Utilization of Carbohydrates by Soft Rot Fungus, *Chaetomium globosum* KUNZE*

Munezoh TAKAHASHI** and Koichi NISHIMOTO**

Abstrract——Utilizing ability of the soft rot fungus, *Chaetomium globosum* KUNZE, to various mono- and polysaccharides, most of them normally occurring in hardwoods as hemicellulose components, was investigated on fungal growth and consumption rate by shake culture. Xylose, mannose, glucose, galactose, maltose and crude xylan were available to the fungus, whereas arabinose, glucuronic acid and galacturonic acid were not utilized at all. Rapid growth and consumption were observed in xylose and xylan media, probably indicating the possible reference to the greater susceptibility of hardwoods to soft rot. Equality of mannose to xylose as carbon source, however, casted the propblem on the relation of mannan utilization to the greater resistance of softwoods to soft rot.

Introduction

It is well known that softwoods are not readily attacked by soft rot fungi as are hardwoods. This is usually attributed to the higher lignin content in softwoods. However, for the completely satisfactory explanation of this phenomenon, different natures of the lignin and hemicelluloses in softwoods and hardwoods could be of importance.[1] Furthermore, difference in ability of soft rot fungi to utilize the hemicelluloses in softwoods and hardwoods may also be a significant factor for the greater resistance of softwoods or the greater susceptibility of hardwoods.

Wood-deteriorating microorganisms, including soft rot fungi, depend upon various carbohydrates in wood cell walls for accomplishing their metabolic activities. Hemicellulose is one of the principal cell wall constituents and the important carbon source as cellulose for the wood-decaying fungi. Hardwoods hemicelluloses are distinctive for high content of xylans, and softwoods hemicelluloses are characterized by a high percentage of mannans. Consequently, utilization of these polysaccharides and their degradation products by soft rot fungi is one of the major interest in studying the different susceptibility of softwoods and hardwoods.

In the present investigation, the utilizing ability to various mono- and polysaccharides, many of these normally occurring in hardwoods, of typical soft rot fungus, *Chaetomium globosum*, has been studied.

Experimental

Carbon source and medium

The following carbohydrates were added as the sole carbon source to the basal nutrient medium;

* Presented at the 18th and 19th Meetings of Japan Wood Research Society, Kyoto, April 1968 and Sapporo, July 1969.

** Division of Wood Biology.

D-arabinose, D-xylose, D-glucose, D-mannose, D-galactose, D-glucuronic acid, D-galacturonic acid, D-maltose, CMC (carboxymethyl cellulose sodium salt), and crude xylan extracted from beech wood (*Fagus crenata* BLUME).

Abram's solution was used as the basal nutrient medium. The composition was as follows;

NH_4NO_3 3.0 g, KH_2PO_4 2.5 g, K_2HPO_4 2.0 g, $MgSO_4 \cdot 7H_2O$ 2.0 g, and distilled water 1000 ml.

Amounts of carbon source were 10 g per litre for xylan and 20 g for other carbohydrates. The carbon sources were sterilized in vapor of propylene oxide and added to the autoclaved Abram's solution. The experiments were mainly carried out in 500 ml shaking flasks containing 50 ml of nutrient solution. Usually three to five flasks per one kind of carbon source were used.

Prior to experiments, the mycelia of the test fungus were transferred to Petri dishes containing malt agar substrate. Then sterilized cellophane disks of 8 mm diameter were laid on the agar plate. After covering the agar plate and cellophane disks by mycelial development, the disks with mycelia were removed and rinsed in sterilized distilled water. The flasks were inoculated with these mycelial disks and incubated at 28±2°C in a shaker for 21 days.

Growth rate and utilizing ability of carbon sources

Growth rate was determined every three days as mycelial dry weight per flask by drying for 20 hours at 90°C after filtering off the nutrient medium. In some experiments with solid agar media containing equal amount of xylose or glucuronic acid as in the nutrient solution, growth was estimated as the radial growth of the mycelium in millimeters per day. In addition, the pH of each culture filtrates was measured every three days.

The culture filtrates were analysed for amount of remnant carbohydrates by methods of BERTLAND[2] and TOLLENS and KROBER.[2] Samples taken from the xylan-containing solutions were hydrolysed with 1N sulfuric acid for 6 hours at 100°C, followed by neutralization with barium hydroxide and passage through Amberlite IR-120 and IR-4B. The effluents and washings containing neutral sugars were concentrated and analysed for pentose content by method of TOLLENS and KROBER.[2]

Usually, *Chaetomium globosum* KUNZE (Strain No. 8059) was used as test fungus. For the purpose of comparison, *Coriolus versicolor* QUÉL. (No. 1030) and *Tyromyces palustris* MURR. (No. 0507) were occasionally used as a white rot and a brown rot fungus, respectively.

Preparation of crude xylan from beech wood

Beech wood sawdust, 40 to 60 mesh, was extracted with ethanol-benzene (1/1). The extractive-free wood (350 g) was suspended in water (3.5 *l*) and treated with glacial acetic acid (20 ml) and sodium chlorite (80 g) by occasionally stirring for 1 hour at 70–80°C. Then without cooling, same amounts of additional reagents were added. The heating was continued at the same temperature range for an additional hour. This treatment was again repeated in the same condition.

The chlorite holocellulose was extracted with 5 % sodium hydroxide in nitrogen gas for one over night at room temperature. The mixture was filtered with suction into a flask containing an excess of glacial acetic acid. 95 % Ethanol was added to the filtrate and washings. The resulting precipitate was collected by centrifuging, followed by washing repeatedly with 5 % ethanol, ethanol and ether and drying on potassium hydroxide *in vacuo*. A crude xylan was obtained as light grayish powder.

The content of lignin and uronic acid of the sample were determined according to TAPPI Standards T 13 m-54 and BROWNING's method,[3] as Klason lignin and uronic anhydride, respectively.

Results and Discussion

Growth of *Chaetomium globosum* on various carbon sources other than crude xylan and consumption rate of some of these sources are shown in Fig. 1 and Fig. 2. Xylose, mannose,

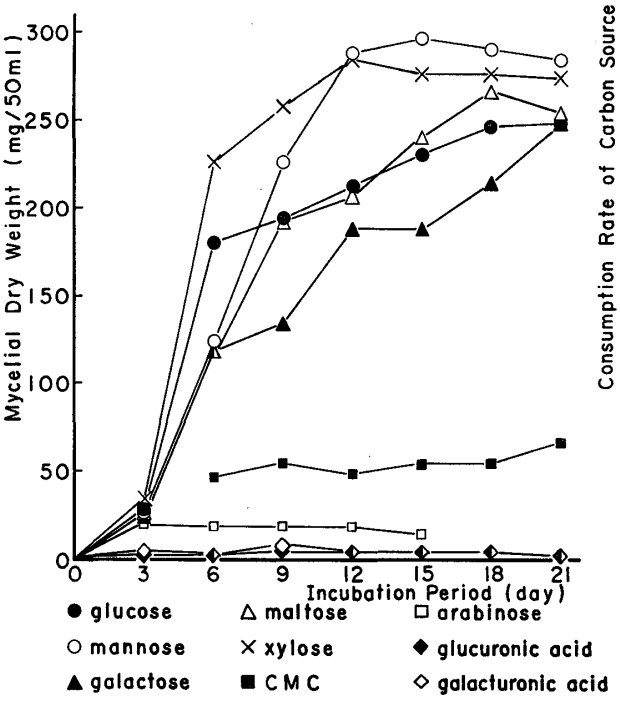

Fig. 1. Mycelial dry weight of *Chaetomium globosum* grown in nutrient medium with various carbon sources.

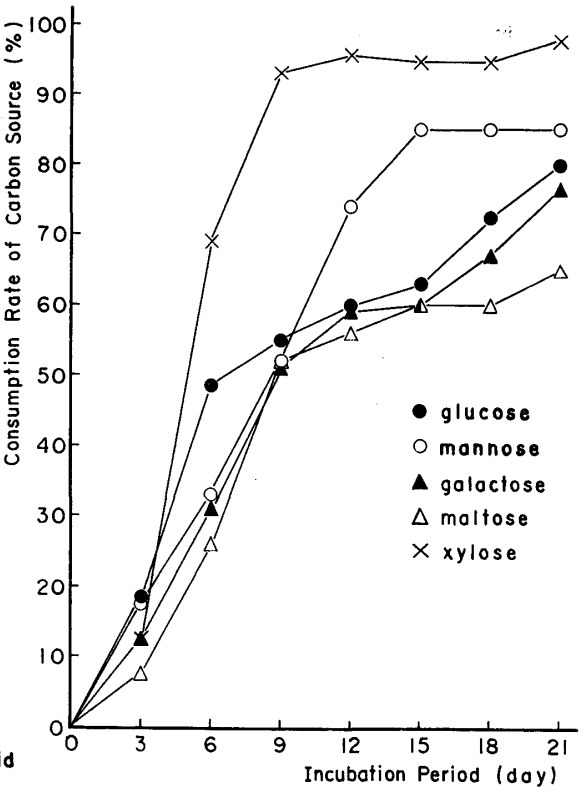

Fig. 2. Consumption rate of five carbon sources by *Chaetomium globosum* grown in nutrient medium.

glucose, galactose and maltose were available to *Ch. globosum*. Among these sources, however, the fungal growth on galactose was apparently slower, although the final mycelial yield was almost equivalent to other sources. COCHRANE[4] reviewed the carbon nutrition of fungi and concluded that galactose was used by most fungi but was not usually so good source as glucose. From the data obtained, it was suggested that adaptive mechanism was at work in utilization of galactose by *Ch. globosum* and this fungus also might not be particular in its metabolic activity to galactose.

There are many reports that glucose is the good carbon source for many fungi including wood-deteriorating fungi and that xylose and mannose are equivalent to glucose.[5,6] In the present investigation, however, glucose was somewhat inferior to xylose and mannose in daily growth rate. In the metabolism of glucose by fungi, three different pathways have been established, namely EMP (Embden-Meyerhof-Parnas) pathway, HMP (hexose monophosphate) pathway and ED (Entner-Doudoroff) pathway. Mannose and galactose are converted into

fructose 6-phosphate via phosphorylated derivatives of these sugars and glucose, and incorporated into the EMP and HMP pathways,[7] whereas xylose is converted into xylulose 5-phosphate via xylulose and connected with HMP pathway to produce fructose 6-phosphate.[8] Rapid growth and rapid consumption in xylose medium seems to reflect active isomerization, phosphorylation and other reactions, leading the major participation of HMP pathway in *Ch. globosum*. On the other hand, the reason for the superiority of mannose to glucose, though the extent was little, is unknown. Or, it may be due to a contamination with metals or growth factors.

Xylose and mannose are the main constituents of wood hemicelluloses. Percentage of xylose in extractive-free basis is ranging 12 to 26 in hardwoods and 5 to 10 in softwoods, whereas that of mannose is less than 3% in hardwoods and about 10% in softwoods.[9] As mannose was the good carbon source as xylose for *Ch. globosum*, utilizing ability of mannan should be investigated in relation to the higher resistance of softwoods, though the lower mannanase activity than the xylanase activity in culture filtrates from *Ch. globosum* has been reported by KEILICH.[10]

Change of economic coefficient in five carbon sources are shown in Fig. 3. Economic coefficient is one of the methods to determine the efficiency of conversion of carbon source to mycelium. It is defined by the formula;

$$\frac{\text{mycelium dry weight (g)}}{\text{amount of carbon compound consumed (g)}} \times 100$$

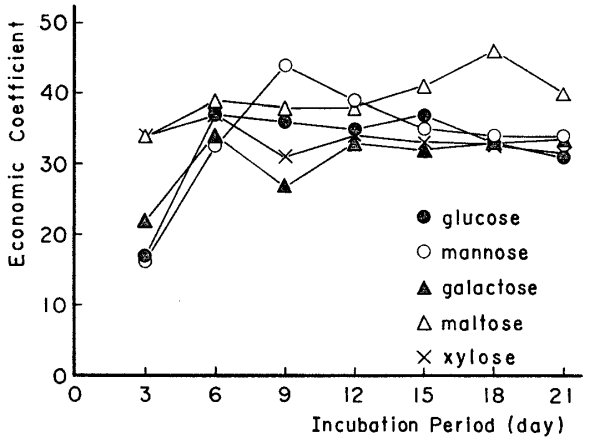

Fig. 3. Efficiency of conversion of five carbon sources to mycelium of *Chaetomium globosum*.

Economic coefficient can be seen by inspection to be maximal when respiratory carbon dioxide and soluble metabolic products are minimal in quantity. Very low values reflect probably the production of significant amounts of soluble compounds in high-carbohydrate media.[11]

As mentioned above, xylose and mannose were utilized more rapidly than other sources, reflecting rapid growth on these sources. However, economic coefficient of xylose maintained the moderately same level throughout the incubation period, whereas that of mannose increased rapidly in early period and then decreased gradually. This suggests that in case of mannose transition from accumlation of metabolic product to mycelial formation was abrupt.

Consumption rate of maltose was slower compared to other sources, however, economic coefficient of the source was generally kept in higher level and mycelial yield was equivalent

to other sources. This indicates the presence of maltase and the effective use of maltose for mycelial formation. However, occurrence of maltose in nature, as a product of starch hydrolysis, is very scarce.

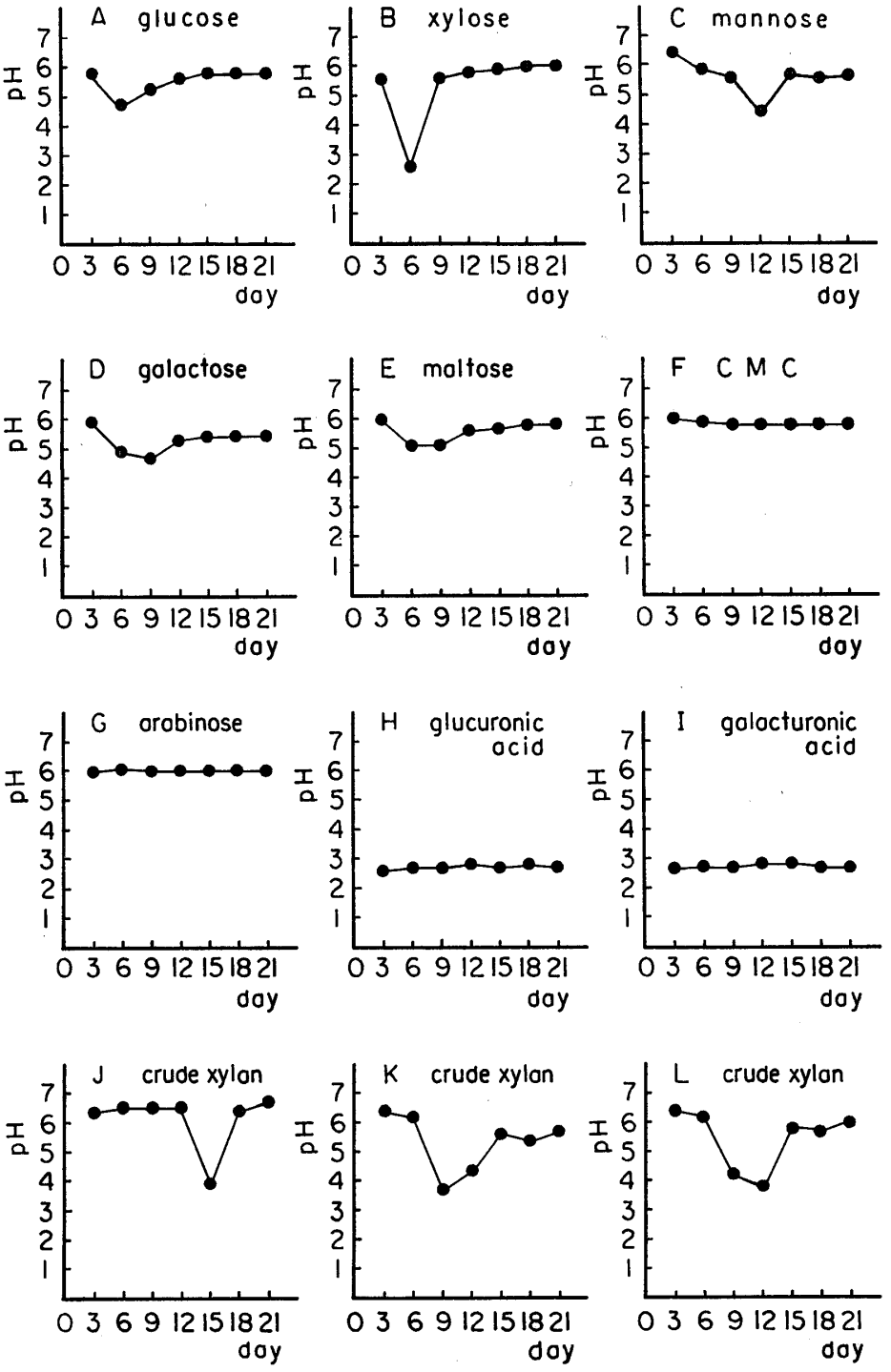

Fig. 4. Change in pH value of nutrient medium with various carbon sources during course of incubation (A–J ; *Chaetomium globosum*, K ; *Coriolus versicolor*, L ; *Tyromyces palustris*).

Arabinose occurs as the hemicellulose component, though the content is very low. In contrast to xylose, this sugar was not utilized at all by *Ch. globosum*, probably indicating the lack of metabolic pathway—arabinose→ribulose→ribulose 5-phosphate→xylulose 5-phosphate.[12] Inability to utilize both isomers of arabinose, especially to D-arabinose, has been frequently reported in many fungi.[13]

Glucuronic acid and galacturonic acid, occurring in wood as a fraction of glucuronoxylan and galactoglucomannan, were also not utilized at all. However, as pH values in these uronic acid media were very low through the incubation period (Fig. 4), the inability to utilize these sources seems to be related to impermeability of carbon sources caused by low pH level. In the experiment with solid agar medium, containing glucuronic acid as the sole carbon source and adjusted to pH 6.5 by sodium hydroxide, poor growth, though better than no carbon medium, was observed, but it was apparently lesser than *Coriolus versicolor* and *Tyromyces palustris* (Fig. 5). From these results, it was suggested that utilizing ability of *Ch. globosum* to the uronic acid in wood hemicellulose was very low.

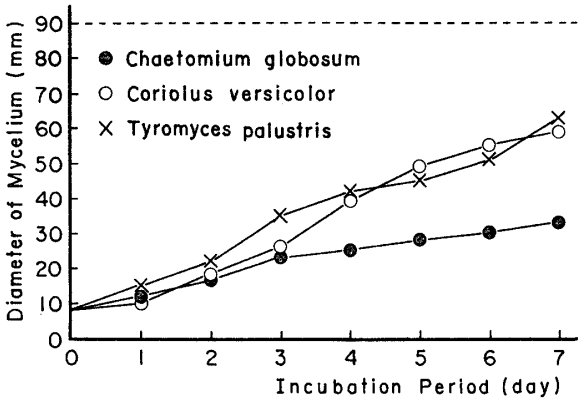

Fig. 5. Radial growth of three fungal species grown on solid agar medium with glucuronic acid as sole carbon source (Broken line shows the diameter of Petri dish.).

Growth on CMC was also very poor but the pH value of the medium was not low as those of glucuronic acid and galacturonic acid (Growth at third day was not measured due to the difficulty of separating the mycelium from the carbon source.). As a cellulolytic activity of *Ch. globosum*, in the present investigation, was not estimated enzymatically, the reason for such poor growth is uncertain. Although *Ch. globosum* is undoubtedly the truly cellulolytic fungus and CMC is one of the commonly used substrates to determine the cellulolytic activity, carboxymethyl group or sodium in the substrate may be supressive to the growth of the fungus. Degree of substitution and degree of polymerization of CMC used in this experiment were 0.5 to 0.7 and 300 to 450, respectively.

Growth, consumption rate and economic coefficient of three fungal species in xylan-containing medium are shown in Figs. 6 to 8. The contents of lignin and uronic anhydride of this source were 6.25 % and 5.56 %, respectively. As mentioned in *Experimental*, determination of pentosan was carried out with the neutral fraction from hydrolyzed culture filtrates. Thus, correction to the furfural yield from uronic acids was not made.

Maximum mycelial yields of test fungi were equivalent as shown in Fig. 6. However, *Ch. globosum* grew more rapidly and supported the same level of growth in longer time period than *Co. versicolor* and *Ty. palustris*. Consequently, economic coefficient in *Ch. globosum*

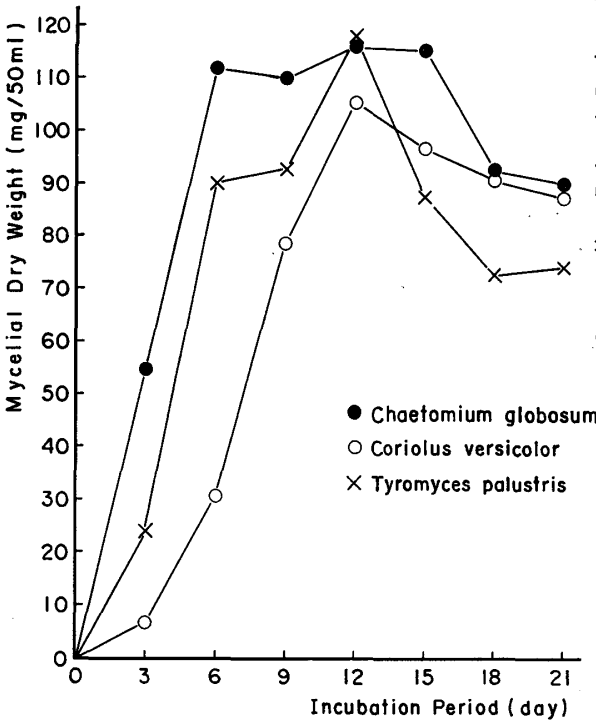

Fig. 6. Mycelial dry weight of three fungal species grown in nutrient medium with crude xylan as sole carbon source.

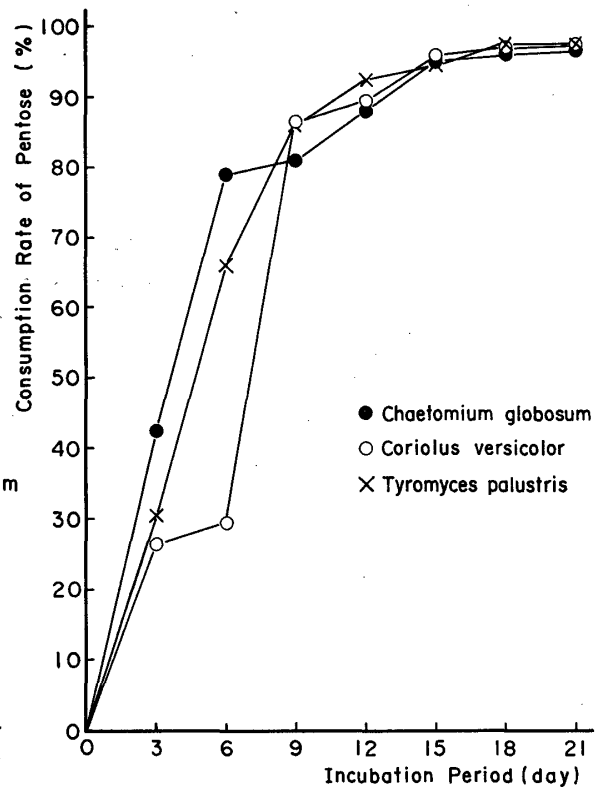

Fig. 7. Consumption rate of pentose by three fungal species grown in nutrient medium with crude xylan as sole carbon source.

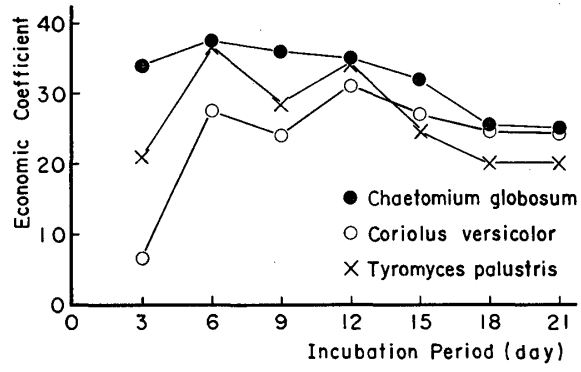

Fig. 8. Efficiency of conversion of crude xylan to mycelium of three fungal species.

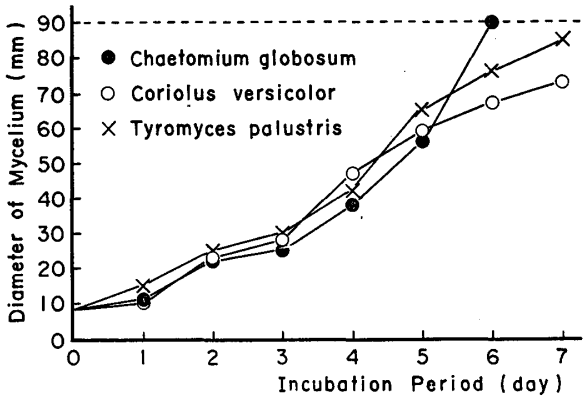

Fig. 9. Radial growth of three fungal species grown on solid agar medium with xylose as sole carbon source (Broken line shows the dimeter of Petri dish.).

kept higher level throughout the incubation period, although the progressive increase of consumption rate showed fairly similar pattern to those of other fungi. Radial growths of these fungi on solid agar medium containing xylose as the sole carbon source were almost same to fifth day, but after that day *Ch. globosum* was superior to other fungi (Fig. 9).

As shown in Fig. 4, the pH value of each nutrient medium of which carbon source was available to test fungi exhibited a similar pattern of transition, that is to say, a considerably

rapid fall and recovery of the value during course of incubation. Particularly in xylose- and xylan-containing media inoculated with *Ch. globosum*, such trend was more prominent. Such pattern of transition in pH value of nutrient medium has been usually obsedved in many fungi and seems to reflect the accumlation of some acidic intermediate products.

In the present experiments, growth factors, e.g. thiamine-HCl, biotin, nicotinic acid, etc., were not added at all to the nutrient medium. Then, rapid decline of mycelial yield, observed in the two species of basidiomycetous fungi, may be related to a defficiency of growth factors or to some inadequacy of medium composition. Thus, metabolic activity of *Ch. globosum* to the hardwood hemicellulose was not discussed here comparing those of other wood-decaying fungi. However, considering the results with the ability of *Ch. globosum* to utilize xylose and xylan, this soft rot fungus has possibly an active pathway to metabolize these mono- and polysaccharide, indicating the possible reference to the greater susceptibility of hardwoods to soft rot fungi.

References

1) Bailey, P. J., W. Liese and R. Rösch, Biodeterioration of Materials, 546, edited by A. H. Walters and J. J. Elphick, Amsterdam, New York and London, Elsevier (1968).
2) T. Miwa and A. Yasumura, Seibutsu-gaku Zikken-ho Koza (in Japanese), **6F**, 13, Nakayama Shoten (1955).
3) B. L. Browning, Methods of Wood Chemistry, **II**, 633, New York, London and Sydney, Interscience (1967).
4) V. W. Cochrane, Physiology of Fungi, 61, New York, John Wiley and Sons (1958).
5) Ibid., 60.
6) D. Perlman, The Fungi, I, 481, edited by G. C. Ainsworth and A. S. Sussman, New York and London, Academic Press (1965).
7) V. W. Cochrane, Physiology of Fungi, 62.
8) H. W. Doelle, Bacterial Metabolism, 188, New York and London, Academic Press (1969).
9) T. E. Timmel, Wood Science and Technology, **1**, 45 (1967).
10) G. Keilich, P. J. Bailey and W. Liese, Wood Science and Technology, **4**, 273 (1970).
11) D. Periman, The Fungi, **I**, 482.
12) H. W. Doelle, Bacterial Metabolism, 184.
13) B. Heningsson, Studia Forestalia Suecica, No. 52 (1967).