Studies on the Physiological Processes During the Growth and Development of *Aspergillus niger*

By

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Recently many studies have been reported on biochemical phenomena during development in multicellular microorganisms. As regards the mold *Aspergillus niger* some analyses of phosphate metabolism have been made by MANN[1,2], BAJAJ *et al.*[3-5] and BELOSERSKY *et al.*[6] BEHAL and EAKIN have reported that some purine and pyrimidine analogs inhibited the development of this organism[7-9]. RAMAKRISHNAN demonstrated the existence of tricarboxylic acid cycle and accumulation of citric acid in the mold[10,11].

At the beginning of our study on the metabolic changes during growth and development of *Aspergillus niger*, the contents of nitrogen compounds, phosphorus compounds, and carbohydrates in mycelium and total amount of organic acids in the medium were determined, and effects of several inhibitors on development were also examined. In this paper, the results of the studies described above on mold cultured normally and under abnormal conditions in the presence of sodium fluoride in culture medium are described.

Methods

Culture of the organism

Aspergillus niger A 1015* was used in this study. The organisms were grown on surface as nonshaking cultures. Usually, 250 ml Erlenmeyer flasks, plugged loosely with cotton wool, were used throughout this study. The basal medium was composed of 5% glucose, 0.5% NH_4NO_3 and suitable amount of boiled extract prepared from 200 g potato and 50 g malt per litre of tap water. The pH of the medium was adjusted to 4.3 by the addition of HCl. Approximate 1 ml of spore suspension (1 mg spores per ml) was inocultated in 50 ml of the medium, and incubated at 30°C for suitable time. The stock

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cultures were the basal potato glucose-malt agar slants and they were subcultured for about 60 days.

Nitrogen

Nitrogen was estimated iodometrically by the method of LEVY and PALMER[12]. The fresh mycelia were homogenated in 20% trichloroacetic acid for about 30 minutes at room temperature. An appropriate volume of the homogenate was used for estimation of total-N. The homogenate was centrifuged and nitrogen of the residual material was regarded as protein-N.

Phosphorus

Phosphorus was determined colorimetrically by the method of ALLEN[13]. The method of SCHNEIDER was used for acid soluble phosphate fractionation[14]. Seven-minute phosphate in acid soluble fraction was calculated from the difference between the orthophosphate before and after hydrolysis for 7 min. with 1 N HCl in boiling water. The difference between the total acid soluble phosphate and seven-minute phosphate was taken as stable phosphate. For nucleic acid fractionation a modification[16] of the method of OGUR and ROSEN[15] was used. RNA and DNA were estimated as RNA-P and DNA-P, respectively.

Glycogen-like polysaccharide in the mycelium

Glycogen-like polysaccharide was hydrolyzed by alkali-method and its hydrolyzate was estimated by the method of BERTRAND[17].

Inhibitors

Monoiodoacetic acid (MIA), sodium fluoride (NaF), methyl alcohol, sodium azide (NaN_3), dinitrophenol (DNP), 8-hydroxyquinoline and salicylaldoxime were used as inhibitor for mold development.

Total-titratable acid and pH measurement

Acids accumulated in culture fluids in various stage of growth were titrated with 0.01 N NaOH by use of pH-meter and initial pH values of these fluids were recorded.

Results and Discussion

Growth processes and effect of several inhibitors on growth and spore formation

Thin mycelium was observable after about 15 hrs. of inoculation and the mycelium further developed within the subsequent 5–7 hrs. into white smooth

pellicles. Usually, at a period of about 22 to 25 hrs. after incubation the first conidiophores made their appearance. Subsequently, the micelium showed a creased yellowish mat. After conidiophore formation, the spore formation was observed. Maximum mycelial growth and spore formation were observed at about 50 hrs. and 90 hrs. of incubation, respectively (Fig. 1).

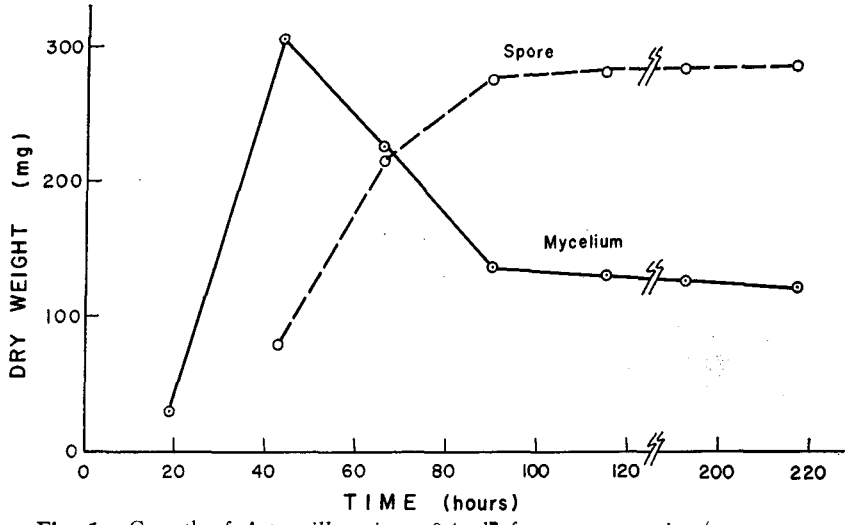

Fig. 1. Growth of *Aspergillus niger* 0.4 ml of spore suspension (mg spores per ml) was inoculated in 20 ml of the medium in this experiment.

MIA arrested completely growth and development of this mold at a concentration of 10^{-3} M, but NaF did not influence the growth even when it was added in large amount (2×10^{-3} M), whereas the concentration suffice to inhibit spore formation completely was 10^{-3} M. Methyl alcohol inhibited spore formation at 4–10%, but at lower concentrations it had no effect on growth. NaN_3 (10^{-3} M) and DNP (10^{-3} M-5×10^{-4} M) exerted a non specific effect on growth and development. 8-Hydroxyquinoline (2×10^{-3} M-10^{-3} M) and salicylaldoxime (5×10^{-3} M-3×10^{-3} M) inhibited spore germination, but did not inhibited mycelial growth and spore formation.

Nitrogen content in mycelium

Changes in the amount of total- and protein-N of mycelium during growth were shown in Fig. 2. Increased amounts of total- and protein-N in mycelium per each flask were paralleled with the growth of mycelium and these increases stopped at the period of spore formation. In the mycelium exposed to 2×10^{-3} M NaF (NaF culture) total-N in the mycelium per each flask increased gradually, but the increase of protein-N stopped at about 40 hrs. of culture.

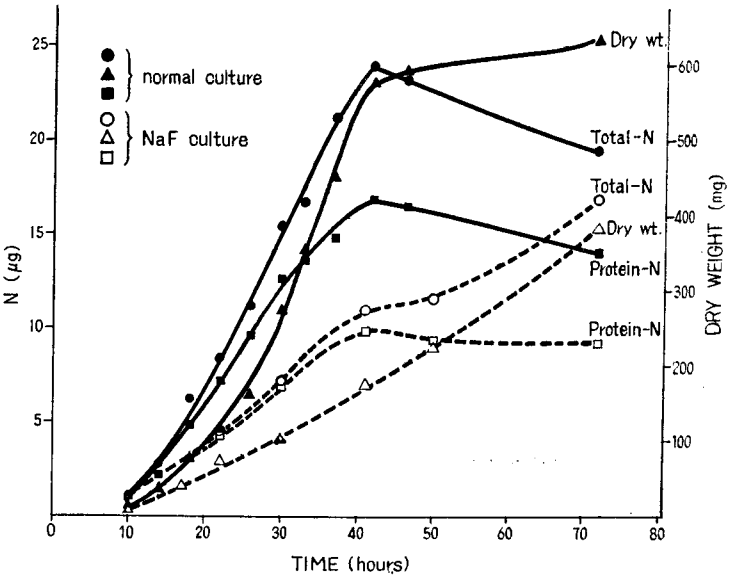

Fig. 2. Changes in amounts of nitrogen in mycelium.

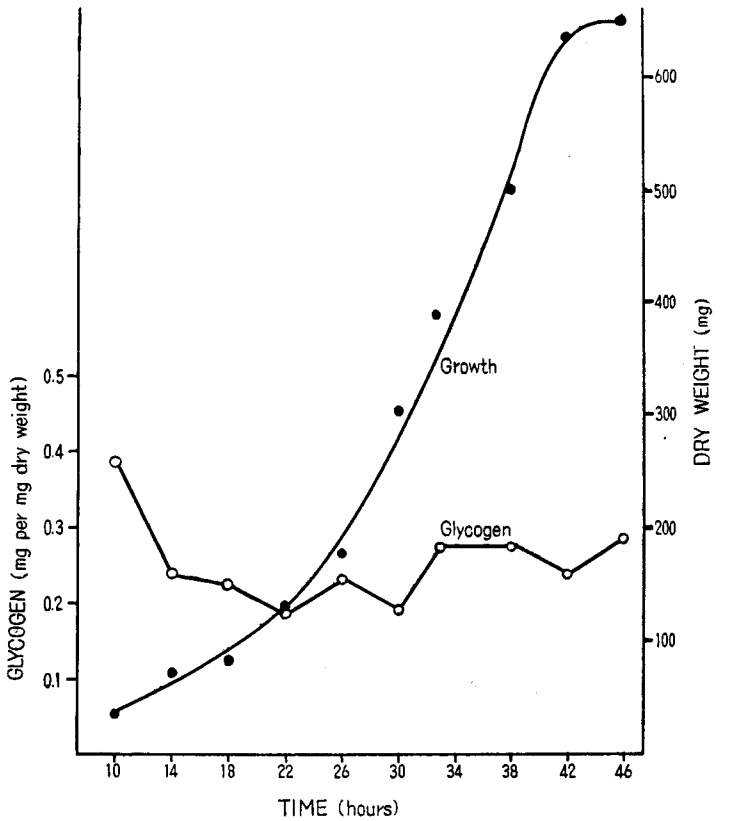

Fig. 3. Content of glycogen-like polysaccharide in mycelium at various stages of culture.

Carbohydrate in mycelium

About 20–30% of mycelial material was composed of a glycogen-like polysaccharide. Its contents in mycelium did not remarkably changed during the growth (Fig. 3). In NaF culture, the content of glycogen-like polysaccharide was low and varied remarkably at an early stage of growth.

Acid soluble phosphate, RNA and DNA contents

The rate of the uptake of phosphate from medium during the growth was high at an early stage of growth and was rather low during spore formation (Fig. 4). The final concentration of phosphorus in mycelium was 0.02–4%

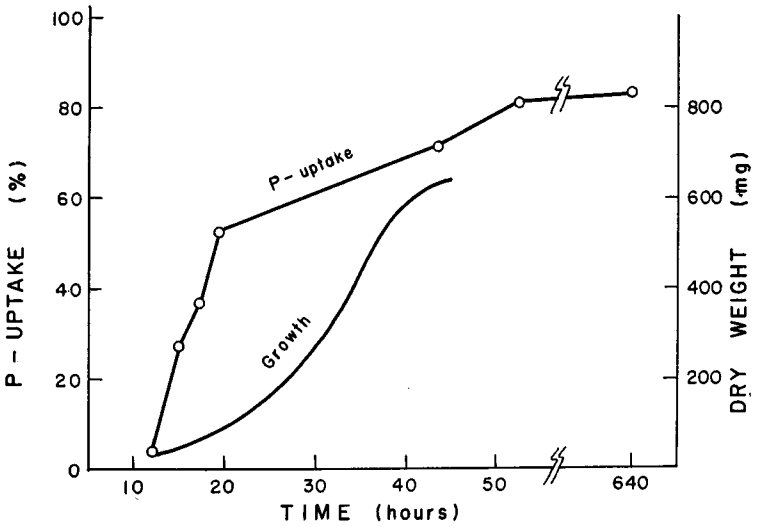

Fig. 4. Uptake of phosphorus from medium.

(dry wt.) through the various stages of growth. Total phosphorus content in the spores was found to be about 0.7% (dry wt.).

Analytical data of acid soluble phosphates and nucleic acids were recorded in Fig. 5. The contents of acid soluble phosphates and nucleic acids in mycelium generally changed remarkably at an early stage of the growth and before conidiophore formation. RNA was contained in mycelium at a higher level as compared with other phosphorus compounds. It was shown that two maximal points of total RNA content existed during the course of culture. Initial peak was observed at an early stage of growth which corresponded to the stage of the initiation of conidiophore formation. Second peak was observed at the logarithmic phase of mycelial growth when the spores were formed. Total DNA in the mycelium increased at an early stage of spore formation.

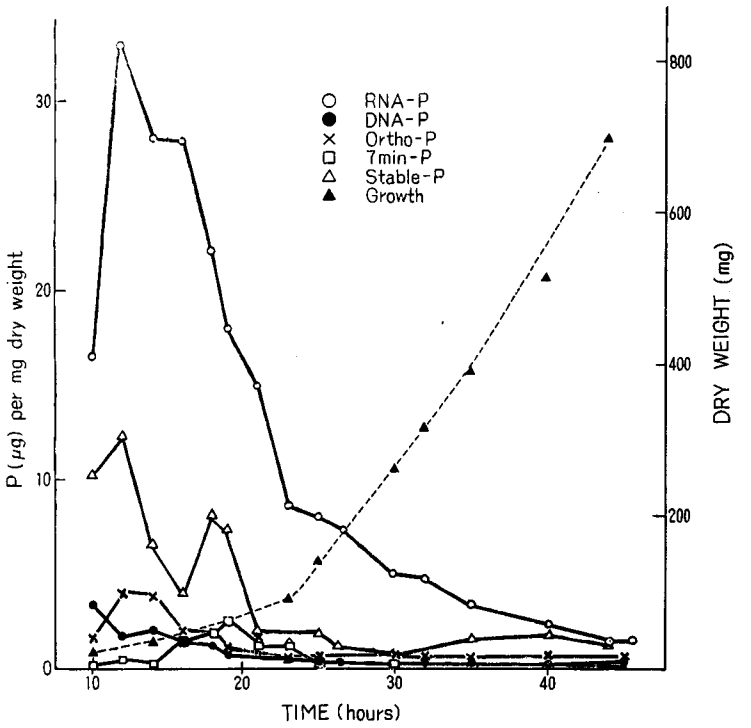

Fig. 5. Changes in amounts of acid soluble phosphates, RNA and DNA in mycelium during various stages of culture.

On the study of metabolic changes at the stage of mycelial growth and spore formation, behaviour of nucleic acids, especially RNA, in the data given in the Fig. 5 is of interest. YANAGITA *et al.*[19] reported that nucleic acid syntheses induce germination of *Asp. niger*. Also, changes in the contents of protein and ribonucleic acid during the formation of chloroplasts in *Euglena gracilis* have been reported by BRAWERMAN *et al.*[20,21] It is probable that RNA may play a role in the metabolic specialization for various physiological processes in *Asp. niger*.

A detailed analysis for stable-P and seven minute-P was not performed, but probably these fractions contained various kinds of sugar phospate esters, polyphosphates and nucleotides, which were related to the synthesis of polysaccharides, nucleic acids and other organic phosphate compounds. In NaF culture, the contents of RNA in mycelium varied irregularly. The results are shown in Fig. 6. On the other hand, KUNINAKA reported that NaF inhibits RNA-depolymerase and RNA-phosphatase[18], but the role of these enzymes in spore formation is not yet clear.

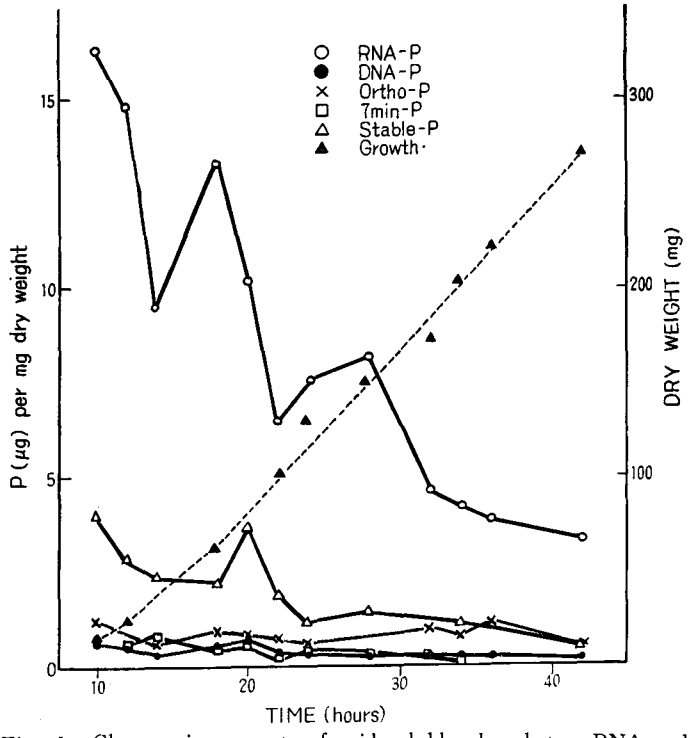

Fig. 6. Changes in amounts of acid soluble phosphates, RNA and DNA in mycelium during various stages of NaF culture.

Acid production and changes of pH value

The total amount of organic acids produced in the culture fluid during mold growth was estimated by the titration. At the same time, changes of pH of culture fluid were recorded. These results were represented in Fig. 7. Acids produced by normal mold culture were gradually accumulated in the culture fluid, the rate of accumulation was high during the period of mycelial growth and at an early stage of spore formation. Some consumption of acids was observed at the later stage of growth. Final pH of culture fluid was shown to be 2.1. Acids produced by NaF-inhibited mold accumulated more slowly in culture fluid as compared with the normal culture and its maximum amount was about 50% of that in normal mold culture fluid. The final pH of culture fluid in this case was shown to be about 3.5.

These experiments were performed in order to obtain some information in regard to the relationship between morphological development and its biochemical basis. Although NaF was used in these experiments as typical inhibitor for spore formation, it inhibited not only spore formation perfectly

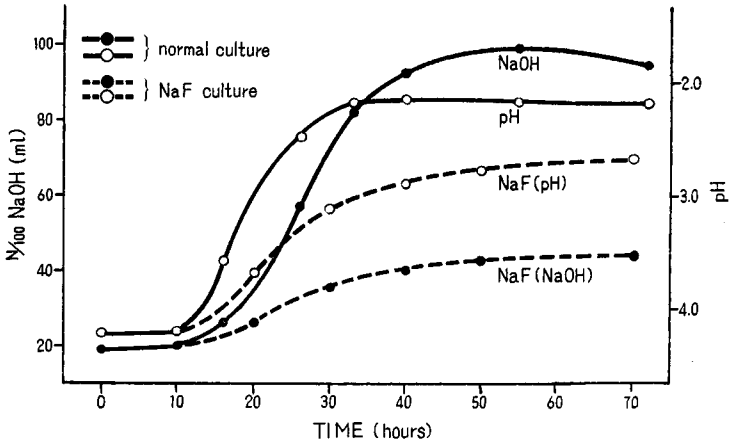

Fig. 7. Acid accumulation and changes of pH value in culture fluid.

but also growth considerably. NaF, therefore, may not be appropriate for analyzing the chemical basis of growth and spore formation. Suitable methods of growth control to separate effectively the process of spore formation from that of mycelial growth will be required. The experiments are in progress in our laboratory.

To Prof. S. USAMI, the authors are greatly indebted for his valuable advice throughout the progress of this study.

Summary

Changes in the amounts of total-N, protein-N, acid soluble phosphates, RNA, DNA and glycogen-like polysaccharide at various stages of growth and development of *Asprigillus niger* were analyzed, and titratable acids and pH in culture fluid were determined simultaneously. The results were compared with those of abnormal growth caused by sodium fluoride.

1. The contents of nitrogen and phosphorus compounds and nucleic acids per mg dry weight of mycelium were generally higher in normal mold culture than in NaF-culture though they were variable at an early stage of mycelial growth. Glycogen-like polysaccharide content was almost constant at various stages.

2. Spore formation was inhibited by NaF. In the presence of NaF, generally, the contents of the various compounds per mg dry weight of mycelium changed irregularly during growth, and the contents of RNA and glycogen-like polysaccharide changed markedly as compared with normal culture without NaF.

3. Amount of titratable acids accumulated in normal culture fluid reached to almost two fold of the amount in NaF-culture. Changes of pH in culture fluid corresponded to the rate of acid accumulation.

4. Effect of some inhibitors besides NaF on growth and development was examined.

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