THE CARBON NUTRITION OF *PIRICULARIA ORYZAE* CAV.*

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Introduction

Nutritional approach to elucidate physiology of parasitism has been attempted. Tochinai and Nakano (1940) first indicated that carbohydrates present in the leaves of the host, namely glucose, fructose, and starch, were all favorable nutrients for the growth of *Piricularia oryzae*. Tomizawa and Koike (1953) studied the nutritional physiology of the fungus and showed that sugars were better sources of carbon than the higher alcohols. The constructive carbohydrates, xylan and cellulose, were also found to be utilized by this fungus (Tanaka and Tsuji, 1952). Recently, it was reported that the isolates of *P. oryzae* differed in their ability to use various carbon sources and this variation in the use of carbon sources resembled the variation in pathogenicity of isolates to different rice varieties (Anonymous, 1964). Chen *et al.* (1964) also revealed that the sensitivity of *P. oryzae* to the nutritional requirement.

In Taiwan, numerous isolates of *P. oryzae* have been characterized into physiologic races by their virulence to the differential rice varieties (Chien *et al.*, 1963; Kou *et al.*, 1963). However, the utilization of certain carbon by the isolates have not been entirely clarified. Studies on the parasitism of this fungus will require the knowledge of its nutrition. Therefore, the ability of selected races of *P. oryzae* to utilize a variety of carbon compounds in defined media was investigated.

Materials and Methods

Five physiologic races of *Piricularia oryzae* Cav., i. e. #1 (2T-82S), #2 (2T-

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32S), #5 (2K-24Sb), #13 (O'S-45Sa), and #17 (2K-82S), were used in the present experiments (Chien *et al.*, 1963). They were kindly furnished by Mr. C.-C. Chien of the Taiwan Agricultural Research Institute. Tanaka's medium B (Tanaka, 1965) without sucrose was used throughout the experiment as the basal medium. Carbon sources tested were prepared in an amount of carbon equivalent to 30 g sucrose per liter of the medium.

The culture medium was aseptically adjusted to pH 6.0 by adding sterilized 0.1 N HCl or 0.1 N NaOH after being autoclaved at 110°C for 10 minutes. Condition for sporulation and method for obtaining spore suspension were described in the previous report (Tseng *et al.*, 1965). A week old cultures grown on Misato's agar medium (Misato and Hara, 1957) were used to obtain spore suspension. The spore concentration was adjusted by centrifugation and resuspension of the washed spore to 5×10^4 spores per ml with an Erma photoelectric spectrophotometer Model No. 4. One ml of adjusted spore suspension was added to each 125 ml Erlenmeyer flask which contained 25 ml liquid medium in question.

Submerged and stationary cultures were compared after 10 days incubation at 26°C. A rotary shaker with 200 revolution per minute was used for submerged culture. The mycelial mats were harvested by pouring cultures in a Buchner funnel with a piece of filter paper and washed with 50 ml distilled water, then they were dried in a hot-air oven at 90°C for 20 hours before measurement of weight. In testing the combination of two carbon sources, the mycelial mass was harvested after 7 days incubation on a rotary shaker at 26°C.

All the results are presented as the average of triplicate and analysed statistically.

Results

Comparison between shaken and stationary cultures

Since the variable effect of shaking was found (Lopez and Fergus, 1965), comparison between shaken and stationary cultures were made with 10 days old cultures of the given physiologic races of *P. oryzae*. The results of the experiment (Fig. 1) indicated that shaking promoted a rapid growth of the races tested though a fairly long lag phase was obtained. However, T-test on the interaction between races and shaken culture, races and stationary culture, showed that most of the races i.e. Races #1, #2, #5, and #13 were statistically significant by the shaken cultures.

Utilization of different carbon sources

Twelve different carbon sources were included in this experiment: glucose, mannose, fructose, galactose, maltose, lactose, sucrose, melibiose, xylose, soluble starch, glycogen, and dextrin.

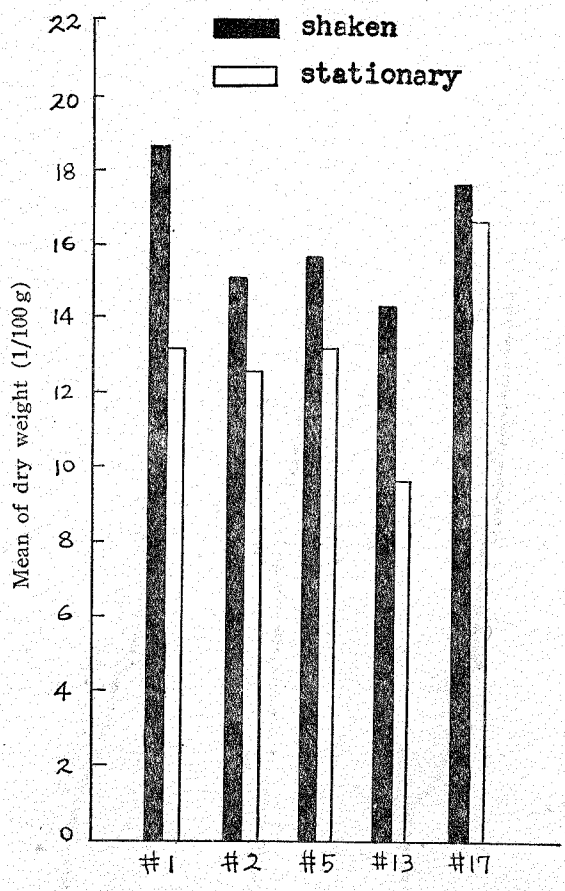

Fig. 1. Comparison between shaken and stationary cultures of 5 races of *Piricularia oryzae*.

As shown in Fig. 2, the ability of the 5 physiologic races to utilize the carbon sources was remarkably different. However, it seemed that most races utilized fructose, glucose, dextrin, and maltose, but utilization of the same compound still varied with different races. Fructose seemed to be the best materials for the mycelial growth of the 5 physiologic races while glucose was readily utilized by Races #5, #13, and #17 but poorly by Races #1 and #2. This variation in the utilization of different carbon sources is similar to the variation observed in virulence of the physiologic races to different varieties.

The data obtained from analysis of variance showed that the effect of physiologic races, culture conditions, and carbon sources were significant at 1% level by F-test. The interactions between physiologic races and culture conditions (S×V); physiologic races and carbon sources (S×C) were also recognized at the same level (Table 1).

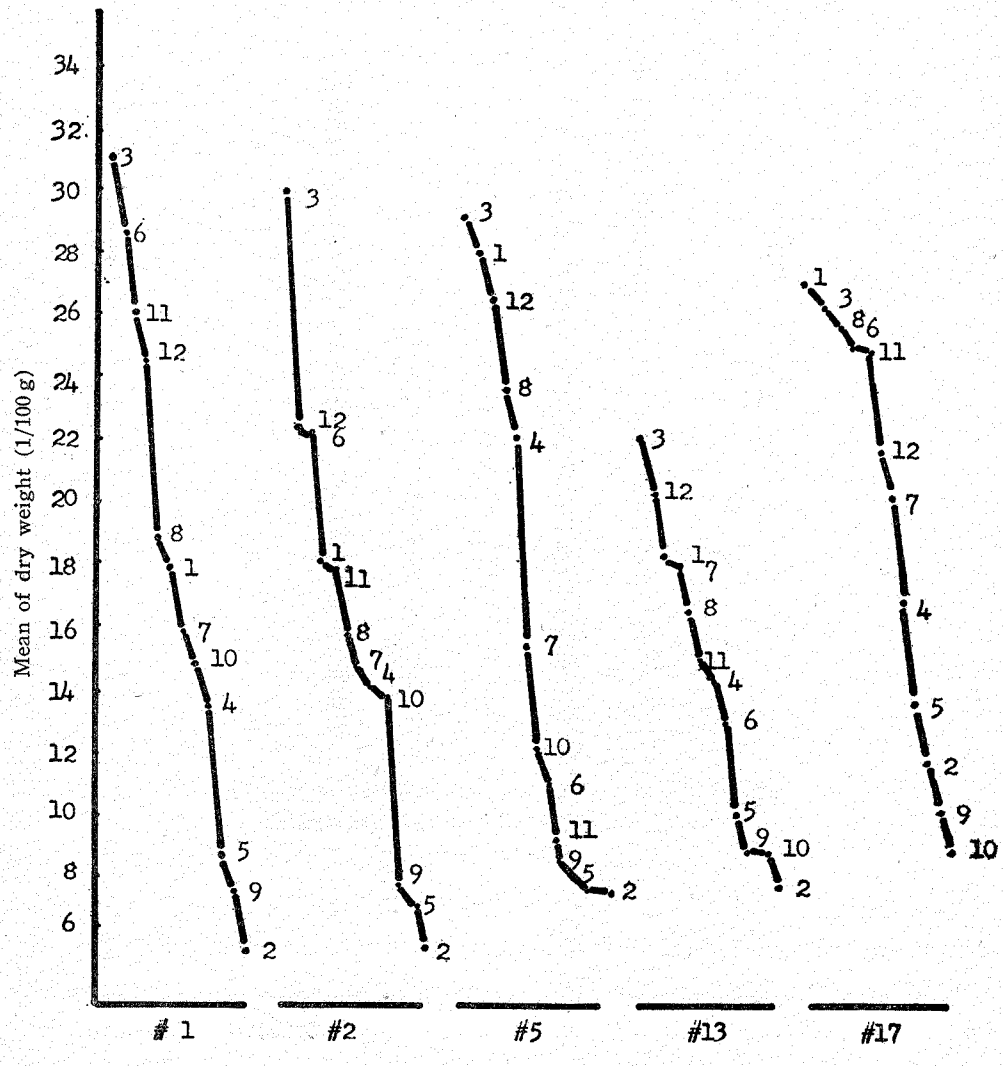

Fig. 2. Utilization of carbon sources by 5 races of *Piricularia oryzae*

1=glucose	2=mannose	3=fructose	4=galactose
5=xylose	6=maltose	7=lactose	8=sucrose
9=melibiose	10=starch	11=glycogen	12=dextrin

Table 1. *Analysis of Variance for 3 factorial in a randomized complete block design*

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Squares	F
Treatments	2.1583	119	0.0181	20.1075**
Strains (S)	0.1072	4	0.0268	29.7118**
Culture condition (V)	0.0912	1	0.0912	101.1086**
Carbon sources (C)	1.2234	11	0.1112	123.3016**
S × V	0.0165	4	0.0041	4.5732**
S × C	0.3721	44	0.0085	9.3758**
V × C	0.1082	11	0.0098	10.9047**
S × V × C	0.2397	44	0.0054	6.0399**
Error	0.1849	205	0.0009	
Total	2.3432	324		

** Significant at 1% level

Combinations of carbon sources

In this experiment, lactose was chosen to be the basic carbon source (G_0, Y_0, C_0=30 g/L) combined with two concentrations of glucose (G_2=2 g/L, G_4=4 g/L); of yeast extract (Y_2=2 g/L, Y_4=4 g/L) and of casein hydrolysate (C_2=

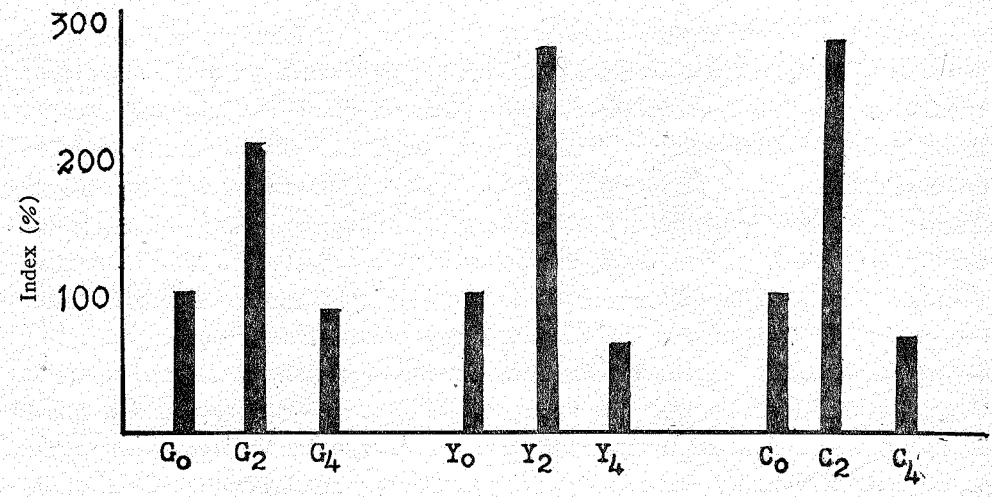

Fig. 3. The effect of lactose, singly and in combination with glucose, yeast extract, casein hydrolysate, upon the amount of growth of *Piricularia oryzae*.

C_0=Y_0=C_0=lactose (30 g/L)
G_2=lactose (30 g/L)+glucose (2 g/L)
G_4=lactose (30 g/L)+glucose (4 g/L)
Y_2=lactose (30 g/L)+yeast extract (2 g/L)
Y_4=lactose (30 g/L)+yeast extract (4 g/L)
C_2=lactose (30 g/L)+casein hydrolysate (2 g/L)
C_4=lactose (30 g/L)+casein hydrolysate (4 g/L)

2 g/L, C_4=4 g/L). Lactose combined with 2g of glucose, yeast extract, or casein hydrolysate was superior to that of the twice weight of any of the aforementioned compounds (Fig. 3)

The data obtained from analysis of variance indicated that effect of physiologic races and treatments were significant at 1% level by F-test while the interactions between physiologic races and treatments were significant at 5% level.

Table 2. *Analysis of Variance for 2 factors factorial in a randomized complete block design*

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Squares	F
Strains (S)	0.0546	4	0.0137	4.720**
Treatments (T)	1.0736	6	0.1789	61.689**
S × T	0.1425	24	0.0059	2.034*
Error	0.1205	42	0.0029	
Total	1.3912	76		

** Significant at 1% level * Significant at 5% level

Discussion

Utilization of the carbon sources by *P. oryzae* have been reported by many workers (Tochinai and Nakano, 1940; Otani, 1953; Tomizawa and Koike, 1953; Otsuka *et al.*, 1957; Anonymous, 1964; Chen *et al.*, 1964). In the present investigation, twelve carbon sources were used to examine their influence on the growth of 5 physiologic races. Most of the races readily utilized fructose, glucose, maltose, and dextrin, while mannose, xylose and melibiose were poorly used. The similar results were also obtained by other workers (Otsuka *et al.*, 1957; Anonymous, 1964). Actually, utilization of different carbon sources by the 5 races was varied. Some races use a carbon source well but others do so poorly. This variation in the use of carbon sources smiliar to the variation in virulence of the races to different rice varieties (Chien *et al.*, 1963). It was concluded that the sensitivity of *P. oryzae* to the nutritional status of the host was partly due to its specific nutritional requirements (Chen *et al.*, 1964).

In nature the fungi usually come in contact with mixed carbon sources rather than a single source of carbon compound. For this reason, utilization of combined carbon sources on the growth of the 5 races of *P. oryzae* was studied. The results revealed that effect of mixed carbon sources was highly influenced by the concentrations of glucose, yeast extract and casein hydrolysate which were combined with lactose in the culture medium.

Summary

Five physiologic races of *P. oryzae* differed in their response to the various carbon sources were studied. The best carbon sources for these races were fructose, glucose, maltose, and dextrin. Mannose, xylose and melibiose were relatively poor sources of carbon. Utilization of mixed carbon sources on the growth of the 5 races was found to be influenced by the concentrations applied.

稻熱病菌與碳素源之關係

曾聰徽 吳龍溪

本實驗之目的在于了解五種稻熱病菌生理小種利用碳素源之情形。由實驗結果悉知，Fructose, Glucose, Maltose 和 Dextrin 爲該五種稻熱病菌生理小種之最佳碳素源，而 Mannose, Xylose, Melibiose 三種爲最劣者。

將該病原菌培養於混合碳素源培養基中，發現混合碳素源可促進菌體之生長，但與碳素源之濃度有關。

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