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PHYSIOLOGICAL STUDIES ON *CYLINDROCARPON TONKINENSE* BUGN. AN ISOLATE FROM DECAYING ARECANUT HUSK

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Summary

The effects of pH, carbon, nitrogen and C/N ratio on growth and sporulation of *Cylindrocarpon tonkinense* isolated from decaying arecanut husk were studied. The fungus was adapted to a wide range of pH though it grew best a pH 7.0. Of the carbon sources tested cellulose gave the maximum mycelial dry weight followed by sucrose, then lactose. Starch was a poor source of carbon. Eleven nitrogen sources were tested. Of these calcium nitrate yielded the greatest mycelial dry weight followed by potassium nitrate, sodium nitrate and peptone, in that order. Ammonium sulphate and ammonium phosphate (dibasic) were the poorest sources of nitrogen.

Seven different concentrations of carbon and nitrogen were studied yielding C/N ratios from 2.4 to 616. The higher concentrations of carbon produced abundant mycelial growth but a significant reduction in sporulation was noticed. The actual concentration of carbon was more significant than the carbon/nitrogen ratio.

Etudes physiologiques sur *Cylindrocarpon Tonkinense* Bugn isolé de la pourriture de cosse de noix d'Arec

On a étudié l'action du pH, du carbone, de l'azote et du rapport C/N sur la croissance et la sporulation de *Cylindrocarpon tonkinense* isolé de cosse de noix d'Arec altérée. Le champignon était adapté à une large gamme de pH bien qu'il pousse le mieux à pH 7.0. Parmi les sources de carbone essayées, la cellulose a donné un poids sec maximum de mycelium, suivie par le sucrose, puis le lactose. L'amidon fut une pauvre source de carbone. On a essayé onze sources d'azote. Parmi celles- ci, le nitrate de calcium a donné le plus grand poids sec de mycelium, suivi par le nitrate de potassium, le nitrate de sodium et la peptone, dans l'ordre. Les plu pauvres sources d'azote ont été la sulfate d'ammonium et le phosphate d'ammonium (dibasique).

Sept concentrations différentes de carbone et azote ont été étudiées conduisant à des rapports C/N de 2, 4 à 616. Les concentrations les plus élevées en carbone ont produit une croissance mycélienne abondante mais on a noté une réduction significative de la sporulation ? La concentration vraie en carbone fut plus significative que le rapport carbone/azote.

Physiologische Untersuchungen an *Cylindrocarpon tonkinense* Bugn, isoliert aus pilzbefallenen Areca-Nußschalen

Der Einfluß des pH-Wertes, des Kohlenstoff- und Stickstoffgehaltes und des C/N Verhältnisses auf das Wachstum und die Sporenbildung von *Cylindrocarpon tonkinense*, isoliert aus pilzbefallenen Areca-Nußschalen, wurde untersucht. Der Pilz tolerierte ein breites pH-Spektrum, obwohl er am besten be pH 7.0 wuchs. Von den untersuchten Kohlenstoff- quellen führte Cellulose zu dem größbten Trockenmycelgewicht, gefolgt von Sucrose und dann Lactose. Stärke erwies sich als schlechte Kohlenstoffquelle. Elf Stickstoffquellen wurden untersucht. Von diesen ergab Kalziumnitrat das größte Trockenmycelgewicht und in abnehmender Reihenfolge Kaliumnitrat, Natriumnitrat und Pepton. Ammoniumsulfat und Ammoniumphosphat (zweibasisch) waren die schlechtesten Stockstoffquellen.

Sieben verschiedene Kohlenstoff- und Stickstoffkonzentratonen wurden geprüft, die C/N Verhältnisse von 2,4 bis 616 ergaben. Die höheren Kohlenstoffkonzentrationen führten zu reichlichem Mycelwachstum, jedoch zu einer deutlich verminderten Sporenbildung. Die tatsächliche Kohlenstoffkonzentration erwies sich als wichtiger als das Verhältnis von Kohlenstoff und Stickstoff.

Estudio fisiologico sobre *Cylindrocarpon tonkinense* bugn aislado de vainas podridas de nuez de Areca

Se estudiarón los efectos de pH, carbono, nitrogeno y la relación C/N sobre el crecimiento y esporulación de *Cilindrocarpon tonkinense* aislado de vaina de areca podrida. El hongo se adapto a un amplio rango de pH aun que crece mejor a pH 7. Al examinar las fuentes de carbono se encuentra que la celulosa da el peso seco maximo de micelia, seguida por sacarosa y lactosa. El almidon fue una fuente de carbono pobre. Se examinaron 11 fuentes de - nitrogeno. De ellos Nitrato de calcio rinde el peso seco de micelia mas grande, seguido por Nitrato de potasio, Nitrato sodico y peptona, consicutivamente. El sulfato amonico y el fosfato amonico dibasico fueron los fuentes de nitrogeno mas pobres.

Se estudiaron 7 concentraciones diferentes de carbono y nitrogeno dando relación C/N de 2,4 a 616. La mas altas concentraciones de carbono produjeron crecimiento abundante de micelio pero una reducción significante en la esporulación. La concentratión real de crbono era mas importante que la relación C/N.

Introduction

Although fungi are broadly similar in their nutritional needs, they express their individuality in their preference for specific substances. Carbon and nitrogen are important components in the functional and structural make up of fungi (Cochrane, 1958) and nutritional and physiological characters of a species will determine to a great extent its ability to co-exist with its neighbours (Alexander, 1961). The present paper deals with physiological studies of *Cylindrocarpon tonkinense* isolated from decaying arecanut husk.

Materials and Methods

A 7 day old monosporic culture of the fungus maintained on 2% potato dextrose agar was used throughout the experiments. To study the effect of pH, the fungus was grown in Czapek's liquid medium adjusted to 8 different levels of pH ranging from 3 - 10 by addition of either 0.1 N HCl or 0.1 N NaOH as required. This experiment was repeated twice. To study the effect of carbon and nitrogen sources Czapek's liquid medium was modified by substitution of either a carbon or a nitrogen source in place of that contained in the basic medium in a quantity of the

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element equal to that which it replaced. Except for pH experiments the pH of the medium was adjusted to 7.0 before autoclaving.

The effect of carbon/nitrogen ratio was studied by adding different concentrations of sucrose or sodium nitrate. For all experiments three replicates were maintained for each treatment. Controls were kept on Czapek's medium from which either carbon or nitrogen was omitted as appropriate. The flask was seeded with a 5 mm fungal disc and incubated at room temperature (28 - 32^{0}C).

Average mycelial dry weight was ascertained by harvesting the fungus on Whatman No.42 filter paper under reduced pressure on a Buchner funnel at the end of the incubation period (20 days). The final pH of the medium in all the experiments was recorded at the time of filtration. Sporulation of the fungus in all the above tests was observed by examining under a low power microscope and ranked:

−	=	no spores
+	=	poor sporulation
++	=	fair sporulation
+++	=	good sporulation
++++	=	excellent sporulation

Results and Discussion

Effect of pH

The fungus appeared to be adapted to a wide range of pH although more growth was observed at pH 7.0. Differences in mycelial dry weight between pH levels were not significant. Sporulation, also, was good at all pH levels except pH 3.0. In all the pH experiments the fungus changed the pH in an alkaline direction although the change was small at the higher initial levels of pH.

Effect of Carbon Source

Among the different carbon sources tested the greatest mycelial dry weight was recorded with cellulose (pure cellulose powder) (Table 1). Agarwal and Sarbhoy (1978) recorded excellent growth and sporulation of *Fusarium oxysporum* with cellulose as the carbon source. Glucose, cellobiose, xylose and galactose were almost on a par with each other in respect of growth and sporulation. Starch did not favour good growth, as has been reported for other fungi (Cochrane, 1958; Lilly and Barnett, 1951). Poor utilization of starch by *C. tonkinense* may indicate an inability to elaborate the enzyme amylase under these, or perhaps any, conditions. It is known that sucrose and maltose are good sources of carbon for most fungi, while lactose favours growth depending upon the length of the incubation period. The good growth obtained with *C. tonkinese* in lactose medium in the present study may be attributable to the fairly long incubation period.

Effect of Nitrate versus Ammonium Nitrogen

The fungus preferred the nitrate form of nitrogen to the ammoniacal form (Table 2). This preferential use of nitrate nitrogen rather than ammonium nitrogen by certain species of *Colletotrichum* has also been reported by Chaturvedi (1964); Kurtz and Fergus (1964); Cochrane (1958) and Srivastava and Saksena (1967). Calcium nitrate gave maximum mycelial growth while little or no sporulation was recorded in ammonium phosphate (dibasic) or ammonium sulphate. There was a drop in pH of the culture medium in all the nitrogen source experiments except where ammoniacal nitrogen was used. This may be due to ammonium assimilation from sulphates, nitrates, or chlorides, which is rapid and is often followed by a large drop in pH resulting in reduced growth (Cochrane, 1958). The fungus grew well with potassium nitrate and sodium nitrate, followed by sodium nitrite and peptone as nitrogen sources.

Effect of carbon and nitrogen concentrations and ratio

Seven different concentrations of each of carbon and nitrogen were used, ranging from one eighth to twice the concentration of each in Czapek's medium. In the basic medium, which contains 12.63 g/litre of carbon and 0.33 g/litre of nitrogen, the C/N ratio is 38.3. The ratios in the experiments ranged from 2.4 to 616. See figure 1. At the lower levels of carbon concentration, differences in growth and sporulation relative to nitrogen concentration were not significant. High concentrations of carbon induced profuse mycelial growth, but sporulation was reduced to a great extent. A similar increase in mycelial weight at higher concentrations of carbon has been reported for *Helminthosporium oryzae* (Das and Baruah, 1946) and for *Fusarium* spp (Agarwal and Sarbhoy, 1978).

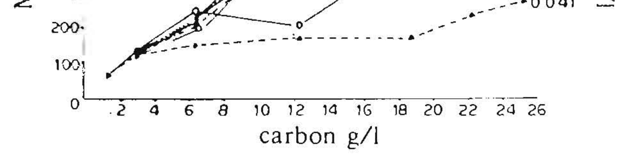

Fig. 1 Effect of C:N ratio on the growth of *Cylindrocarpon tonkinense*

The fungus produced abundant four celled spherical spores at higher concentrations of carbon or nitrogen. The growth and sporulation of the fungus were altered more by carbon than by nitrogen concentrations. This indicates that the variation in growth and sporulation of the fungus may be due more to the actual concentrations of carbon and nitrogen than to their

Table 1

Effect of carbon source on growth and sporulation of *Cylindrocarpon tonkinense*

Carbon source	Dry wt of mycelium milligrams (mean of three replicates)	Sporulation (for code see text)
Glucose	178.3	+++
Galactose	175.0	+++
Xylose	176.7	+++
Cellobiose	173.3	+++
Lactose	204.3	++++
Maltose	198.3	+++
Sucrose	206.7	++++
Cellulose	258.3	+++
Starch	86.3	++
Control (no added carbon)	16.0	+
S.E.	14.61	
L.S.D. (P = 0.05)	25.07	

Table 2

Effect of nitrogen source on growth and sporulation of *Cylindrocarpon tonkinense*

Nitrogen source	Dry wt of mycelium milligrams (mean of three replicates)	Sporulation (for code see text)
Ammonium phosphate (monobasic)	138.3	+
Ammonium phosphate (dibasic)	88.3	-
Ammonium sulphate	70.0	-
Ammonium tartrate	143.0	+++
Calcium nitrate	200.0	+++
Potassium nitrate	191.7	++++
Sodium nitrate	188.3	++++
Sodium nitrite	186.0	+++
Urea	135.0	+++
Peptone	175.0	++++
Control (no added nitrogen)	41.3	-
S.E.	17.51	
L.S.D. (P = 0.05)	25.07	

ratio. This may be explained by the fact that when carbon concentration is high, other constituents of the medium, especially nitrogen, become limiting. Within this limit, growth is increased by higher levels of carbohydrate, provided that adequate nitrogen is supplied (Cochrane, 1958).

Utilization of cellulose by the fungus as carbon source, and its ability to grow at high concentrations of carbon, suggest that *C. tonkinense* may have potential for use as a biodegradation agent for arecanut husk which contains about 46% cellulose (Baruah *et al.* 1957). Further work on utilization of different sources of cellulose with special reference to the degradation of arecanut husk by *C. tonkinense* is in pogress,

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