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Research Article

A Simple and Economical Method to Induce Sporulation of *Pyricularia oryzae*

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Abstract

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It requires a large number of conidia not only for pathogenic races identification of *Pyricularia oryzae* (the causal agent of rice blast) but also for disease resistance assessment of rice varieties against the pathogen. For stable test result, the same age of conidiospores are also essential for these pathogenicity tests. A mycelial homogenate method (called "liquid spawn method") modified from the IRRI (International Rice Research Institute) protocol was used to induce simultaneous conidia production of rice blast fungus. The processes are: (1) 4-d-old colony of *P*. *oryzae* isolate was cut into numerous pieces and cultured in prune broth for 4 d; (2) homogenizing the whole broth, dispersing the liquid spawn evenly on oat flour agar (OFA) medium plate, air-drying the excess water on the plate, and then culturing the medium at room temperature with natural light illumination for 4 d for conidia production. By use of this method, the conidia yield of several tested isolates of *P*. *oryzae* were much higher (2−31 folds) than those with IRRI protocol. In another test, 14 *P*. *oryzae* isolates which had very poor conidia yields with regular methods produced significantly higher number of conidia by the method. The results showed that the liquid spawn method could be used for simultaneous and massive sporulation of *P*. *oryzae*.

Key words: *Pyricularia oryzae*, Sporulation, Conidia, Liquid spawn, Oat flour agar.

INTRODUCTION

Rice is the third most important staple food crop in the world, and also the main source of dietary energy for more than half of the world's population. There are approximately 271,506 ha of rice-cultivated area in Taiwan producing an annual output value over 42.9 billion NT dollars. Rice blast disease, caused by *Pyricularia oryzae* Cavara (Teleomorph: *Magnaporthe oryzae* B.C. Couch), is one of the major fungal diseases in various rice-growing regions worldwide (Agrios 1997). If the damage occurs at the flowering stage, the rice yield will be severely affected. Average annual production loss due to rice blast disease is ranged from 10% to 30% globally, depending on the differences in the planting environment and cultivars (Talbot 2003; Skamnioti & Gurr 2009). In some areas of the world, even 50% to 90% yield loss has been reported (Hajanoe *et al*. 2011).

P. *oryzae* has many known races, which can affect different rice cultivars. To avoid severe damages due to *P*. *oryzae*, it is imperative to monitor the pathotypes and distribution of the virulent rice blast fungus in fields (Chen *et al*. 2013). In principle, to maintain the objectivity

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and credibility of the pathotypes distribution, different *P. oryzae* isolates must be collected from various production areas for pathogenicity evaluation. However, some *P. oryzae* isolates have poor sporulation capability to produce sufficient conidia for inoculation tests (Koga *et al*. 2008). Therefore, developing a reliable method to stimulate the conidia production of *P. oryzae* is critical for the evaluation of pathogenicity of rice blast fungus.

Many environmental factors including nutrients, temperature, humidity, and light conditions may affect the sporulation of *P. oryzae* (Hemmi & Imura 1939; Namai & Yamanaka 1985; Lee *et al*. 2006; Rajput *et al*. 2017). The reported optimal temperature suitable for *P. oryzae* conidial production is around 28℃ to 30℃ and the relative humidity higher than 93% (Hemmi & Imura 1939; Netam *et al*. 2013). Several media, including potato dextrose agar (PDA), oatmeal agar (OMA), rice flour agar, yeast extract starch agar (YSA) and prune agar (PA) are commonly used for *P. oryzae* to stimulate sporulation (Hayashi *et al*. 2009; Lan *et al*. 2012; Hajano *et al*. 2013). Although the light was not an essential factor for *P. oryzae* sporulation (Chakrabarti & Wilcoxson 1970), exposure of fungal cultures to light during the first few days can stimulate conidiophore formation (Namai *et al*. 1977) and conidia production. Further studies have found that *P. oryzae* isolates kept under 8/16 h of light/dark photoperiod could significantly increase the conidia production (Hajano *et al*. 2013). However, none of the reported methods is suitable for all *P. oryzae* isolates.

The mycelial plug method is the most commonly used method for many fungi to obtain spores (Hau & Rush 1980; Lan *et al*. 2012; Chaudhary *et al*. 2018). The drawback of this method is that spores in a cultural plate are produced at varying time. Lack of uniformity in spore ages may impact the results of fungal pathogenicity, let along many isolates of *P. oryzae* fail to produce spores using the mycelial plug method. A stimulating sporulation method originally reported by Hayashi *et al*. (2009) is commonly used by researchers in the International Rice Research Institute (IRRI). Although the IRRI protocol improves the conidial yield and reduces the age variation of spore, it has a risk of contamination during the culture process.

The main objective of this study was to develop a universal protocol suitable for conidia production by most field-collected isolates of *P. oryzae*, and the two methods mentioned above were included as references in this study.

MATERIALS AND METHODS

Comparison of sporulation among the liquid spawn, IRRI protocol, and the agar plug method

Three *P. oryzae* isolates with different sporulation abilities based on the conventional agar plug method were used in this study. Isolates YL2a3-1603 and TJ4a3-1603 from diseased rice seedlings were collected from Yuanli and Tianjung Townships, respectively, in March 2016. PT5a5-1205, which sporulated poorly was originated from the infected leaf of Pitou Township at vegetative stage in May 2012. The average conidia yields of YL2a3-1603, TJ4a3-1603 and PT5a5-1205 were 2.0×10^5, 1.7×10^5 and 4.0×10^4 conidia per 9-cm diameter plate, respectively. All isolates were preserved on filter papers and stored at -20℃. The production of conidia was carried out by growing fungal isolates from the stock culture.

In IRRI protocol, *P. oryzae* mycelium cultured on the PA slant for 10 d and a 2-mL of the mycelial suspensions from the PA slants was uniformly spread on a new PA medium plate and incubated at 27℃ for 7 d. After aerial hyphae were scrapped by sterilized glass slides, the plates were left open under the fluorescent lamp at about 27℃ for 4 d to induce sporulation. The tested method (called “liquid spawn method”) used in this study was modified from the IRRI protocol (Hayashi *et al*. 2009). This method allowed the fungal isolates to produce a large number of mycelium in a shorter time. Fungal isolates preserved on desiccated filter papers were revived on V-8 juice agar plate (each liter containing V-8 juice 100 mL, $CaCO_3$ 0.2 g, agar 17 g, pH 6.5) at room temperature. The 4-d-old colony was cut into pieces, transferred to a 125-mL flask containing 50 mL of prune broth (each liter containing prune 15 g, starch 2.5 g, yeast extract 0.5 g, pH 6.5). Fungal isolates were cultured in the prune broth on a shaker (130 rpm) at 28℃ for 4 d in the dark. Mycelial pellets and culture broth were homogenized in a sterile blender (Oster Blender 6640-022, Oster, Chicago, IL, USA) at low speed for 15 s to produce liquid spawn, from which 2-mL liquid spawn was dispersed evenly on a 9-cm diameter oat flour agar (OFA) plate (each liter containing oat flour 10 g, agar 17 g) and air dried to remove excess water in a laminar flow. The plates were placed in a zipper bag and incubated at 28℃ with a 12 h photoperiod fluorescent light for 4 d without sealing to induce sporulation. Conidia were harvested by flooding the plate with 10 mL of 0.025% Tween 80 solution and gently scrapping with a glass slide. The number of conidia was visually counted on the haemocytometer (Hausser Scientific, Horsham, PA, USA) under a light microscope (100×). The yield of conidia produced by the liquid spawn method was compared with those produced by the IRRI protocol and the conventional agar plug method. Rice seedlings were inoculated according to Hayashi *et al*. (2009), to prove the pathogenicity of the conidia collected by these methods.

For each sporulation treatment, fungal isolate was inoculated on four OFA medium plates. Oat flour was prepared by grinding the whole oat groats (from local market) in a grinder in the laboratory.

Effect of sunlight and fluctuating temperature conditions on sporulation of *P. oryzae*

Effect of natural sunlight and fluorescent light with either fluctuation temperature or constant temperature on conidiation were evaluated and compared to identify the optimal conditions for sporulation in *P. oryzae*. The OFA medium plates inoculated with fungal isolates obtained from liquid spawn as described above were incubated in different combinations of light and temperature treatments. In the first experimental design, two treatments were fixed temperatures, the plates were incubated under a 12-h diurnal fluorescent light condition either 24℃ or 28℃, the other 2 treatments with fluctuated temperatures either 24−28℃ or 28−33℃. In the second experimental design, the plates were placed at room temperature (28−33℃) and exposed to 24-h constant fluorescent lights (LL), 24-h constant dark (DD), 12-h daily illumination (LD), or natural sunlight near the laboratory windows (SL). The laboratory was located in Wufeng District, Taichung City, Taiwan (24.029197N, 120.695850E), and the windows were facing south. On average, sunshine duration was 6.5 h per day in summer and 4.3 h per day in winter. After 4-d incubation, the number of conidia was determined using a haemocytometer. Each treatment has four replicates and the experiments were repeated twice.

Effect of the liquid spawn method on sporulation of different *P. oryzae* field isolates

In addition to YL2a3-1603, TJ4a3-1603 and PT5a5-1205 isolates, another 14 isolates collected in Taiwan fields failed to sporulate effectively using the agar plug method were evaluated for conidiation using the liquid spawn method. Conidiation by these fungal isolates were evaluated on OFA medium by the liquid spawn method as described above. The conidial yield of *P. oryzae* isolates cultured on agar plug method on PDA medium were used as the control. Each treatment contained 4 replicates, and the experiment was repeated twice. The number of conidia was determined using a haemocytometer.

Statistical analysis

Statistical analysis was performed by the R package, Agricolae (version 3.6.0) (De Mendiburu 2015) via analysis of variance (ANOVA) followed by Fisher's least square difference

(LSD) test at $P < 0.05$ significance level. Data were expressed as mean ± standard deviation of at least four replicates.

RESULTS

Sporulation yields of the liquid spawn, IRRI protocol, and the agar plug method

To compare the effects of each factor in different sporulation methods, ANOVA was used to analyze the data (Table 1). Results indicated that isolate and method significantly impacted sporulation, and there was a significant interaction between isolates and methods ($P < 0.001$). Sporulation of all isolates was significantly ($P < 0.001$) affected by the methods used, in which the tested isolates tended to produce higher number of conidia when using the liquid spawn method than the agar plug method ($P < 0.001$) (Fig. 1). Using the liquid spawn method, isolate YL2a3-1603 produced 68.5×10^5 conidia plate^{-1} and TJ4a3-1603 40.3×10^5 conidia plate^{-1}, which were approximately 27-fold and 31-fold increases compared to the IRRI protocol. However, PT5a5-1205 isolate only increased 2-fold. Conidia of three isolates obtained by the liquid spawn method were used to inoculate rice seedlings of 'Tainan 11' cultivar. Results confirmed that the spores were still pathogenic and all could cause disease on the rice seedlings.

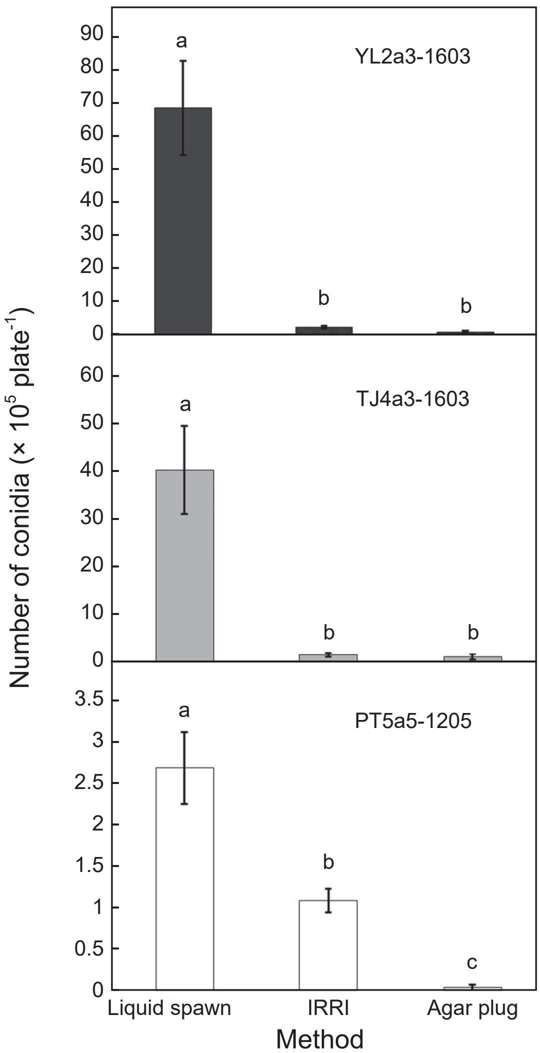

Fig. 1. The number of conidia of three *Pyricularia oryzae* isolates YL2a3-1603, TJ4a3-1603, and PT5a5-1205 using liquid spawn method, IRRI (International Rice Research Institute) protocol, and agar plug method on the oat flour medium. Error bar is the standard error of the mean ($N = 4$). Means with the same letter are not significantly different at 5% level by the least significant difference (LSD) test.

Table 1. The ANOVA result of the effects of different culture methods on the spore yields of 3 rice blast isolates[z].

Treatment	*df*	*MS*	*F*
Method	2	52051000000000	161.36***
Isolate	2	15359000000000	47.61***
Method × isolate	4	14123000000000	43.78***
Error	27	322580000000	

[z] ANOVA: analysis of variance; *df*: degree of freedom; *MS*: mean square. $N = 4$.
***Significant at 0.1% level.

Influence of sunlight and fluctuating room temperature condition on sporulation of *P. oryzae*

To further optimize culture conditions for sporulation, fungal isolates were grown under different combination of environmental factors and conidia were quantitatively compared. ANOVA was applied to analyze the data revealing that sporulation varied significantly among all isolates tested. Conidiation by *P. oryzae* was also impacted by light and temperature ($P < 0.001$) (Tables 2–3). There were significant interactions between isolates and light, as well as between isolates and temperature ($P < 0.001$). Temperature was set as the variable factor in the first experiment, revealing that the best temperature conditions for conidiation by isolates YL2a3-1603 and TJ4a3-1603 were at 28℃ and 24−28℃, respectively, under 12-h daily illumination. Fungi cultured in 28−33℃ produced the least conidia.

In the second experiment, fungal isolates were grown in 28−33℃ with different light sources and durations, revealing that *P. oryzae* isolates produced the most abundant conidia under natural sunlight. The results of experiments 1 and 2 showed that the combination of room temperature (28−33℃) and 12-h diurnal fluorescent light resulted poor sporulation of *P. oryzae*. However, at the room temperature condition, all 3 isolates showed higher conidial yield under the natural sunlight.

Effect of the liquid spawn method on sporulation of *P. oryzae* among different isolates

ANOVA was used to analyze the effects of different culturing methods on sporulation among different isolates (Table 4). Isolates and methods had significant effects on sporulation, and there was a significant interaction between isolates and methods ($P < 0.001$). Sporulation of all isolates was significantly ($P < 0.001$) affected by culturing methods. The conidial yield of 17 isolates inoculated with the liquid spawn method on OFA medium were all higher than those obtained by using agar plug PDA medium method ($P < 0.001$) (Table 5). As assessed using the agar plug method, BP2a2-1904 isolate produced the most abundant conidia, reaching 0.63×10^5 conidia plate^{-1}, but 9 out of 17 tested isolates of *P. oryzae* did not sporulate. In the liquid spawn method treatment, YL2a3-1603 isolate had the

Table 2. The ANOVA result of the effects of the temperature on the spore yield of 3 rice blast isolates under 12-h diurnal fluorescent light condition^{z}.

Treatment	*df*	*MS*	*F*
Isolate	2	70881000000000	133.99***
Temperature	3	4964500000000	9.38***
Temperature × isolate	6	3738100000000	7.07***
Error	36	529010000000	

^{z} ANOVA: analysis of variance; *df*: degree of freedom; *MS*: mean square. $N = 4$.
***Significant at 0.1% level.

Table 3. The ANOVA result of the effects of different light conditions on the spore yields of 3 rice blast isolates at the room temperature (28−33℃)^{z}.

Treatment	*df*	*MS*	*F*
Isolate	2	38323000000000	98.90***
Light	3	10012000000000	25.84***
Light × isolate	6	2619700000000	6.76***
Error	36	387520000000	

^{z} ANOVA: analysis of variance; *df*: degree of freedom; *MS*: mean square. $N = 4$.
***Significant at 0.1% level.

Table 4. The ANOVA result of the effects of different culture methods on the spore yields of 17 rice blast isolates[z].

Treatment	*df*	*MS*	*F*
Method	1	41118000000000	330.90***
Isolate	16	1135600000000	9.14***
Method × isolate	16	1110200000000	8.93***
Error	102	124260000000	

[z] ANOVA: analysis of variance; *df*: degree of freedom; *MS*: mean squares. $N = 4$.
*** Significant at 0.1% level.

Table 5. The conidia spore yields of 17 rice blast isolates culturing in liquid spawn or agar plug methods.

	Number of conidia (× 10^5 plate^{-1})	
Isolate	Liquid spawn	Agar plug
YL2a3-1603	29.4 ± 2.8 a[z]	0.04 ± 0.08 b
TJ4a3-1603	12.1 ± 1.4 a	0.08 ± 0.09 b
PT5a5-1205	7.9 ± 0.3 a	0.04 ± 0.08 b
BP2a2-1904	19.8 ± 4.2 a	0.63 ± 0.95 b
EM3a1-1904	1.4 ± 0.9 a	0 ± 0 b
XIH2a4-1904	7.6 ± 1.7 a	0.38 ± 0.48 b
SY3a3-1905	21.4 ± 17.5 a	0 ± 0 b
TL2a3-1905	16.6 ± 3.5 a	0.08 ± 0.09 b
XG2a2-1904	6.6 ± 0.5 a	0 ± 0 b
MS1a2-1903	13.5 ± 1.8 a	0 ± 0 b
DS1a1-1904	13.1 ± 7.3 a	0 ± 0 b
LIY2a2-1904	1.3 ± 0.3 a	0 ± 0 b
WD2a1-1904	2.4 ± 0.6 a	0 ± 0 b
Sx1a2-1905	9.5 ± 1.5 a	0.08 ± 0.09 b
XC1a(1)-1904	8.3 ± 1.3 a	0 ± 0 b
GS4a3-1905	11.1 ± 3.3 a	0.13 ± 0.25 b
TD1a3-1905	6.5 ± 0.4 a	0 ± 0 b

[z] Means with the same letter within each row are not significantly different at 0.1% level by the least significant difference (LSD) test.

highest conidial yield reaching 29.40 × 10^5 conidia plate^{-1}, and LIU2a2-1904 isolate was the lowest, which was 1.30 × 10^5 conidia plate^{-1}. Not only all tested isolates produced conidia using the liquid spawn method, some isolates increased at least 20-folds conidia production when compared to the agar plug method.

DISCUSSION

The liquid spawn method proposed by this study shortens the harvest time for *P. oryzae* conidia spores with less operation steps. The agar plug method requires 2 steps and at least 18 d to obtain conidia; 3 steps and 12 d were required using the liquid spawn method; while the IRRI protocol needs 4 steps and 25 d. The liquid spawn method was apparently the superior method to prepare conidia as this method produced abundant conidia in the shortest time period.

The conventional mycelium agar plug method takes about 2 wk for rice blast fungus to fully grown in a 9-cm diameter plate. The spore age obtained in this method varies greatly, in which the differences could maximum up to more than 10 d, and might indirectly affect the inoculation results. The IRRI protocol shortens the culturing time by dispersing the hyphae agar fragments with sterile water on the medium surface, so the rice blast fungi could quickly fully grow on the medium plates. This protocol can keep the spore age differences within 4 d, reducing the pathogenicity instability (Darby & Mandels 1955).

In our study, three isolates had poor conidial yield on agar plug medium, of which the highest sporulation was only 3.3 × 10^5 conidia plate^{-1} on PDA (Fig. 1). If such an isolate is necessary to be included in a study, huge amount of preparation works need to be done to ensure enough conidia yield for the following experiments. As such, the results obtained may not be able to objectively represent the real pathogenicity distribution of rice blast fungus populations in the field. The IRRI protocol can only slightly improve the conidial yield compared to the mycelium plug method. However, the IRRI protocol still has a risk of contamination during the process of scraping aerial hyphae and incubation without lids. The liquid spawn method proposed in this study has solved the above-mentioned problems by homogenizing hyphae in a sterile blender instead of scrapping. The described method not only saves the intensive labor work, but also avoids the risk of medium dehydration and contamination of other microorganism. Based on the experiences of handling hundreds of rice blast fungi isolates, this method could greatly increase the sporulation of about 73% of the rice blast fungus field isolates in our laboratory, and thus shortens the time for the whole process. Although the increase in sporulation of some strains is still limited (Table 5), 9 isolates that could not sporulate using agar plug method were all able to produce spores using the liquid spawn method. We speculated that differences of the improving sporulation may be due to the differences in nutrient requirements or sporulation ability between strains. The other advantage of the liquid spawn method is relatively cheap by using the oat flour for the OFA medium. The oat flour is grounded with whole oat groats in the laboratory, which greatly reduce the cost of OFA medium to only USD $1 per liter.

In experiments with different temperatures and light conditions, the three tested isolates had the lowest sporulation under 12-h diurnal fluorescent light at room temperature (28−33℃) (Fig. 2). When the light treatment changed to the sunlight near the window, the sporulation of the three isolates increased significantly by 2.9−11.4 folds (Fig. 3). Previous studies have indicated that environmental factors have different effects on sporulation. Our data also support the notion that the combination of light and temperature is important for good conidia yield, and sunlight apparently is the key factor for *P. oryzae* sporulation. The results indicated that when *P. oryzae* is incubated under natural environment, most of isolates could sporulate. At present, light is the only known signaling factor that stimulates the conidiation of rice blast fungus (Deng *et al*. 2009). In contrast, the occurrence of asexual reproduction of *P. oryzae* is mainly stimulated by environmental light-to-dark transition rather than circadian clock (Lee *et al*. 2006). Abundant aerial hyphae and conidiophore development are observed in the dark period but not in the light period when cultured under the LD cycles, which demonstrated the aerial hyphae development in *P. oryzae* is suppressed by blue light (470 nm). *P. oryzae* has long been known to sporulate at the

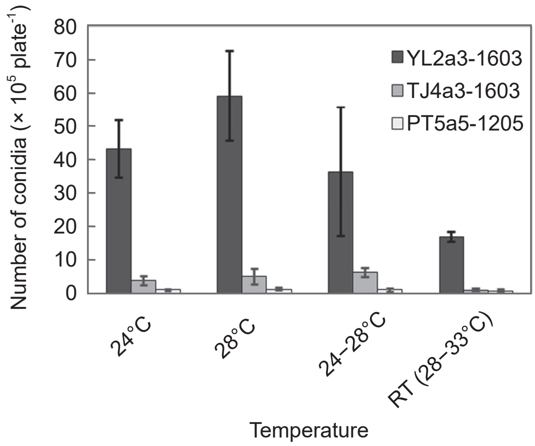

Fig. 2. The conidia yield of *Pyricularia oryzae* isolates YL2a3-1603, TJ4a3-1603, and PT5a5-1205 under 12-h diurnal fluorescent light condition at different temperatures, 24℃, 28℃, daily fluctuation ranging from 24–28℃, or 28–33℃ (room temperature; RT). Refer to Materials and Methods for details of experimental conditions. Error bar is the standard error of mean (*N* = 4).

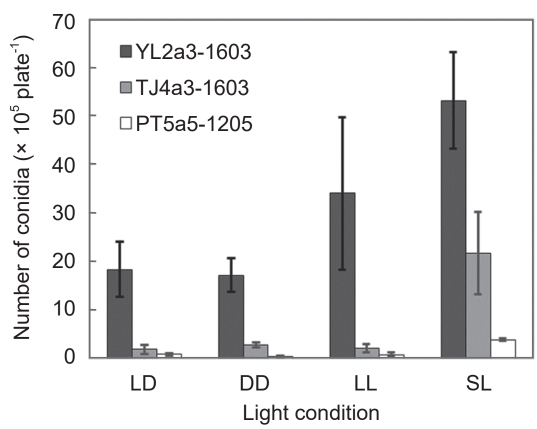

Fig. 3. The conidia yield of *Pyricularia oryzae* isolates YL2a3-1603, TJ4a3-1603, and PT5a5-1205 cultured at the room temperature (28–33℃) under different light conditions. LD: 12-h daily illumination; DD: 24-h constant dark; LL: 24-h constant fluorescent lights; and SL: natural sunlight near the laboratory windows. Refer to Materials and Methods for details of experimental conditions. Error bar is the standard error of mean (*N* = 4).

temperature range from 15℃ to 35℃, and the optimum temperature is 25−28℃ (Kuribayashi 1928). Under natural environment, there are no constant light, darkness, temperature, and humidity. Most studies often use constant or artificial control equipment (such as fluorescent lamps). Thus, the data obtained may not be able to reflect the true behavior of the organism in natural environment. Whether or not different wavelength compositions and other factors exist in the sunlight that affect the sporulation of *P. oryzae* isolates remains to be determined. If the asexual generation of rice blast fungus is actually affected by environmental light and temperature changes rather than the circadian clock, finding the genetic factors affected by light and temperature might help solve the conidia production problem of *P. oryzae*.

In nature, after rice blast fungus successfully penetrates rice tissue, 5−7 d later, conidiophores developed to bear conidia, which are released on the surface of the lesions in the night environment when temperature drops and humidity increases. The improved liquid spawn method as described in this study simulates the growth and sporulation ecology of *P. oryzae* in fields. The medium plate without sealing and incubating under room temperature and sunlight is to create a culture condition similar to the natural environment in the field to induce sporulation of *P. oryzae*.

The combination of injured mycelial fragments as the inoculation source, culturing *P. oryzae* on low-nutrient source (oat flour), and keeping in the natural ventilation petri dishes were to avoid the fungus undergo excessively vegetative growth. These treatments allow most of the *P. oryzae* isolates start to produce a large quantity of conidia within 24 h after inoculation into the OFA medium. However, differences in the sporulation ability and nutrient requirement were observed among some isolates. Thus, the medium formula must be adjusted to effectively increase the sporulation for certain isolates with specific nutrient requirements (Ou 1985).

The production of a good quantity of quality inoculum is crucial for studying many aspects of fungal species (Rodrigues *et al*. 2010). The combination of many factors, such as light, temperature and nutrition, seems to affect the sporulate behavior of *P. oryzae*. The liquid spawn method overcomes the problems of slow growth and unstable conidia production of *P. oryzae*. Further research needs to be done to clarify the effect of light wavelength on *P. oryzae* sporulation.

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簡易經濟的稻熱病菌 (*Pyricularia oryzae*) 產孢誘導方法

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摘要

陳繹年、吳東鴻、陳美君、陳珮臻。2021。簡易經濟的稻熱病菌 (*Pyricularia oryzae*) 產孢誘導方法。台灣農業研究 70(1):1–10。

稻熱病菌 (*Pyricularia oryzae*) 的生理小種鑑定與水稻品種的抗性評估，都需要大量病原孢子作為接種源進行接種檢定。為獲得穩定的檢定結果，接種源分生孢子的齡期必須具一致性。本研究改良國際稻米研究所常用的稻熱病菌產孢誘導程序 [IRRI (International Rice Research Institute) protocol]，利用菌絲體均質方式 (稱「液態菌種法」) 來誘導稻熱病菌在短時間內同時大量產孢。操作程序為：(1) 將 4 日齡的稻熱病菌落以滅菌解剖刀切細碎後，移入加州梅養液中震盪培養 4 d；(2) 以滅菌均質瓶將菌絲體與養液一起均質作為液態菌種，取適量液態菌種接種於燕麥粉平板培養基 (oat flour agar; OFA) 上，於無菌操作台中風乾培養基表面多餘水分後，置於室溫、窗邊自然光環境下培養 4 d 進行產孢誘導。供試菌株採用液態菌種法後的分生孢子產量，明顯高於採用 IRRI protocol 的孢子產量 (2–31倍)。另外，14 株於傳統菌絲塊培養法下產孢不良的稻熱病菌株，在採用液態菌種法培養後，所有菌株的產孢量都有顯著提昇。本研究結果顯示，液態菌種法可用於誘導稻熱病菌同時大量產孢。

關鍵詞：稻熱病、產孢、分生孢子、液態菌種、燕麥粉培養基。

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