The effects of various media on sporulation and growth of *Pyricularia oryzae* Cavara

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The authors have investigated the effect of many media on the development of 10 isolates of *Pyricularia oryzae* in order to obtain abundant sporulation, to facilitate the determination of physiological races and preparation of phytopathological tests.

INTRODUCTION

Pyricularia oryzae Cavara, the organism which causes rice blast disease was isolated from diseased rice leaves and panicles collected from various varieties of the trap nursery grown at different locations in Egypt.

The effect of certain nutritional factors on the sporulation and growth of 10 isolates of the fungus was studied in the present investigation. This was found to be of great importance since good sporulation is required for the identification of the physiologic races and other pathological tests (Ashour et al. 1973).

MATERIALS AND METHODS

P. oryzae was isolated from diseased rice leaves and panicles on oat meal agar medium. The cultured fungi were examined microscopically from pieces of mycelium added to 1 ml of sterile water, poured on 10 ml of plain agar in petri dishes. Single conidia were isolated and transferred to potato dextrose agar slants, after 12 hrs incubation at 28°C.

For the growth rate, uniform mycelial mats (4 mm in diameter) of the different isolates were seeded centrally in petri dishes containing 20 ml of the 17 media. Growth was measured after 14 days incubation at 28°C.

Amount of growth was determined by inoculating 20 ml of the liquid

media in Erlenmeyer flasks with mycelial mats as mentioned before. These were collected after 14 days incubation at 28°C on filter paper, washed, dried in the oven at 70°C for 24 hrs, and then weighed.

The amount of sporulation was determined in the previous plate cultures by adding 20 ml of water to dislodge the spores from the culture, after which spore counts were made under the microscope as follows:

- +++ more than 50 spores per microscopic field (low power).
- ++ 30-50 spores per microscopic field.
- + less than 30 spores per microscopic field.
- no spores.

RESULTS AND DISCUSSION

Media. The effects of 17 different media on the growth rate of ten isolates of *P. oryzae* is shown in Tables 1-4. All the isolates failed to grow on synthetic media, and gave good growth on natural media. Simi-

Table 1

Effect of different media on the rate of growth of ten isolates of *P. oryzae* Cav.

Media	Serial numbers of the isolates									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Barley extract agar	+	-	-	+	+	-	+	-	+	+
Wheat extract agar	-	-	-	-	-	-	-	-	-	-
Rice extract agar	-	-	-	-	-	-	-	-	-	-
Barley decoction agar	-	-	-	-	-	-	-	-	-	-
Wheat decoction agar	-	-	-	-	-	-	-	-	-	-
Rice decoction agar	-	-	-	-	-	-	-	-	-	-
Potato dextrose agar	++	++	++	++	++	++	++	++	++	++
Oat meal agar	++	++	++	++	++	++	++	++	++	++
Corn meal agar	++	++	++	++	++	++	++	++	++	++
Oat meal + Sorghum 1:1	++	++	++	++	++	++	++	++	++	++
Polished rice agar 1%	+	+	+	+	+	+	++	+	+	+
Rice decoction straw agar	+	++	+	+	+	+	+	+	++	++
Yeast starch agar	+	+	+	+	++	+	++	+	+	+
Sucrose potato decoc- tion agar	++	++	++	++	++	++	++	++	++	++
Glucose peptone	+	+	++	+	++	+	++	+	+	+
Czapek's	-	-	-	-	-	+	-	-	-	-
Takahashi's A	+	++	++	+	+	++	++	++	++	++

++ more than 70 mm, + from 50-70 mm, and - less than 50 mm diameter of the mycelial growth.

Table 2

Effect of different media on spore production of ten isolates of *P. oryzae* Cav.

Media	Serial numbers of the isolates									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Barley seeds	++	++	+	++	++	+	++	+	+	++
Wheat seeds	—	+	+	+	+	+	—	—	+	—
Rice seeds	++	+	++	+	++	++	+	++	+	+
Barley stem nodes	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Wheat stem nodes	++	++	++	+++	++	++	++	++	+++	+++
Rice stem nodes	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Banana stalk	++	++	+++	++	+++	+++	+	++	+	++
Water hyacinth	+	+	—	+	+	++	+	—	+	+
Barley extract agar	++	+	+	+	+	+	++	+	++	+
Wheat extract agar	+	+	+	+	—	—	+	+	++	—
Rice extract agar	++	+	++	+	+	+	+	++	++	+
Barley decoction agar	+	—	—	—	—	+	+	+	+	—
Wheat decoction agar	—	—	—	—	—	—	—	—	+	—
Rice decoction agar	+	+	—	—	—	—	+	+	+	+
Potato dextrose agar	+++	+++	+++	++	+	++	++	++	++	+++
Oat meal agar	++	+	+++	++	+++	+++	+	++	++	++
Corn meal agar	+++	+	++	++	+	++	++	++	++	++
Oat meal+sorghum 1:1	+	+	++	+	+	+	+	++	+	+
Polished rice agar 1%	++	+	+	—	+	+	—	+	+	++
Rice decoction										
straw ag.	+	+	++	+	+	+	+	+	+	++
Yeast starch agar	++	++	+	+	++	++	++	++	+	+
Sucrose potato										
decoction agar	+++	+++	+++	++	++	++	++	+++	+++	++
Glucose peptone	++	+	+	+	+	+	+	+	+	++
Czapek's	—	—	—	—	—	—	—	—	—	—
Takahashi's A	++	+++	++	++	+	+	+	++	+	+

+++ more than 50 spores, ++ from 30-50 spores + less than 30 spores per optical field, and — no spores.

lar results were presented by Henry and Anderson (1948). Abdel-Hak et al. (1970) concluded that isolates of *P. oryzae* grow best on Takahashi's and potato sucrose agar media.

The isolates differed in their ability to grow on the different media. Isolates VII and IV gave the highest and lowest rates of growth, respectively. In addition, isolates II, III, VI and VIII showed poor growth on barley extract agar medium, while the other isolates gave satisfactory growth on the same medium. Hence, the variation in the rate of growth was found to be specific for each isolate/medium.

Spore production was studied on 8 different natural media, in addition to the above 17 agar media. The medium was considered suitable for sporulation if the spore count was 30 or more per optical field under

a low power microscope, and classed as most suitable if the count of spores was 50 or more (Table 1).

Spore production was found to be very high on natural media such as rice, barley and wheat stem nodes. Sanchez and Ou (1967) found also that the best spore production was obtained on similar media.

Non-synthetic agar media were very suitable for sporulation of the fungus. Potato dextrose agar, oat meal agar, sucrose potato decoction

Table 3
Effect of different carbon sources on the growth and sporulation of ten isolates of *P. oryzae* Cav.

Carbon sources		Serial numbers of the isolates									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Glucose	(G)	++	++	++	++	++	++	++	++	++	++
	(S)	++	+++	+++	+++	+++	+++	+++	++	+++	+++
Arabinose	(G)	+	+	-	-	+	+	+	-	+	+
	(S)	+	+	+	+	+	+	+	++	++	++
Xylose	(G)	-	-	-	+	-	-	-	+	-	-
	(S)	+	+	+	+	+	++	+	+	+	+
Fructose	(G)	+	+	+	+	+	+	+	+	+	+
	(S)	++	+++	+++	+++	++	+++	+++	+++	+++	+++
Mannose	(G)	+	+	+	-	-	-	+	+	+	+
	(S)	++	++	++	+	+	+	++	++	+	++
Galactose	(G)	-	-	-	-	-	-	-	-	+	-
	(S)	++	+	+	+	++	+	++	++	++	+
Lactose	(G)	-	-	-	-	-	-	-	-	-	-
	(S)	++	++	++	+	++	++	+	+	+	+
Sucrose	(G)	+	+	+	+	+	+	+	+	+	+
	(S)	++	+++	+++	+++	+++	+++	+++	+++	+++	++
Maltose	(G)	+	+	+	+	+	+	+	+	+	+
	(S)	++	+	++	+	++	+	+	+	+	++
Raffinose	(G)	+	+	+	+	+	+	+	+	+	+
	(S)	+	+	+	+	+	+	+	+	++	+
Starch	(G)	++	++	++	++	++	++	++	++	++	++
	(S)	-	-	+	-	-	-	-	-	-	-
Inulin	(G)	+	+	+	+	+	++	+	+	+	+
	(S)	+	+	-	-	-	-	+	-	-	+
Glycogen	(G)	+	+	+	+	+	+	+	+	+	+
	(S)	+	-	-	+	-	-	+	-	+	-
Dextrin	(G)	++	++	++	++	++	++	++	++	++	++
	(S)	+	+	+	+	+	+	+	+	-	-

(G) = amount of growth: ++ more than 500 mg, + from 300-500 mg, and - less than 300 mg dry wt. of mycelium.

(S) = Spore production: +++ more than 50 spores, ++ from 30-50 spores, + less than 30 spores per optical field, and - no spores.

Table 4
Effect of different nitrogen sources on the growth and sporulation of ten isolates of *P. oryzae* Cav.

Nitrogen sources		Serial numbers of the isolates									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Potassium nitrate	(G)	+	+	++	+	+	+	+	+	++	+
	(S)	++	++	++	++	++	++	+	+	+	+
Sodium nitrite	(G)	-	-	-	-	-	-	-	-	-	-
	(S)	-	-	-	-	-	+	-	-	-	+
Sodium nitrate	(G)	+	-	-	-	+	+	+	-	+	+
	(S)	-	-	-	-	-	-	-	-	-	-
Ammonium nitrate	(G)	+	+	+	-	-	+	-	-	+	-
	(S)	++	+	+	+	+	+	+	+	+	++
Ammonium sulphate	(G)	++	+	-	-	+	+	+	-	-	-
	(S)	+	++	+	++	+	+	+	+	+	+
Ammonium chloride	(G)	-	-	-	-	-	-	-	-	-	-
	(S)	-	-	-	-	-	-	-	-	-	-
Aspartic acid	(G)	++	++	++	++	++	++	++	++	++	++
	(S)	+++	+++	+++	++	++	+++	+++	++	+++	+++
Glutamic acid	(G)	++	++	++	++	++	++	++	++	++	++
	(S)	++	++	++	++	++	++	+	+	+	+
Glycine	(G)	++	++	++	++	+	++	++	++	++	++
	(S)	+++	+++	+++	+++	++	+++	+++	++	+++	+++
Alanine	(G)	+	+	+	+	+	+	+	+	+	+
	(S)	+	+	+	++	+	++	+	+	+	+
Cystine	(G)	+	+	+	+	+	-	-	+	+	+
	(S)	++	+	+	++	++	++	++	++	++	+
Histidine	(G)	+	+	-	-	-	-	-	+	-	+
	(S)	+	+	+	+	+	+	+	++	+	+
Peptone	(G)	++	++	++	++	+	++	++	+	++	++
	(S)	+++	++	++	+++	+++	+++	++	++	+++	+++
Urea	(G)	+	+	-	-	-	-	-	+	+	+
	(S)	-	-	-	+	-	-	+	-	+	-

(G) = amount of growth: ++ more than 500 mg, + from 300-500 mg, and - less than 300 mg dry wt. of mycelium.

(S) = Spore production: +++ more than 50 spores, ++ from 30-50 spores, + less than 30 spores per optical field, and - no spores.

agar and corn meal agar gave high sporulation, but other media namely wheat extract agar, barley, wheat and rice decoction agar and polished rice agar gave poor sporulation. On the other hand, all the isolates failed to sporulate on Czapek's medium. This is in agreement with the findings of Otsuka (1961) who concluded that potato sucrose agar, yeast extract agar, Koji agar were suitable for the sporulation of the fungus.

It has also been found that the sporulating ability of the fungus differed with the medium under consideration; while isolate I had the highest spore production followed by isolates III, isolates IV and VII had the smallest spore number.

Carbon sources. Growth and spore production of the isolates of the fungus were studied on media differing in carbon sources by adding different sugars according to their molecular weights using the glucose peptone medium. Results are shown in Table 3.

The best growth was obtained with glucose, fructose, sucrose, starch and dextrin, while xylose, mannose, galactose and lactose were found to be poor sources for all the isolates. Tochinal and Nakano (1940) in this respect, concluded that carbohydrates stored in leaves of the plants, namely glucose, fructose, sucrose and starch were all favourable for the growth of this fungus.

The ten isolates sporulated well with glucose, fructose and sucrose but not with starch, inulin and glycogen.

Similar results were reported by Tseng and Wu (1967), who found that simple sugars were more favourable for spore production than the complex ones.

Nitrogen sources. Peptone in glucose peptone medium was replaced by other nitrogen sources according to their molecular weights. Amounts of growth and rates of sporulation of the isolates of *P. oryzae* are given in Table 4.

The best amount of growth was found when glutamic and aspartic acids were used, followed by glycine, peptone and potassium nitrate in descending order. The other sources of nitrogen, however, were found to be unsuitable for maximum growth.

Spore production was very satisfactory with glycine, aspartic acid, peptone, cystine, glutamic acid and potassium nitrate, while no sporulation occurred in any of the isolates when ammonium chloride or sodium nitrate were used. Similar result was obtained by Lee and Wu (1967). Abdel-Hak et al. (1970) however, showed peptone to be favourable for the growth and sporulation of this fungus.

SUMMARY

Potato dextrose agar, oat meal agar, corn meal agar and sucrose potato decoction agar were found to be the best media for maximum growth of *Pyricularia oryzae* Cavara.

On natural media, the best spore production for the ten isolates was on rice, barley and wheat stem nodes, while wheat seeds and water hyacinth were found to be unsuitable for maximum sporulation.

The highest amount of growth was recorded with glucose, starch and dextrin for all the isolates. Glucose, fructose and sucrose gave the maximum rate of sporulation, and starch was found to be a poor carbon source in this respect.

Glutamic and aspartic acids were found to be the best nitrogen sources for maximum growth, glycine and aspartic acid for maximum sporulation of the all isolates. No spores were produced with ammonium chloride or sodium nitrate by any of the isolates.

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Wpływ różnych pożywek na wzrost i zarodnikowanie
Pyricularia oryzae Cavara

Streszczenie

Autorzy — mając na celu uzyskiwanie obfitej sporulacji *Pyricularia oryzae* Cavara dla łatwiejszego oznaczania ras fizjologicznych i przygotowywania testów fitopatologicznych — przebadali wpływ kilkunastu pożywek na rozwój 10 izolatów wymienionego grzyba.