Polysaccharide synthesis *in vitro* from cellulose-producing model organisms

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Cellulose is synthesized from terminal complexes (TCs) on the plasma membrane. Globules of TCs are supposed to be cellulose synthase, CesA. However the composition and mechanism of cellulose synthase or TCs are poorly understood. In order to elucidate them, trails to synthesize cellulose *in vitro* were made.

The originality of this experiment lies on the manipulation of membrane-bound cellulose synthase, and its application to *in vitro* synthesis of cellulose. For establishing methods for the *in vitro* synthesis of cellulose, microsomal fraction was collected from different model organisms – tobacco BY2 cells, *Gluconacetobacter*, *Ciona intestinalis*, *Saprolegnia monoïca* and *E. coli* heterogously expressing cellulose synthase of *Gluconacetobacter* (BcsA). In all the cases, cells were disrupted and subjected to differential centrifugation to collect the microsomal fraction. Membrane-bound proteins were further extracted by detergent, for example digitonin, to which UDP-glucose was added to conduct *in vitro* synthesis of cellulose. The product was characterized by electron microscopy, FT-IR and X-ray diffraction, as well as radio isotope assay with UDP-[^{14}C]-glucose. Incorporation of radioactivity to the *in vitro* product was observed for each detergent extract. This confirmed that the analyzed polysaccharides were newly synthesized and not arising from endogenous sources like cell wall.

In the case of BY2, two types of *in vitro* products were observed: fibers and spindles (Figure 1a and 1b). These products were analyzed with ATR-FTIR and X-ray diffraction, and most of them were revealed as (β1→3)-D-glucan. The *in vitro* product from *Ciona intestinalis* was detected only before detergent extraction, and fibers of 5-12 nm in width and indefinite in length were observed (Figure 1c). The *in vitro* product from *Saprolegnia monoïca* was microfibrils, 4-6 nm or some of them are 10-20 nm in width (Figure 1d) as seen in the previous study [1]. The characterization of these products and optimizing parameters for cellulose synthesis *in vitro* are in progress.

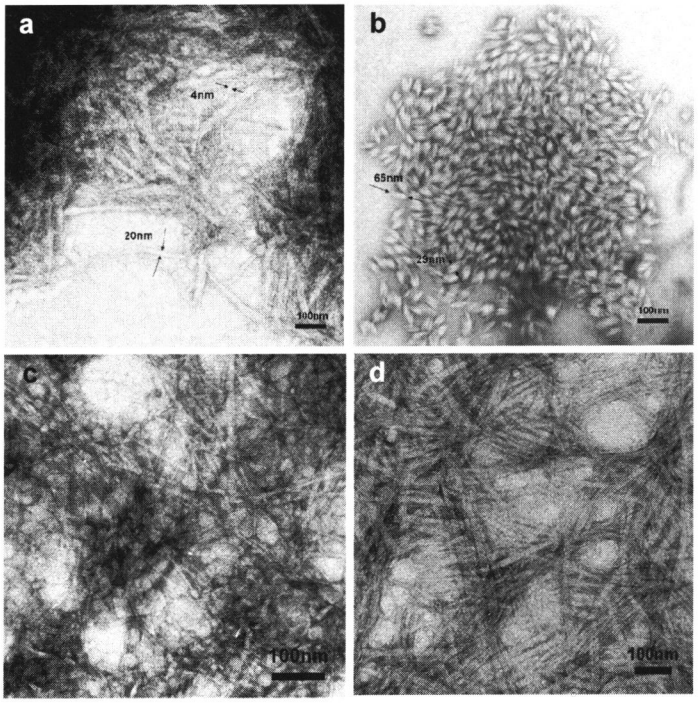

Figure 1
The *in vitro* products from: (a) BY-2 (microfibrils), (b) BY-2 (spindles), (c) *Ciona intestinalis* and (d) *Saprolegnia monoïca*

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REFERENCE

[1] Pelosi, L. *et al.* (2003) *Biochemistry* **42**, 6264-6274