CARBON-13 NMR SPECTROSCOPIC STUDY OF A THERMOPHILIC FUNGUS—*THERMOMYCES LANUGINOSUS*

A. K. RAJASEKARAN† and S. DIVAKAR*

**Molecular Biophysics Unit, †Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India.*

ABSTRACT

Natural abundance ^{13}C NMR spectra of mycelia and ethanol extracts of *Thermomyces lanuginosus* grown at 30°C indicated the presence of mannitol. In mycelia grown at 50°C, trehalose was also detected.

INTRODUCTION

NATURAL abundance ^{13}C NMR has been used in the investigation of carbon metabolism of many microbial systems[1–3]. However, only a few reports are available on filamentous fungi[4–6]. In this paper the results obtained from *in vivo* natural abundance ^{13}C NMR of a thermophilic fungus, *Thermomyces lanuginosus* grown at two different temperatures are reported.

MATERIALS AND METHODS

T. lanuginosus was grown in a glucose-asparagine medium[7] in shake cultures at 30°C and 50°C. At the mid-log phase of growth (72–84 h of growth at 30°C and 18 h at 50°C) the mycelia were harvested by filtration, washed with distilled water and pressed between filter papers. Approximately, 3–4 g of wet fungal cells were packed inside a 10 mm NMR tube such that there was very little air space in the medium. Spectra of the concentrated ethanol extracts of the mycelia were recorded. About 2–3 ml of cell extract were taken in a 10 mm NMR tube for obtaining the spectra.

Natural abundance ^{13}C NMR was run on an NMR spectrometer (Varian FT80A, pulse fourier transform) operating at 20 MHz for carbon. Both cell extracts and in tact cell samples were locked externally and the probe temperature maintained at 30°C. Samples were spun at 20 rps. Proton noise-decoupled spectra (^{13}C-{^{1}H}), showing full decoupling with the nuclear Overhauser effect (NOE) were obtained. Samples were subjected to 60° pulses with a recycle time of 10 s for the cell extracts and 2 s for the in tact cells. A spectral width covering 0–200 ppm was employed. At least 6000 scans were accumulated for in tact cells. For cells extracts about 4000 scans were collected. A sensitivity enhancement parameter corresponding to 1 Hz line broadening was employed. A small amount of dioxane was used as an internal standard and its value at 67.4 ppm with respect to TMS was taken as reference for all the signals observed from the sample. All carbon chemical shifts were measured within ±0.05 ppm.

RESULTS AND DISCUSSION

Intense carbon signals were observed from cells grown at 30°C, namely at 64.4, 70.4 and 72.4 ppm. A less intense signal at 61 9 ppm (figure 1) was also detected in the spectra of cell extracts obtained from fungal cells grown at 50°C. Extracts of cells grown at 50°C along with the above mentioned signals also exhibited signals at 94.4, 74.6, 73.3, 70.4, 71.9 and 61.4 ppm (figure 1).

Natural abundance ^{13}C spectra of intact cells obtained both at 30°C and 50°C were slightly broader than those obtained with cell extracts. Better signal-to-noise ratio was observed with in tact cells than with cell extracts. The signals observed from the mycelia grown at 30°C and 50°C were the same as mentioned above (figure 1 and table 1). Normally in a ^{13}C NMR spectrum compounds containing –CHOH– groups, especially sugars, give rise to signals in the region 60–100 ppm exclusively. Chemical shift values observed with the cells grown at 30°C were identified as due to mannitol. Although these signals possibly arise due to the presence of other sugars, all the observed signals could be clearly accounted for by mannitol[8] which gives rise to three signals at 64.6, 70.7 and 72.2 ppm corresponding to C_1 and C_6, C_3 and C_4, and C_2 and C_5 carbons respectively. The carbon chemical shift values of the signals observed from fungus grown at 50°C indicated the presence of trehalose as well. This was confirmed from ^{13}C spectra of authentic trehalose. Comparison of the chemical shift values obtained from the samples (table 1) with the values for some commonly occurring sugars in fungus[8] indicates that α-D-glucose can give rise to signals

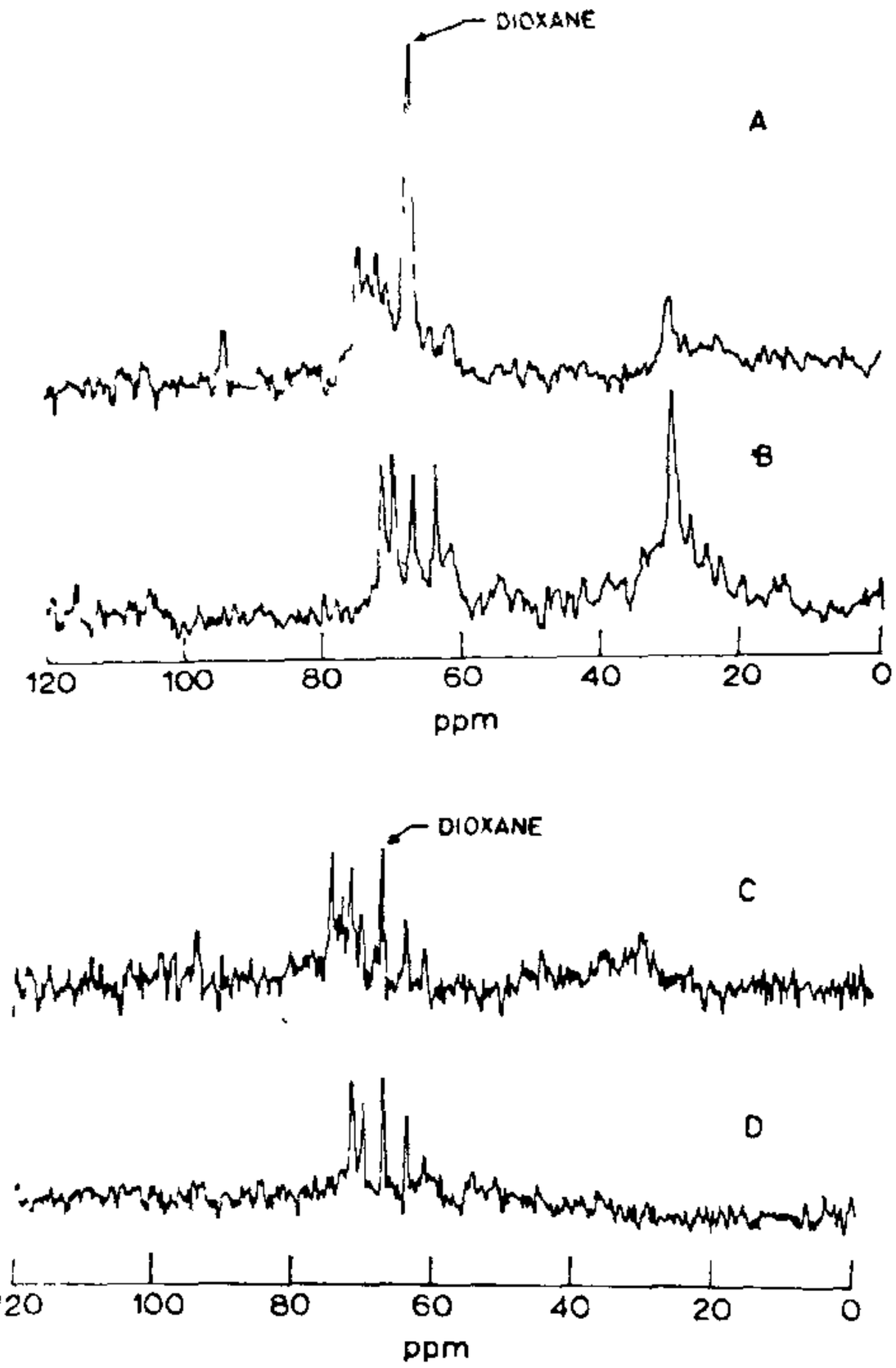

Figure 1A–D. Natural abundance ^{13}C NMR spectra of *T. lanuginosus*. **A, B.** Intact cells grown at 50°C; and 30°C respectively. **C, D.** Extracts of fungus grown at 50°C; and 30°C respectively.

observed for trehalose in the sample. However, paper chromatography of cell extracts did not reveal α-D-glucose. Mannitol and trehalose formed at the respective temperatures were also detected by paper chromatography (unpublished results).

Around 30 ppm, intense signals were observed in the spectra of in tact cells grown at 30°C. A much less intense signal was observed at this region in the sample of the cells grown at 50°C. This and other unidentifiable signals observed around this region may arise from membranes and other components of the cells. The peak at 61.9 ppm in the spectra of cells grown at 30°C may be due to C_6 carbon signal of glucosamine that is commonly found in the cell walls of fungi.

^{13}C NMR studies indicate that *T. lanuginosus* produces mannitol both at 30°C and 50°C. In addition to mannitol, a high level of trehalose is formed at 50°C. Trehalose is possibly formed at a very low level at 30°C which could not be detected by natural abundance ^{13}C NMR. In *T. lanuginosus* trehalose was the major sugar present in spores and mycelia when grown[9] at 50°C. Trehalose accumulation in fungi generally appears to be associated with periods of reduced growth rate like differentiation and starvation[10]. In yeast cells, trehalose accumulated to a substantial quantity when grown at higher temperatures[11,12]. However, in these cases the growth was slower compared to the growth at normal temperatures. It is interesting to note that *T. lanuginosus* accumulate trehalose at high temperature (50°C) in actively growing cells and the absence of trehalose at 30°C suggests that trehalose accumulation in *T. lanuginosus* is probably a temperature-related phenomenon. A possible role for trehalose at high temperatures may be to prevent desiccation and to protect membranes and other essential cellular components[13,14]. It has been indirectly shown that a high level of trehalose enhances the resistance of spores under extreme

Table 1 *Carbon chemical shift values of sugars present in Thermomyces lanuginosus*

Sugars	Source	Chemical shift values					
		C_1	C_2	C_3	C_4	C_5	C_6
Mannitol[a,c]	Cell extracts	64.4	72.4	70 4	70.4	72.4	64.4
Mannitol[a]	In tact cells	64.2	71.83	70 22	70.22	71.83	64.2
Trehalose[b,c]	Cell extracts	94 35	73.3	74.6	70 35	71 9	61.4
Trehalose[b]	In tact cells	93.7	73.2	74 2	70 6	71.9	61.7
Trehalose	authentic	94 1	73.03	73.52	70 6	72 0	61.5

[a] Fungal cells grown at 30°C. At this temperature a signal at 61.9 ppm was also detected, [b] Fungal cells grown at 50°C. Along with trehalose peaks, mannitol C_6 signal was detected at 64.4 ppm; [c] Assignments based on Hocking and Norton[8].

environmental conditions such as high and low temperatures, desiccation, etc.[11].

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1. Evans, J. N. S., Raleigh, D. P., Tolman, C. J. and Roberts, M. F., *J. Biol. Chem.*, 1986, **261,** 16323.
2. Ugurbil, K., Brown, T. R., den Hollander, J. A., Glynn, P. and Shulman, R. G., *Proc. Natl. Acad. Sci. USA*, 1978, **75,** 3742.
3. den Hollander, J. A., Brown, T. R., Ugurbil, K. and Shulman, R. G., *Proc. Natl. Acad. Sci. USA*, 1979, **76,** 6096.
4. Eakin, R. T., Morgan, L. O. and Matwiyoff, N. A., *Biochem. J.*, 1975, **152,** 529.
5. Matsunaga, T., Okubo, A., Fukami, M., Yamazaki, S. and Toda, S., *Biochem. Biophys. Res. Commun.*, 1981, **102,** 524.
6. Dijkema, C., Kester, H. C. M. and Visser, J., *Proc. Natl. Acad. Sci. USA*, 1985, **82,** 14.
7. Prasad, A. R. S. and Maheshwari, R., *Proc. Indian Acad. Sci.*, 1978, **B87,** 231.
8. Hocking, A. D. and Norton, R. S., *J. Gen. Microbiol.*, 1983, **129,** 2915.
9. Suryakrishna Prasad, M., Dissertation for Diploma in Molecular Biochemistry, IISc., 1973.
10. Thevelein, J. M., *Microbiol. Rev.*, 1984, **48,** 42.
11. Grba, S., Oura, E. and Soumalainen, H., *Eur. J. Appl. Microbiol.*, 1975, **2,** 29.
12. Savioja, T. and Meittinen, J. K., *Acta Chem. Scand.*, 1966, **20,** 2444.
13. Crowe, J. H., Crowe, L. M. and Chapman, D., *Arch. Biochem. Biophys.*, 1984, **232,** 400.
14. Madden, T. D., Bally, M. B., Hope, M. J., Cullis, P. R., Schieren, H. P. and Janoff, A. S., *Biochem. Biophys. Acta*, 1985, **817,** 68.

ANNOUNCEMENT

SIXTH ANNUAL CONVENTION OF THE INDIAN SOCIETY FOR MEDICAL STATISTICS AND NATIONAL SEMINAR ON STATISTICS IN MEDICINE, HEALTH AND NUTRITION

The Indian Society for Medical Statistics will be organizing its Sixth Annual Convention and National Seminar on Statistics in Medicine, Health and Nutrition at National Institute of Nutrition, Hyderabad (India) from 27–29th October, 1988. Medical teachers, Medical research workers, Nutritionists, Medical statisticians, Demographers, Anthropologists, Social Scientists and Computer-personnel, both from India and abroad are invited to attend the 3-day programme. The conference will concentrate on the following aspects.

A. *Symposium on "Data-base and analysis in health and nutrition — Present and future"*

(i) Nutrition; (ii) Epidemiology, hospital statistics, Registration system; (iii) Survey and data collection methods; Methods of assessment of health and nutrition-relative merits and critical limits for use; (iv) Ecological methods; Secular trends in health; Time series models; Economic analysis in health; Environmental pollution; (v) Operational research, systems analysis and computer applications; (vi) Fertility differentials and health.

B. *Other topics*

(i) Bio-assay methods; (ii) Statistical models and Bayesion methods; (iii) Multivariate methods; (iv) Genetic models and growth studies; (v) Training in and curriculum of Biostatistics.

Contributions from the above and other related topics are welcome. Three copies of an abstract of the paper, not exceeding 300 words are to be submitted by **31 May 1988.** Original paper typed as per suggested guidelines may be submitted by **31 July 1988.** Further details of seminar and convention can be had from: Dr K. Visweswara Rao, Organising Secretary, National Seminar on Statistics in Medicine, Health and Nutrition, Department of Statistics, National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania, Hyderabad 500 007.