

## Metabolic status of temperature induced swollen conidia of *Aspergillus nidulans*

Rajendra K. Saxena and Umakant Sinha<sup>1</sup>

Department of Botany, University of Delhi, Delhi-110007 (India), 13 May 1977

**Summary.** There is an active synthesis of DNA, RNA, protein, and carbohydrate in conidia incubated at 48°C. DNA increases more or less 3 times; while RNA, protein and carbohydrate are synthesized at a much faster rate. Conidia do not germinate at this temperature.

In an earlier communication<sup>2</sup> it was reported that when conidia of *riboA<sub>1</sub>*, *biA<sub>1</sub>* (a riboflavin and biotin requiring) strain of *Aspergillus nidulans* were incubated at 48°C under submerged culture conditions, there was an approximately 5 to 6 fold increase in their diameter, although conidia did not germinate at this temperature. When these swollen conidia were further incubated under optimal growth conditions, that is at 37°C, conidia germinated and reproduced asexually with a limited vegetative growth. Microscopic observations of these larger conidia obtained at 48°C revealed that the conidial wall remained thick. This indicated that extension of the wall should be accompanied by wall synthesis. The present study was undertaken to determine the changes in the levels of macromolecules in enlarging conidia in order to have an idea of their metabolic status.

A riboflavin- and biotin-requiring, green conidial (*riboA<sub>1</sub>*, *biA<sub>1</sub>*) strain, from the departmental stock, was used in the present investigation. A thick conidial suspension was inoculated in 100 ml of minimal medium<sup>3</sup>. Cultures were incubated at 48°C for 24 and 48 h in a New Brunswick Gyrotory Incubator Shaker at 150 rpm. Conidia were harvested after desired incubation periods and extracts were prepared in distilled water and stored at about 4°C, until used. Deoxyribo- and ribo-nucleic acids were estimated using diphenylamine and orcinol reagents, respec-

tively<sup>4</sup>. Anthrone reagent<sup>5</sup> was used for determining carbohydrates, and proteins were estimated by using Folin's phenol reagent<sup>6</sup>.

It is evident from the observations presented in figure 1, that there was an active synthesis of different macromolecules within the conidia at 48°C, accompanied by an increase in weight. In order to have a better idea about the levels of different macromolecules within the conidia, the amount per conidium was calculated (figure 2).

These observations show clearly that the amount of DNA per conidium was approximately tripled within 48 h during the temperature treatment. RNA, proteins and carbohydrates were synthesized at a much faster rate within the conidium during the same period. In 48 h, there was a 7 fold increase in the amount of RNA, whereas there were almost 12 fold increases in the amounts of total proteins and soluble carbohydrates.

We can summarise our data as follows: Incubation at 48°C leads to an increase in conidial diameter and subsequent conidiation with limited vegetative growth when pretreated conidia are incubated at 37°C. Rapid and tremendous increases in the levels of DNA, RNA, proteins, and carbohydrates, in the conidia incubated at 48°C, clearly indicate that the conidia at this temperature are at a high state of metabolic activity. Perhaps these larger conidia have attained the status of maturity required for conidiation and this is one of the reasons why they conidiate profusely without extensive vegetative growth. In other words, during the metabolically active period within the conidium incubated at 48°C, the enzyme or a group of enzymes responsible for conidiation are synthesized or are switched on, which in the normal course are synthesized or are switched on only after a certain amount of vegetative growth. Certain bacteria have been found to behave in a similar fashion. It has been reported, that the bacterial spores that lead to microcyclic sporulation have a higher concentration of DNA<sup>7</sup>. In general, conidiation with less or no vegetative growth seems to be similar to normal sporulation, with the exception that the macromolecular synthesis required for this process gets switched on in the conidium itself or soon after it germinates. Studies with *Aspergillus niger*<sup>8</sup>, *Neurospora crassa*<sup>9,10</sup> and *Blastocladiella emersonii*<sup>11</sup> support this view.

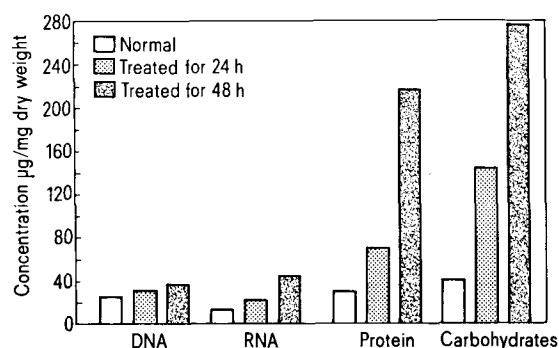


Fig. 1. Macromolecular levels (DNA, RNA, proteins and carbohydrates) in conidia of the *riboA<sub>1</sub>*, *biA<sub>1</sub>* strain of *Aspergillus nidulans* incubated at 48°C for 0, 24 and 48 h.

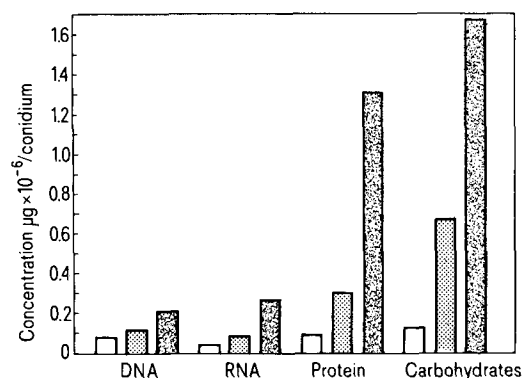


Fig. 2. Levels of macromolecules (DNA, RNA, proteins and carbohydrates) per conidium, in the *riboA<sub>1</sub>*, *biA<sub>1</sub>* strain of *Aspergillus nidulans*, treated at 48°C for 0, 24 and 48 h.

1. Acknowledgments. Grateful thanks are due to Professor B. M. Johri for valuable suggestions. R. K. S. acknowledges with thanks a Senior Research Fellowship of the Council of Scientific and Industrial Research, New Delhi.
2. R. K. Saxena and U. Sinha, Trans. mycol. Soc. (Japan), in press (1977).
3. U. Sinha, Genetics 62, 495 (1969).
4. W. E. Schneider, Methods in Enzymology, vol. 3; p. 680. Ed. S. P. Colowick, and N. O. Kaplan. Academic Press Inc., New York 1957.
5. F. A. Loewus, Analyt. Chem. 94, 219 (1952).
6. O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, J. biol. Chem. 193, 265 (1951).
7. S. D. Rodenberg, D. J. O'Kane, R. A. Hackel and E. Cocklin in: Spores, vol. 5; pp. 197. Ed. H. O. Halvorson, R. Hanson and L. L. Campbell, Washington 1972.
8. J. G. Anderson and J. E. Smith, J. gen. Microbiol. 69, 185 (1971).
9. M. N. Ojha and G. Turian, Archs. Microbiol. 63, 232 (1968).
10. M. Cortat and G. Turian, Archs. Microbiol. 95, 305 (1974).
11. S. W. Hennessy and E. C. Cantino, Mycologia, 64, 1066 (1972).