THE NITROGEN NUTRITION OF *PIRICULARIA ORYZAE* CAV.*

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Introduction

Rice blast, incited by *Piricularia oryzae* Cav. has been known to be worldwide in distribution and generally causes severe damage. Study of nitrogen nutrition of this pathogen is fundamental to an understanding of the nature of rice blast. Otsuka *et al.* (1965) proposed that the utilization of nitrogen compounds by this fungus varied considerably with the strain and that the difference in utilization correlated with the differences in pathogenicity. Lopez and Fergus (1965) showed that two different isolates differed in their response to the various nitrogen sources. Thiamine and biotin were required for growth of *P. oryzae* (Leaver *et al.*, 1947) and Tanaka (1965) said thiamine was available only when the biotin was present. Wu *et al.*, (1966) also pointed out that differences in specific activity of aldolase and lactic dehydrogenase of the five races of blast fungus were in existence. Garber (1956) suggested that a plant was susceptible to a pathogen when the suscept provided a suitable nutritional environment and failed to present an inhibitory environment for growth of the pathogen.

The present studies is intended to provide some information concerning the physiology of rice blast. The specific purpose of this investigation is to determine the nitrogen requirements of some physiologic races of *P. oryzae.*

Materials and Methods

Five physiologic races of *Piricularia oryzae* Cav. previously used for the sporulation study (Tseng *et al.* 1965) were applied in the present experiment.

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They are Race #1 (2T–82S), #2 (2T–32S), #5 (2K–24Sb), #13 (O'S–45Sa), and #17 (2K–82S) (Chien *et al.*, 1963). These cultures were kindly provided by Mr. C.-C. Chien of the Taiwan Agricultural Research Institute. Tanaka's medium B (Tanaka *et al.*, 1965) without KNO_3 and urea was utilized as the basal medium in the present investigation.

The inorganic and organic compounds examined were prepared to yield the weight of nitrogen equivalent to the two nitrogen sources mentioned above. The hydrogen ion concentration of the medium was adjusted to pH 6.5 by adding 0.1 N HCl or 0.1 N NaOH before sterilization. To each 125 ml Erlenmeyer flask, 25 ml of the adjusted medium were distributed and autoclaved at 10 pounds for 15 minutes. When the media were cooled down to the room temperature, they were readjusted aseptically with sterilized 0.1 N HCl or 0.1 N NaOH to obtain the appropriate hydrogen ion concentration.

The spore suspension was obtained from one-week-old culture on Misato's medium (Misato and Hara, 1957) and filtered through a column with 0.1 g cotton in 15 mm height to get rid of the mycelial fragments. The spore was collected by centrifuge at 1,700 × g for 20 minutes and resuspended into an adequate amount of sterile water. These processes were repeated three times to wash off the nutrients carried over. Finally, the spore concentration was adjusted to reach 5×10^4 spores per ml or thereabout at 420 mμ with an Erma photoelectric spectrophotometer Model No. 4. One ml of thus prepared spore suspension was added to each flask containing 25 ml medium.

The cultures were set on a rotary shaker with 200 revolution per minute at 26° C. A week later, the cultures were poured into a Buchner funnel with a piece of Toyo filter paper No. 5, washed three times with distilled water, and dried at 90° C for 20 hours before measurement. All the figures were the average of triplicate. In order to obtain a comparable data, cultures grown on the basal medium or adding proper amount of glycine was run together each time and the dry weight of mycelium was calculated on this base.

Results

Growth as a function of time

When either potassium nitrate or ammonium chloride was added to the basal medium as a sole nitrogen source, maximum growth of mycelium was obtained in 10 days after inoculation. Thereafter, the dry weight of mycelium declined significantly (Fig. 1). Although Lopez and Fergus (1965) pointed out that the erratic results were obtained from the cultures by using spores as inoculum, this was not the case in the present experiments. Therefore, spores

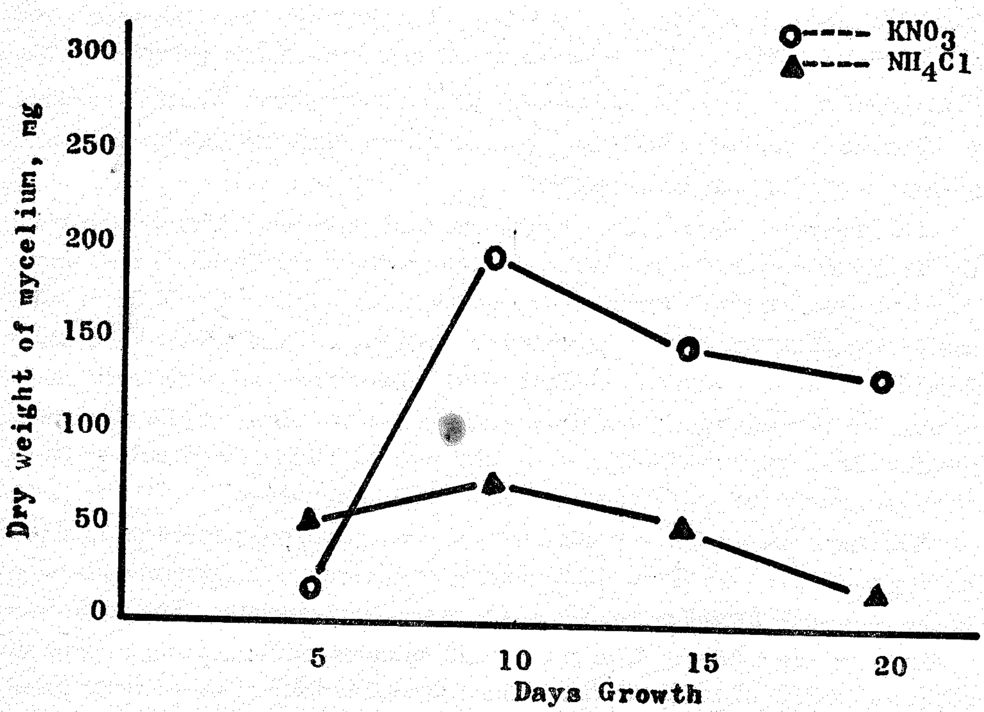

Fig. 1. Growth of *Piricularia oryzae* as related to time

were used as inoculum and the cultures were incubated for a week throughout the experiments.

Utilization of nitrogen compounds

Nineteen nitrogen sources were used to compare the growth response of five physiologic races of *P. oryzae*. They were KNO_3, $NaNO_3$, $NaNO_2$, NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$, alanine, arginine, aspartic acid, citrulline, glutamic acid, glycine, leucine, lysine, methionine, ornithine, phenylalanine, urea, and valine.

Utilization of inorganic and organic nitrogen sources varied in different races and different compounds. Race #1 utilized glycine, DL-alanine, DL-aspartic acid, L(+)glutamic acid as well as urea with KNO_3 while Race #2 readily utilized alanine, asparic acid, NH_4NO_3, urea with KNO_3 (Fig. 2). The results obtained in the present experiment is similar to that from elsewhere (Anonymous, 1964). Among the amino acids tested, glycine, aspartic acid, and glutamic acid were thought to be the best which was also found by other workers (Leaver *et al.*, 1947; Tanaka, 1965). Next came to leucine, valine, phenylalanine, lysine, and methionine whereas arginine, ornithine and citrulline were poorly utilized for the mycelial growth of the fungus. As to the response of the fungus to inorganic nitrogen compounds, KNO_3 and NH_4NO_3 yielded rather high dry weight of mycelium. It is very interesting that urea was more

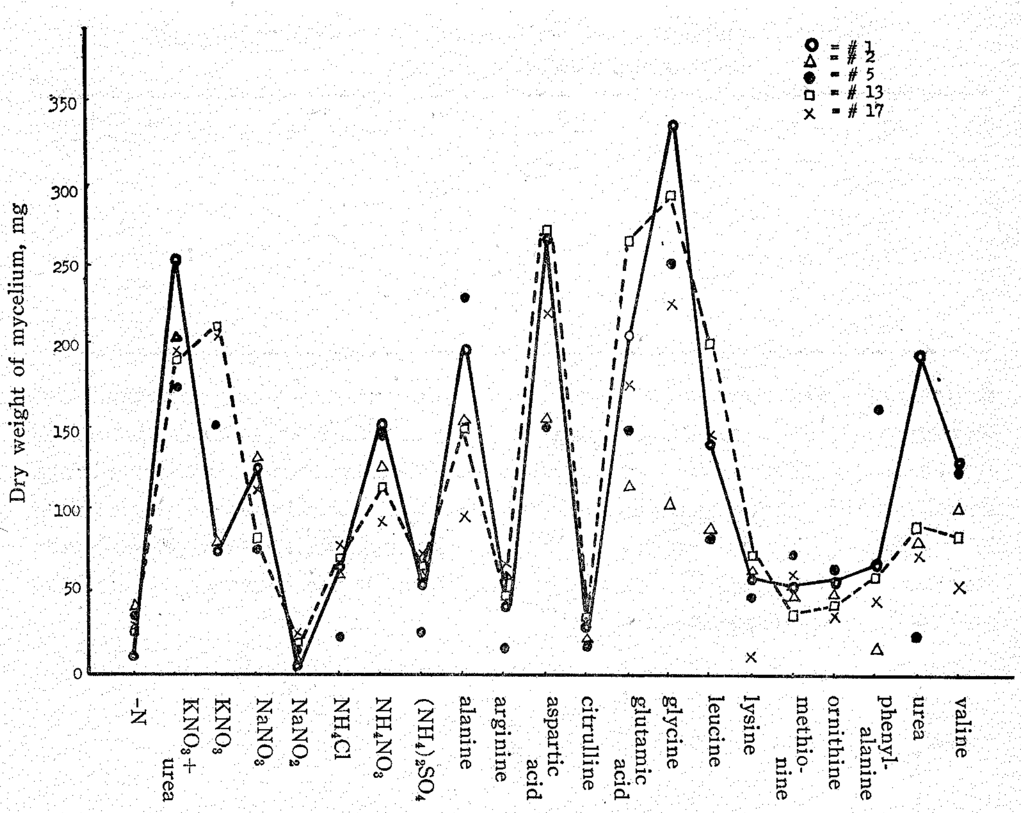

Fig. 2. Utilization of nitrogen sources by 5 races of *Piricularia oryzae* Cav.

readily utilized than arginine, citrulline, and ornithine. Presumably, this was simply due to the easy uptake of urea into the fungal cell since urea was absorbed as an intact molecule and formed free urea pool in the cell before being metabolized (Webster, 1957).

Utilization of ammonium or nitrate by some fungi was thought to be largely dependent on the hydrogen ion concentration of the media used (Foster, 1949). When $NaNO_3$, NH_4NO_3 $(NH_4)_2SO_4$, NH_4Cl, and $NaNO_2$ were compared, $NaNO_3$ and NH_4NO_3 produced more dried mycelium whereas $(NH_4)_2SO_4$ and NH_4Cl produced less. And $NaNO_2$ seemed to be inhibitory in comparison with that of basal medium without any nitrogen source. (Fig. 2). This was found in some other fungi (Kurtz and Fergus, 1965). When NH_4Cl and $(NH_4)_2SO_4$ were used as nitrogen source, the fungus grew well in the first few days and further growth was almost impaired thereafter. This might be due to the drop of pH during the growth since the pH of the medium with NH_4Cl or $(NH_4)_2SO_4$ at the end of growth was as low as 3.0 (Table 1).

Utilization of combined nitrogen sources

In the present experiment, three races, i. e. Race #5, #13, and #17 were used and three combinations were determined: KNO_2 and KNO_3, arginine and glycine,

Table 1. *Comparison of the utilization of inorganic nitrogen sources by **Piricularia oryzae***

Nitrogen source	Dry weight of mycelium, mg	pH of medium in harvest time
$NaNO_3$	88.8	6.7
NH_4NO_3	83.8	3.1
$(NH_4)_2SO_4$	59.9	3.1
NH_4Cl	57.7	2.8
$NaNO_2$	15.1	6.6

aspartic acid and methionine (Fig. 3). Different races seemed to respond differently with different nitrogen sources. Sodium nitrite unfavorably influenced the growth of this fungus even when it was combined with potassium nitrate. Failure of the fungus to utilized nitrite was well documented on the basis of its toxicity (Lilly and Barnett, 1951; Cochrane, 1957). Both glycine and aspartic acid promoted the growth of the fungus in the presence of arginine and methionine, respectively.

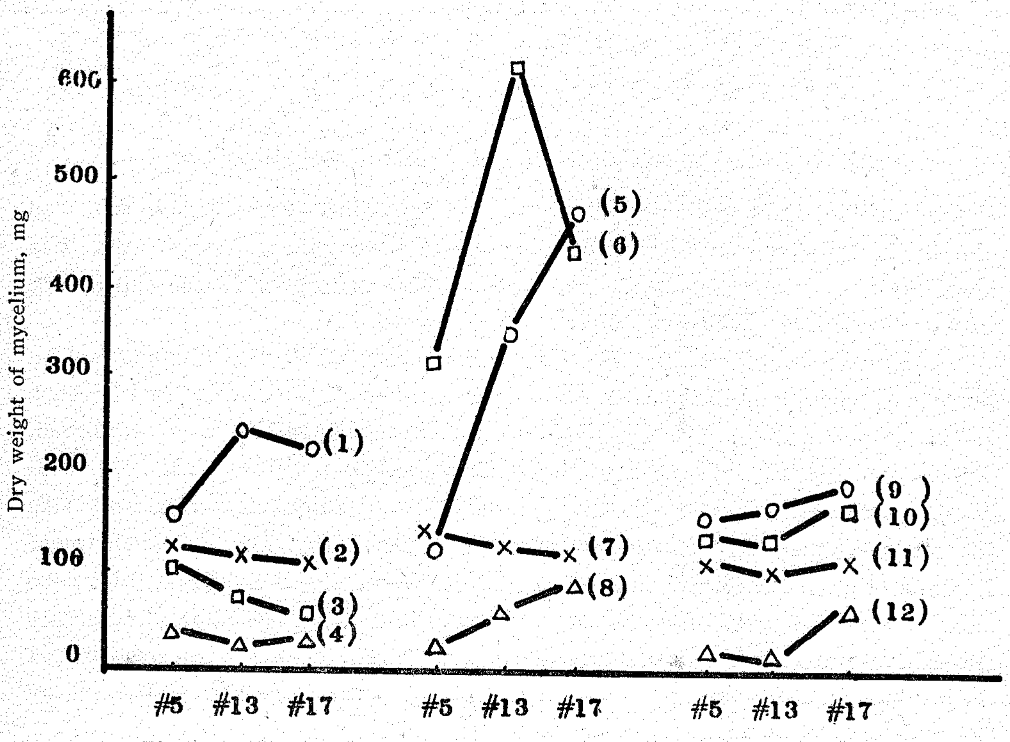

Fig. 3. Utilization of combined nitrogen sources by *Piricularia oryzae*

(1) KNO_3
(2) ½ KNO_3
(3) KNO_3+NaNO_2
(4) $NaNO_2$
(5) glycine
(6) glycine+arginine
(7) ½ glycine
(8) arginine
(9) aspartic acid
(10) aspartic acid+methionine
(11) ½ aspartic acid
(12) methionine

Discussion

For utilization of nitrogen sources by this pathogen., glutamic acid and aspartic acid were found to be very good ones (Leaver *et al.*, 1947; Tanaka, 1965). In the present experiments, the same results were obtained, that is, glutamic acid, aspartic acid, glycine were the best. Although the acidic amino acids were utilized very well by the fungus, the reason of maximum susceptibility of rice plant to blast was at the lower content of aspartic acid in the rice plant (Tanaka and Katsuki, 1952). The host and pathogen have close relationship between utilization of nitrogen compounds and pathogenicity (Otsuka *et al.*, 1965). The pathogenicity of this fungus seemed very complicated. The pathogen might use the other non-essential amino acids in the plant at first and change them into its favorable one.

Nitrate and ammonium sources were good nitrogen sources for many microorganisms. But their nutrition values were most variable with the different species. Some workers demonstrated that ammonium salts were more utilizable inorganic nitrogen sources than nitrates for the rice blast (Chen *et al.*, 1964). The others also proposed that potassium nitrate was good one for growth and sporulation. (Otani, 1952; Apparo,' 1962). Here we confirmed again that nitrates were better than ammonium salts. With ammonium chloride and ammonium sulfate as nitrogen sources, the fungus grew well in the first few days but did not grow further. The hydrogen ion concentration of the medium in the harvest time increased to below pH 3. The higher concentration of hydrogen ion might be inhibitory to the growth of this pathogen in later period of growth. Utilization of ammonium by some fungi was known to depend upon pH (Foster 1949) and the blast fungus used ammonium salts well if the medium was maintained at pH 7 (Tanaka, 1965).

Some combinations of two inorganic nitrogen sources did not seem to induce growth of *P. oryzae* well. Inhibitory effect of nitrite was explained on the basis of its known toxicity, some organic nitrogen sources combined could greatly enhance the growth. Lewis (1957) indicated that combinations of two amino acids that single enhanced growth might further increase growth when present together or might give less growth than did either one of the amino acids.

Summery

Nineteen different nitrogen sources were tested for the present studies. Prior to the nutritional study, the time for harvesting culture of this fungus were studied. The most favorable nitrogen sources for *P. oryzae* were found to be glycine, glutamic acid, aspartic acid, KNO_3. Urea, leucine, valine, phenylalanine, lysine, methionine, $(NH_4)_2SO_4$, and $NaNO_3$ were next. Arginine, ornithine, citrulline, NH_4Cl, and $NaNO_2$ were poor. Some combinations of two nitrogen sources seemed to induce better growth and some did not.

稻熱病菌氮素營養之研究

李義雄　吳龍溪

本實驗係利用十九種不同氮素源培養稻熱病菌。先決定本菌之收穫期。對稻熱病菌最好之氮素源爲 glyine, glutamic acid, aspartic acid, 硝酸鉀。尿素，leucine, valine, phenylalanine, lysine, methionine, 硫酸銨，硝酸鈉次之。arginine, ornithine, citrulline, 氯化銨，亞硝酸鈉最差。混合二種不同氮素源有些可促進生長，有些則否。

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