SPORULATION BY PHYSIOLOGIC RACES OF *PIRICULARIA ORYZAE* CAV.[1]

TSUNG-CHE TSENG, YIH-SHYONG LEE and LUNG-CHI WU[2]

(Received April 6, 1965)

Introduction

Studies on the rice blast fungus, *Piricularia oryzae* Cav., have been made by many workers. Nishikado (1917) described his work on *Piricularia* strains from rice and some gramineous plants. Tochinai and Shimamura (1932) studied physiologic specialization of the same fungus and classified them into nine physiologic forms by relative difference of the cultural characters on four artificial media. The similar results were also reported by Konishi (1933). However, Hemmi (1949) indicated the dissimilarity of the cultural types and the physiologic races on the basis of their pathogenicity to selected rice varieties. Chien *et al.* (1963) also found that the pathogenicity of the physiologic races was not entirely associated with the cultural characters.

In physiologic race studies, a large number of the spores of individual race must be produced on artificial media of which the compositions, if possible had better be chemically defined. As with many other fungi, various methods of artificial culture have been devised to induce growth and sporulation by isolates obtained from the lesions of rice blast (Tochinai and Nakano, 1940; Henry and Andersen, 1948; Misato and Hara, 1957; Yamanaka *et al.*, 1961; Apparao, 1962; Chen, 1963). However, one can hardly expect to apply a single condition for different physiologic races of different localities since the races of the fungus occurring in the different countries are different (Ou, 1963).

Objectives of the present investigation are to determine substrate effect on the spore formation of five physiologic races by mycelium and to find a suitable condition for preservation of these spores to provide desired quantities of spores for the studies of rice blast fungus.

(1) Paper No. 42. The authors wish to express their hearty thanks to Dr. H. W. Li, Director of the Institute of Botany, Academia Sinica and Dr. S. H. Ou, plant pathologist of the International Rice Research Institute for their valuable suggestions and critical reading of the manuscript.
Research was partly supported by the International Rice Research Institute under the cooperative research project entitled "Physiology of *Piricularia oryzae* Cav."

(2) Respectively, Research Assistants and Research Fellow of the Institute of Botany, Academia Sinica.

Material and Methods

Cultures of five physiologic races, i. e. #1 (2T-82S), #2 (2T-32S), #5 (2K-24Sb), #13 (0'S-45Sa), and #17 (2K-82S), were kindly furnished by Mr. C. C. Chien of the Taiwan Agricultural Research Institute. Stock cultures were maintained on potato dextrose agar (PDA) at 5°C. The inocula were prepared each time from stock cultures by plating a bit of mycelium at the approximate center of PDA on Petri-dish and incubated at 28°C for a week. Inoculation was made by cutting the margin of a week old mycelial colonies and placed with the mycelium uppermost on the substrate in ordinary test tubes of 18XI. 6 cm with cotton plugs.

Media for sporulation of the fungus were PDA (Riker and Riker, 1936) modified by adding 20 g/1 baby foods of bananas, beans, garden vegetales, or peas; 2 g/1 yeast extract (Difco) or 10 pieces/1 yeast tablet (Taiwan Sugar Cane Co.); 10 g/1 soluble starch; 2 g/1 yeast extract (Difco) with 20 ml/1 of V-8 juice; decoction from 200 g/1 zizania stem (*Zizania aquactia* L.); or 5 μg/1 biotin with 5 mg/1 thiamine. Baby foods and V-8 juice were respectively the products of Beech-Nut (Baby Foods Division) MFR., Canajoharie, N. Y. and Campbell Soup Co., General offices, Camden, N. J., U. S. A. The eleven modified PDA were compared with rice straw dextrose agar, decoction from 200 g/1 rice straw with 20 g/1 dextrose; Tochinai's medium (Tochinai and Nakano, 1940); and Misato's medium (Misato and Hara, 1957). Fifteen grams of agar powder were used for each liter of media throughout the experiments.

For the studies on the effect of hydrogen ion concentration on sporulation, PDA modified by adding yeast extract or biotin and thiamine; rice straw dextrose agar; and Misato's medium were used. The nine pH values, from 5.0 to 9.0 at 0.5 intervals were obtained by adding 0.1 N HCl or NaOH to the agar media for adjustment of pH value.

Spore suspensions for counting were prepared by adding sterile water to a week old agar cultures incubated at 26°C and lightly scraping the surface with a wire loop. Spore counting was made by haemocytometer. Count for each tube was repeated three times and the figure was computed.

The longevity of the spores were determined by transferring a loopful of spore suspensions stored in a low temperature incubator (at 5°C) to PDA slants at a week interval for 15 weeks. The PDA slants smeared with above-mentioned spore suspensions were incubated at 28°C for the determination of the growth of the fungus in a week later.

Triplicate cultures for each physiologic race were used to obtain an average number of spores produced. PDA plus yeast extract medium was run

together with other media as the control of computation. All the experiments wee repeated at least twice.

Results

Effect of substrate on sporulation——Growth and sporulation took place on the media tested except a few cases. In general, modified PDA strikingly increased spore formation, e. g. the addition of zizania docoction, yeast extract and V-8 juice, or biotin and thiamine. However, addition of baby foods e. g. beans, garden vegetables, beef, peas, and bananas was not of much help. The synthetic medium devised by Tochinai and Nakano (1940) supported the abundant mycelial growth with sparse sporulation. On the other hand, the semisynthetic medium of Misato and Hara (1957) was fairly favorable for the most of the physiologic races to produce spores. Some races produced abundant spores in most of the media applied, some others produced less spores regardless of the media used. Apparently, different media had different influence on the sporulation of the five physiologic races as illustrated in Table 1.

Effect of hydrogen ion concertration on sporulation——Hydrogen ion concertration of the substrate did not seem to be much influential with the sporulation by the five physiologic races within the range tested (Fig. 1). The

Table 1. *Sporulation by 5 physiologic races of Piricularia oryzae on 14 media at 26°C for a week.**

Medium	Physiologic Race				
	#1	#2	#5	#13	#17
PDA+Boby food (beans)	1.41	5.58	0.08	0.24	0.20
PDA+Baby food (garden vegetables)	16.00	0.60	0.35	4.25	0
PDA+Baby food (beef)	0.10	0	0.08	0.93	0
PDA+Baby food (peas)	1.85	0	0.20	0.60	0.10
PDA+Baby food (bananas)	0.43	0.86	2.15	0	0.64
PDA+Yeast extract	76.08	54.58	2.80	37.31	3.83
PDA+Yeast extract+V-8	56.41	34.16	3.33	30.45	1.33
PDA+Soluble starch	1.75	0.08	0	0	0
PDA+Zizania	129.12	3.50	12.00	30.25	19.25
PDA+Yeast tablet	15.60	21.36	3.29	48.66	0.58
PDA+Biotin+Thiamine	262.07	2.37	9.36	43.56	2.84
Rice straw dextrose agar	18.11	51.00	1.94	6.00	1.16
Tochinai's medium	1.16	0.58	0.58	0.41	0
Misato's medium	143.38	8.34	33.10	318.05	16.35

* Data presented in 10 thousands of condia per test tube culture. Each figure represents the average of 3 cultures.

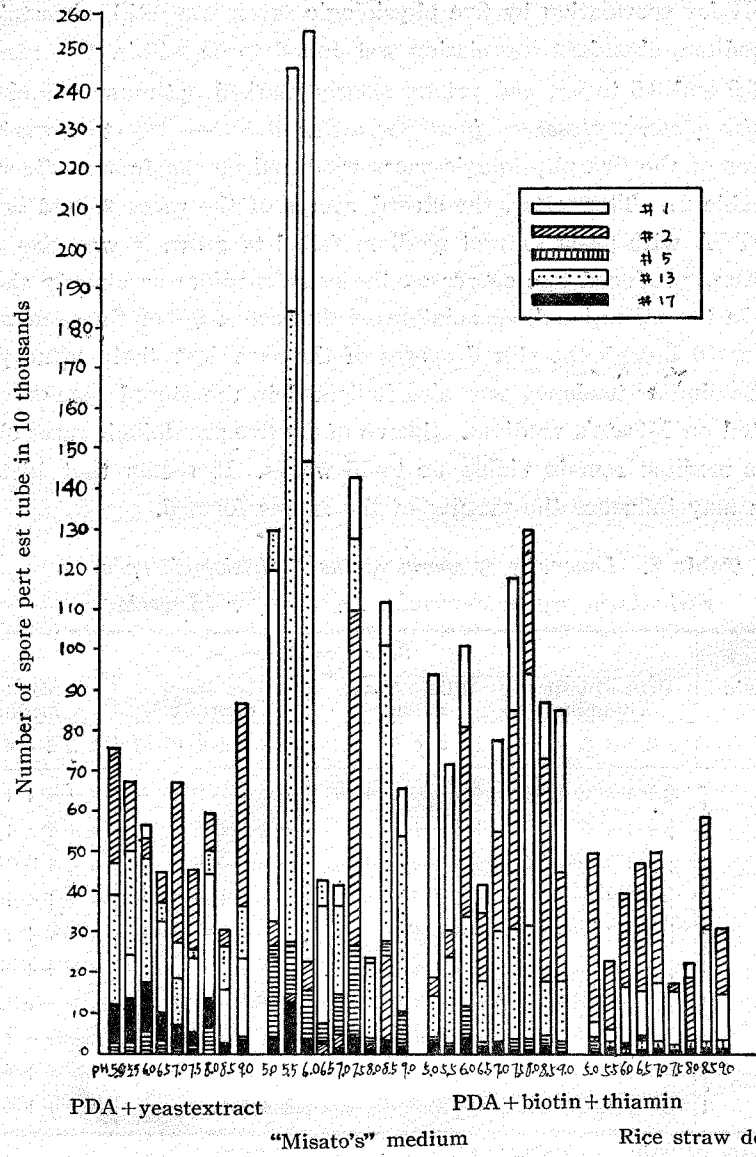

Fig. 1. Effect of hydrogention concention on the sporulation of 5 physiologic races of *Piricularia oryzae* on 4 media at 26°C for a week.

same might be true for the other genera of fungi (Lilly & Barnett, 1951; Cochrane, 1958). However, Henry and Andresen (1948) reported that growth and sporulation occurred from pH 4.1 to 8.8 with maximum spore formation at between pH 4.9 and 7.5. The fact was attributed to the result of the effect on vegetative growth. Using Misato's medium, optimum pH range was found to be from 5.6 to 5.8 (Misato and Hara, 1957).

Because of the fluctuation of data were random with no consistent patterns,

optimum pH for sporulation by five physiologic races was still uncertain. On Misato's medium, abundant sporulation was found to be within the ranges of pH 5.0 to 6.0 and 8.5 to 9.0, and yet no clearly marked optimum was observed.

Longevity of spores preserved in sterile water at 5°C——Up to 4 weeks, the stored spores of the five physiologic races produced on the four media remained vital (Table 2). Thereafter, the stored spores of the races #5 and #17 obtained from PDA with yeast extract medium failed to grow. It was also evident that both races produced less spores on the same medium as already shown in Table 1. On the other hand, sporulation of the race of #13 on the same medium was fairly good though the stored spores of the race lost their vitality in 10 weeks. The similar tendency was also indicated in the stored spores of race #5 sporulated on Misato's medium. Spores of the five physiologic races obtained on Misato's medium remain viable up to 15 weeks. It seems that history of sporulation may influence the vitality of the spores formed.

Table 2. *Longevity of spores of the 5 physiologic races of* ***Piricularia oryzae*** *determined weekly for 15 weeks.**

Physiologic race	Spores produced on			
	PDA+Biotin+Thiamine	PDA+Yeast extract	Rice straw dextrose	Misato's medium
	1 2 3 4 6 10 15	1 2 3 4 6 10 15	1 2 3 4 6 10 15	1 2 3 4 6 10 15
# 1	++++++ + − +++++	++++++ + + ++++	++++++ + + ++++++ + +	++++++ + + ++++++ + +
# 2	++++++ + + +++++	++++++ + + +++++	++++++ + + ++++++ + +	++++++ + + ++++++ + +
# 5	++++++ − − +++	++++− − − ++++	++++− − − +++	++++++ + + ++++++
#13	++++++ + + ++++	++++++ − − ++++	++++++ + + ++++++ + +	++++++ + + ++++++ + +
#17	++++++ + − ++++	++++− − − ++++	++++++ + + ++++	++++++ + + ++++++ + +

* $\overset{+}{+}$=abundant growth +=sparse growth −=no growth

Discussion

Nutrition is the most important factor to control the growth and sporulation of the fungi. Concentration of nutrients, carbon and nitrogen sources, carbon—nitrogen ratio, minerals, vitamins, and unidentified reproductive factors are particularly influential with the sporulation (Lilly and Barnett, 1951; Hawker, 1957; Cochrane, 1958).

Sporulation by *P. oryzae* was also found to be a process distinct from vegetative growth in many respects (Henry and Andersen, 1948). In the present

investigation, the similar results were obtained, substrate supported luxuriant growth of mycelium was not always followed by the abundant sporulation while a certain amount of vegetative myclium was prerequisite for sporulation. For instance, PDA modified by adding baby foods did not induce the five physilolgic races to produce abundant spores though they stimulated the luxuriant growth of vegetative mycelium. It was also evident that different media supported the sporulation of the five physiologic races differently as already described by Henry and Andersen (1948). Longevity of spores was nearly controled by the medium on which spores were produceed. In view of the epidemic of the rice blast disease, inoculum potential of the fungus might be influenced quantitatively and qualitatively by the history of spore formation by individual race.

Summary

Fourteen media were used for comparing the sporulation of the five physiologic races of *Piricularia oryzae*. No single medium allowed all physiologic racesto produce the same amount of spores. Vitality of the spore stored in sterile water at 5°C remained vital for 15 weeks. History of sporulation was influential with the longevity of spores. Misato's medium (soluble starch 10 g, yeast extract 2 g, per liter) was suitable for sporulation of the five physiologic races examined.

稻熱病菌生理小種之胞子形成

曾聰徽　李義雄　吳龍溪

五種稻熱病菌生理小種，分別培養在14種培養基上，比較其胞子形成，沒有一種培養基可使各種生理小種產生同量的胞子。貯于無菌水中的胞子放在5°C 下，可保存15個星期。所用培養基明顯地影響胞子壽命。見里培養基（其成份爲可溶性澱粉10克，酵母抽出物 2 克，配成一公升）對于 5 種生理小種的胞子形成爲最適合。

Literature Cited

APPARAO, A. Nutritional factors governing sporulation of *Piricularia oryzae* in culture. J. Indian Bot. Soc. **41**: 604-622. 1962. (Abstr. in Rev. Appl. Mycol. 43: 17).

CHEN, M. J. Studies on rice blast disease. (I). Suitable media for the sporulation of the causal organism, *Piricularia oryzae*. Bull. Soc. Plant Path. Taiwan **1**: 21-24, 1963. (In Chinese)

CHIEN, C. C., S. Y. LIN, and S. C. JONG. Studies on the physiologic races of *Piricularia oryzae*. Cav. (II). Agr. Res. Taiwan **12**: 29-39, 1963.

COCHRANE, V. W. Physiology of fungi. John Wiley & Sons, Inc. 524 p., 1958.

HAWKER, L. E. The physiology of reproduction in fungi. Cambridge Univ Press. 128 p., 1957.

HEMMI, T. Studies in the rice blast. Asakura Book Co. Tokyo, 347 p. 1949. (In Japanese)

HENRY, B. W. and A. L. ANDERSEN. Sporulation by *Piricularia oryzae*. Phytopathology **38**: 265-278, 1948.

KONISHI, S. On physiologic specialization in the rice blast fungus, *Piricularia oryzae* Br. et Cav. Forsch. auf dem Geb. der Pflanzenkrankh. (Kyoto) **2**: 55-77. 1933. (In Japanese)

LILLY, V. G. and H. L. BARNETT. 1951. Physiology of the fungi. McGraw-Hill Book Co., Inc. 464 p., 1951.

MISATO, T. and K. HARA. On a medium for the sporulation by rice blast fungus. Agriculture and Harticulture **32**: 797-798, 1957 (In Japanese)

NISHIKADO, Y. Studies on the rice blast fungs. I. Berichted. Ohara Inst. f. landw. Forsch. **1**: 171-218, 1917.

OU, S. H. Varietal reactions of rice to blast. p. 223-234. In The rice blast disease, Proceedings of a symposium at the International Rice Research Institute, July, 1963. The Johns Hopkins Press, Baltimore, Maryland, 1965.

RIKER, A. G. and R. S. RIKER. Introduction to research on plant disease. John S. Swift Co., St. Lonis, 117 p., 1936.

TOCHINAI, Y. and T. NAKANO. Studies on the synthetic nutrient solution being suitable for the mycelial growth of *Piricularia oryzae* Cav. Ann. Phytopath. Soc., Japan **10**: 110-118. 1940. (In Japanese)

TOCHINAI, Y. and M. SHIMAMURA. Studies on the physiologic specialization in *Piricularia oryzae* Br. et Cav. Ann. Phytopath. Soc. Japan **2**: 414-441, 1932.

YAMANAKA, T., N. KOBAYASHI, K. SINDO and M. IKEDA. Spore formation and preservation of rice blast fungus. Ann. Phytopath. Soc. Japan **26**: 56, 1961. (Abstr. in Japanese).