



Preliminary communication

Production of cellulose II by *Acetobacter xylinum* in the presence of 2,6-dichlorobenzonitrile

Xiaochun Yu^{*a,b}, Rajai H. Atalla^{a,b}

^aU.S.D.A., Forest Service, Forest Products Laboratory, One Gifford Pinchot Dr., Madison, WI 53705, USA
^bDepartment of chemical Engineering, University of Wisconsin, Madison, WI, USA

Received 30 January 1996; accepted 26 March 1996

Abstract

This report provides X-ray diffraction and Raman spectral evidence that, when 2,6-dichlorobenzonitrile is present in the culture medium, *Acetobacter xylinum*, which is a model system for investigation of the biosynthesis of native cellulose, produces cellulose II, as well as cellulose I. The significance of the observations with respect to the mechanism of biosynthesis of cellulose is discussed briefly.

Keywords: *Acetobacter xylinum*; 2,6-Dichlorobenzonitrile; Cellulose II

2,6-dichlorobenzonitrile (DCB) is a herbicide, which has been shown to be a specific and effective inhibitor for cellulose synthesis in algae and higher plants [1-5] and also in *Acetobacter xylinum* [6]. The extent of the inhibition is dependent on the concentration of DCB. Here we present evidence that DCB, in addition to inhibiting the synthesis of cellulose in the *Acetobacter xylinum* system, also initiates changes in the crystalline allomorph produced in its presence.

The bacterium *Acetobacter xylinum* has been used as a model system for investigation of the biosynthesis of cellulose because it produces cellulose as an extracellular product [6,7]. Normally, the cellulose produced is in the cellulose I allomorph, which is the predominant native form. However, in the presence of 12 μ M DCB, we found that cellulose II, as well as cellulose I, was produced.

The first indication of the production of cellulose II is in the X-ray diffractogram of the cellulose sample produced by *Acetobacter xylinum* in the presence of 12 μ M DCB, as shown in Fig. 1.

The strong diffraction peak at $20.3^\circ 2\theta$ and a shoulder at around $12^\circ 2\theta$, which correspond, respectively to the $10\bar{1}$ and 101 lattice planes of cellulose II, clearly indicate the presence of cellulose II in the sample [8]. While the diffraction peaks at 14.5° and $16.5^\circ 2\theta$, which

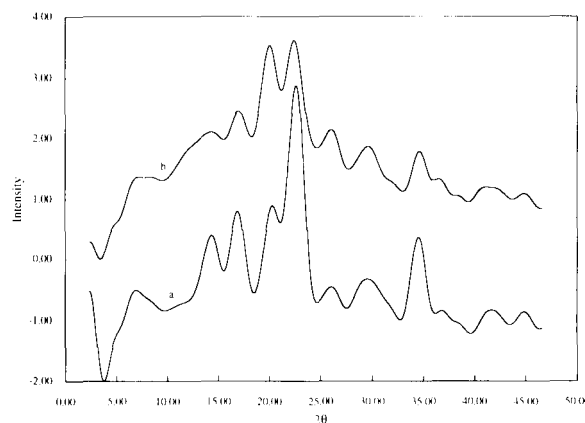


Fig. 1. Transmission X-ray diffractogram of bacterial cellulose (a) normal bacterial cellulose, (b) bacterial cellulose produced in the presence of 12 μ M DCB.

* Corresponding author, U.S.D.A., Forest Service, Forest Products Laboratory, One Gifford Pinchot Dr., Madison, WI 53705, USA.

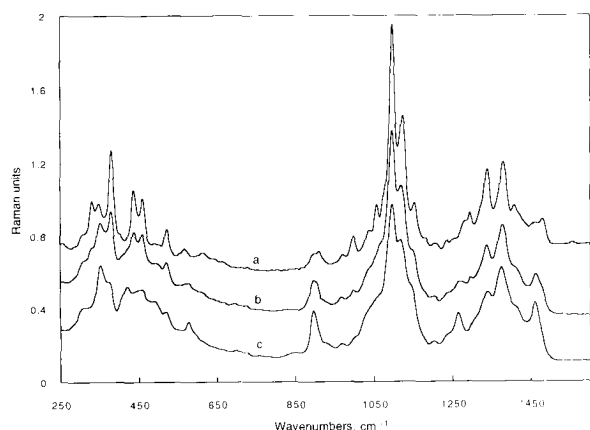


Fig. 2. Raman spectra of cellulose: (a) cellulose I, normal bacterial cellulose, (b) cellulose produced by *Acetobacter xylinum* in the presence of 12 μM DCB, (c) cellulose II, mercerized bacterial cellulose.

correspond, respectively to the 101 and $10\bar{1}$ lattice planes of cellulose I [8], suggest that cellulose I is also present.

Further supporting evidence of the production of cellulose II by *Acetobacter xylinum* in the presence of DCB comes from the Raman spectra shown in Fig. 2.

The Raman spectra of cellulose I and cellulose II have significant differences [9] in the region between 250 and 1600 cm^{-1} , particularly between 250 and 700 cm^{-1} . The occurrence of cellulose II in the cellulose sample produced by *Acetobacter xylinum* in the presence of 12 μM DCB can be seen from the following evidence:

(i) The position, shape and intensity of the peaks in the region of 300–700 cm^{-1} , especially 300–400 cm^{-1} , which is very sensitive to differences between the allomorphs of cellulose. For example, the peak at 352 cm^{-1} , which in cellulose I is at 347 cm^{-1} and in cellulose II is at 353 cm^{-1} , and its intensity is stronger than that in cellulose I, although it is still weaker than that in cellulose II. Also a small shoulder at 420 cm^{-1} , where cellulose I does not have a band, and cellulose II has a medium band; and a weak peak at 577 cm^{-1} , where cellulose I does not have a band, and cellulose II has a medium peak.

(ii) The intensity of the band at about 900 cm^{-1} is much stronger than it is for cellulose I, and its position also is shifted toward the position for cellulose II.

(iii) The intensities of peaks at 1339 cm^{-1} , 1380 cm^{-1} and 1463 cm^{-1} are significantly different from those for cellulose I, and have a similarity to those in the spectrum of cellulose II.

The production of cellulose II by *Acetobacter xylinum* in the presence of DCB raises a significant question about the mechanism of the biosynthesis of cellulose. Native cellulose, as produced by *Acetobacter xylinum* as well as most algae and all higher plants, is normally the cellulose I allomorph. Cellulose II is usually the form which occurs upon regeneration from solution or mercerizing in strong alkaline solution. The mechanism of aggregation into the cellulose I, both in *Acetobacter xylinum* and in other organisms remains unknown.

Haigler [10] demonstrated that *Acetobacter xylinum* extrudes cellulose from the cell in the form of 1.5 nm microfibrils. These microfibrils have no crystallinity, but appear to have the correct conformation for them to crystallize into cellulose I. This crystallization process was assumed to be cell-directed self assembly. There is no direct evidence concerning the manner of aggregation of the microfibrils. The production of cellulose II by *Acetobacter xylinum* in the presence of DCB indicates that the process of aggregation of the 1.5 nm microfibrils was modified in a manner not yet well defined and the subject of further investigation. The effect of DCB that we report is clearly in addition to the well established inhibition of the biosynthesis of cellulose by DCB.

Acknowledgements

This work is supported by the USDA Forest Service and the Division of Energy Biosciences of the Office of Basic Energy Sciences of the US Department of Energy.

References

- [1] Montezinos, D. and Delmer, D.P. *Planta* 1980; 148: 305.
- [2] Shedletzky, E., Shmuel, M., Trainin, T., Kalman, S. and Delmer, D. *Plant Physiol.* 1992; 100: 120.
- [3] Shedletzky, E., Shmuel, M., Delmer, D.P. and Lampert, D.T. *Plant Physiol.* 1990; 94: 980.
- [4] Wells, B., McCann, M.C., Shedletzky, E., Delmer, D. and Robert, K. *J. Microscopy* 1994; 173(2): 155.
- [5] Delmer, D.P., Read, S.M. and Cooper, G. *Plant Physiol.* 1987; 84: 415.
- [6] Aloni, Y. and Benziman, M. In: *Cellulose and other Natural Polymer Systems* (R.M. Brown, ed.), Plenum Press, 1982, pp. 356.
- [7] Delmer, D.P. *Annu. Rev. Plant Physiol.* 1987; 38: 259.
- [8] Krassig, H.A. *Cellulose: Structure, Accessibility and Reactivity*, Gordon and Breach Science Publishers, 1993, pp. 98.
- [9] Wiley, J.H. and Atalla, R.H. In: *The Structure of Cellulose* (R.H. Atalla, ed.), ACS Symposium Series, Chicago, 1985, 340, pp. 151.
- [10] Haigler, C.H., Brown, R.M. and Benziman, M. *Science* 1980; 210: 903.