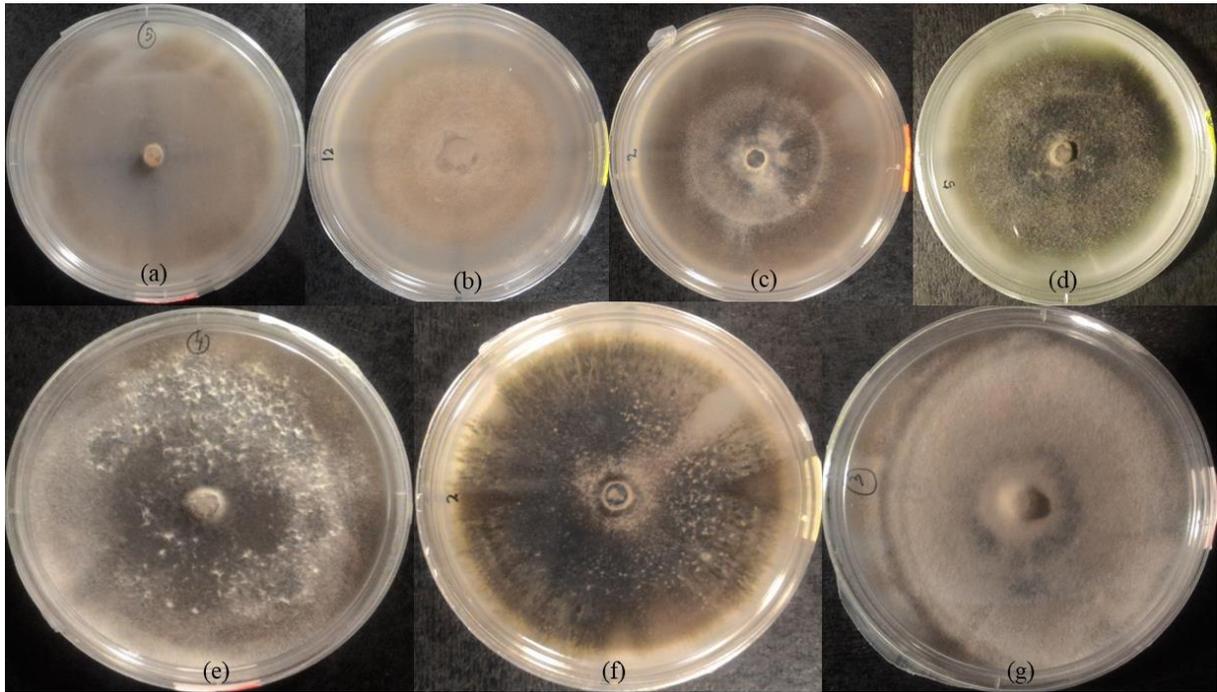


**Figure 2:** Spores of 12 isolates under light microscope at 40x magnification (a) POS2 (b) POSA1 (c) POK4 (d) POK5 (e) POK6 (f) POSA2 (g) POS3 (h) POMI2 (i) POK2 (j) POS1 (k) POSA3 (l) POK3. Scale bar, 10µm.

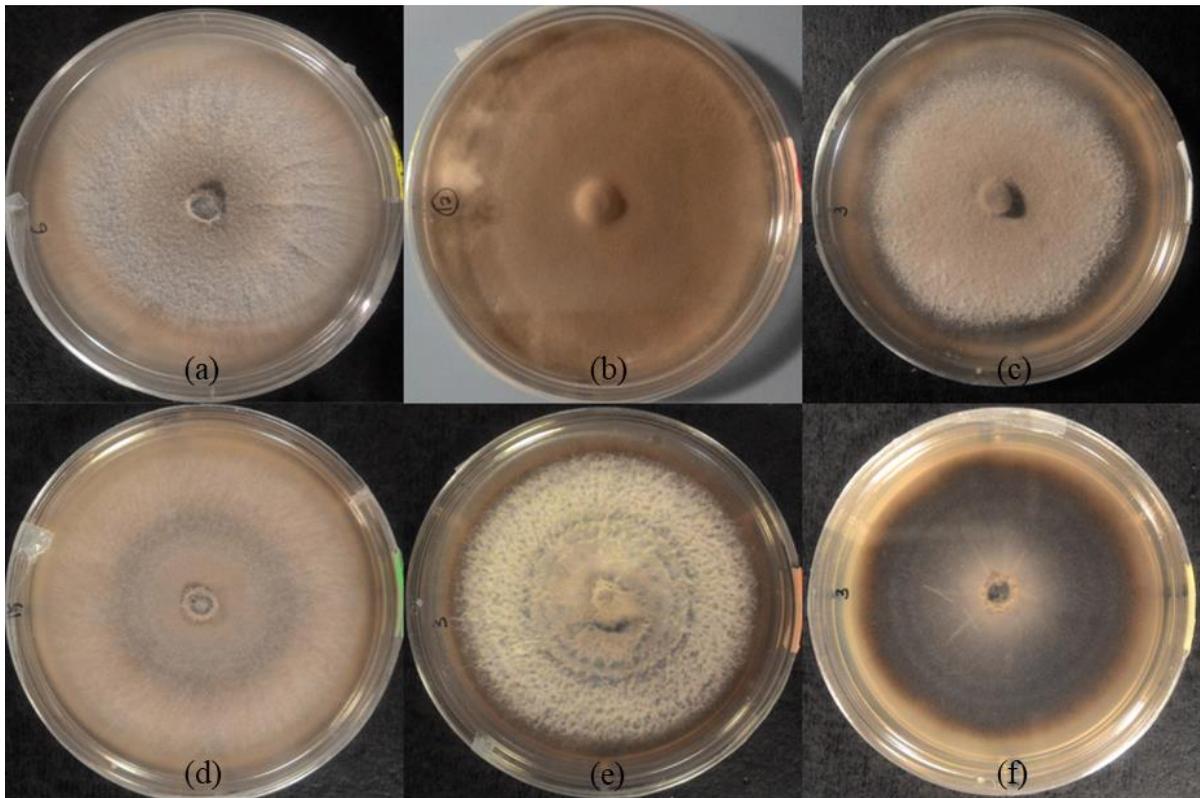
**Table 3:** Summary of the morphological characteristics of *P. oryzae* on filtered oatmeal agar (FOMA) and potato dextrose agar (PDA).

| Media | Group | Isolate | Division | Color           |             | Elevation | Surface description  |  |
|-------|-------|---------|----------|-----------------|-------------|-----------|--|--|
|       |       |         |          | Front           | Reverse     |           |  |  |
| FOMA  | 1     | POS1    | Serian   | Olivaceous grey | Black       | Flat      | Smooth flattened mycelia   |  |
|       |       | POMI2   | Miri     | Light brown     | Light brown |           |  |  |
|       | 2     | POS2    | Serian   | Grey            | Light grey  | Raised    | Smooth fluffy mycelia at the center, flattened mycelia at the edges                              |  |
|       |       | POK4    | Kuching  | Grey            | Black       |           |  |  |
|       |       | POK6    | Kuching  | Light grey      | Light brown |           |  |  |
|       | 3     | POS3    | Serian   | Grey            | Black       | Raised    | Smooth fluffy mycelia with concentric ring, flattened mycelia after formation of ring            |  |
|       |       | POK5    | Kuching  | Light grey      | Black       |           |  |  |
|       | 4     | POSA1   | Sri Aman | Olivaceous grey | Black       | Raised    | Smooth thinned mycelia   |  |
|       |       |         |          | Grey            | Black       |           |  |  |
|       | 5     | POK2    | Kuching  | Grey            | Black       | Raised    | Smooth fluffy mycelia with white aerial mycelia  |  |
|       | 6     | POSA3   | Sri Aman | Grey            | Black       | Raised    | Rough thinned mycelia  |  |
|       | 7     | POSA2   | Sri Aman | Grey            | Black       | Raised    | Smooth fluffy mycelia  |  |
|       | PDA   | 1       | POS1     | Serian          | Light grey  | Black     | Raised   | Smooth fluffy mycelia at the center, aerial mycelia at the edges |
|       |       | 2       | POSA2    | Sri Aman        | Grey        | Black     | Raised   | Smooth fluffy mycelia  |
| POK2  |       |         | Kuching  | Grey            | Black       |           |  |  |
| POS3  |       |         | Serian   | Grey            | Black       |           |  |  |
| 3     |       | POSA1   | Sri Aman | Grey            | Black       | Raised    | Smooth fluffy mycelia at the center, flattened mycelia at the edges                              |  |
|       |       | POK4    | Kuching  | Light grey      | Black       |           |  |  |
| 4     |       | POMI2   | Miri     | Light grey      | Black       | Raised    | Smooth fluffy mycelia at the center with concentric ring, aerial mycelia after formation of ring |  |
|       |       | POS2    | Serian   | Grey            | Black       |           |  |  |
| 5     |       | POK3    | Kuching  | Grey            | Black       | Raised    | Aerial mycelia with concentric rings   |  |
|       |       | POK5    | Kuching  | Grey            | Black       |           |  |  |
| 6     |       | POK6    | Kuching  | Light grey      | Black       | Flat      | Smooth flattened mycelia   |  |
|       |       | POSA3   | Sri Aman | Light brown     | Black       |           |  |  |

Note: Form and margin for all isolates are circular and entire respectively.



**Figure 3:** Colony morphology of isolates on FOMA based on groups; (a-g) represent isolates from group 1 to 7.



**Figure 4:** Colony morphology of isolates on PDA based on group; (a-f) represent isolates from group 1 to 6.

**Table 4:** Growth rate and sporulation rate of different isolates on two types of media: FOMA, and PDA.

| Isolates | Growth rate (cm/day) |                |                     |                | Sporulation rate (10 <sup>5</sup> ) in 5mL of spore suspension |                |                   |                |
|----------|----------------------|----------------|---------------------|----------------|--|----------------|-------------------|----------------|
|          | FOMA                 | Standard error | PDA                 | Standard error | FOMA   | Standard error | PDA               | Standard error |
| POS3     | 0.32 <sup>a</sup>    | ± 0.0030       | 0.33 <sup>a</sup>   | ± 0.0045       | 0.33 <sup>a</sup>  | ± 0.0629       | 0.15 <sup>a</sup> | ± 0.0382       |
| POSA2    | 0.32 <sup>a</sup>    | ± 0.0015       | 0.37 <sup>def</sup> | ± 0.0012       | 1.24 <sup>ab</sup>   | ± 0.0333       | 0.31 <sup>a</sup> | ± 0.0315       |
| POK5     | 0.33 <sup>ab</sup>   | ± 0.0018       | 0.36 <sup>bcd</sup> | ± 0.0014       | 0.43 <sup>a</sup>  | ± 0.1797       | 0.16 <sup>a</sup> | ± 0.0042       |
| POSA1    | 0.34 <sup>bc</sup>   | ± 0.0025       | 0.36 <sup>bc</sup>  | ± 0.0020       | 0.59 <sup>a</sup>  | ± 0.0402       | 0.23 <sup>a</sup> | ± 0.0315       |
| POK4     | 0.34 <sup>cd</sup>   | ± 0.0054       | 0.39 <sup>f</sup>   | ± 0.0011       | 2.63 <sup>c</sup>  | ± 0.3403       | 1.39 <sup>c</sup> | ± 0.0629       |
| POK3     | 0.36 <sup>de</sup>   | ± 0.0039       | 0.36 <sup>bcd</sup> | ± 0.0040       | 2.08 <sup>bc</sup>   | ± 0.1585       | 0.95 <sup>b</sup> | ± 0.0870       |
| POMI2    | 0.36 <sup>e</sup>    | ± 0.0010       | 0.35 <sup>b</sup>   | ± 0.0014       | 0.00   | -              | 0.00              | -              |
| POK2     | 0.36 <sup>e</sup>    | ± 0.0017       | 0.36 <sup>bcd</sup> | ± 0.0021       | 0.56 <sup>a</sup>  | ± 0.0696       | 0.24 <sup>a</sup> | ± 0.0217       |
| POS2     | 0.36 <sup>e</sup>    | ± 0.0010       | 0.44 <sup>h</sup>   | ± 0.0015       | 6.22 <sup>d</sup>  | ± 0.9813       | 1.35 <sup>c</sup> | ± 0.0723       |
| POSA3    | 0.37 <sup>e</sup>    | ± 0.0018       | 0.37 <sup>cde</sup> | ± 0.0014       | 0.00   | -              | 0.00              | -              |
| POK6     | 0.37 <sup>e</sup>    | ± 0.0030       | 0.42 <sup>g</sup>   | ± 0.0041       | 3.08 <sup>c</sup>  | ± 0.1630       | 1.44 <sup>c</sup> | ± 0.1740       |
| POS1     | 0.38 <sup>f</sup>    | ± 0.0014       | 0.38 <sup>ef</sup>  | ± 0.0042       | 0.00   | -              | 0.00              | -              |

Note: ANOVA followed by Tukey HSD post hoc test was used to compare the difference in growth rate and sporulation rate between isolates in each media ( $p < 0.05$ ). The same alphabet within column indicates the variances do not differ significantly. Mann-Whitney test was used to compare the growth rate of each isolate on the two media. Bold font indicates significant different in growth rate on the different media for each isolate.

## CONCLUSION

In conclusion, 12 isolates were successfully isolated from infected leaves through spore drop isolation method. This preliminary study showed that there were variations observed based on morphological characters for the different isolates collected in Sarawak, Malaysian Borneo. There were no morphological characters unique to a specific location. The growth rate and sporulation rate of the different isolates varied on different media. This study is the first step towards understanding variations in the population of *P. oryzae* from Sarawak. It would be interesting to isolate more *P. oryzae* from different locations to have a better representation of the population. Further study on the genetic variations and pathogenicity are also significant.

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