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Experimental Induction of Altered Nonmicrofibrillar Cellulose

Abstract. *Cellulose produced by* Acetobacter xylinum *was experimentally modified during its biosynthesis. In the presence of fluorescent brightening agents, such as Calcofluor White M2R or Tinopal LPW, nonmicrofibrillar sheets of cellulose were synthesized by the bacteria. These sheets could then be converted to fibrils by washing with distilled water. Possible mechanisms for these modifications of cellulose assembly are discussed.*

The gram-negative bacterium *Acetobacter xylinum* normally synthesizes an organized, twisting ribbon of cellulose I (Fig. 1A) (*1*). This ribbon is a composite of microfibrils assembled in association with an array of particles and extrusion pores in the bacterial outer membrane (*2*). When fluorescent brightening agents are added, they bind to small groups of glucan chains as they are polymerized and extruded through the pores (*3*). These agents prevent the cooperative crystallization of glucan chains required for the assembly of the organized ribbon of cellulose. The altered cellulose appears as a band of fine fibrils after negative staining (*3*).

We report the synthesis of a second type of altered cellulose that appears as nonmicrofibrillar sheets with no detectable substructure after negative staining (Fig. 1B). The assembly of sheet or fibrillar forms of altered cellulose in the presence of fluorescent brightening agents is dependent on strain variations among the bacteria and the concentration of the brightener. Variants of *A. xylinum* occur spontaneously during growth. Sheet cellulose is synthesized by a variant that characteristically produces a "rough" colony on agar and a thick cellulosic pellicle in liquid medium. In the presence of brightener at concentrations above 8 μM, this variant consistently synthesizes sheets of nonmicrofibrillar cellulose (*4*). Fibrillar cellulose is synthesized in the presence of lower concentrations of brightener (2 to 4 μM) (*4*). Fibrillar and sheetlike celluloses are synthesized sequentially when the strain forming thick pellicles is exposed to a series of increasing concentrations of the brightener (Fig. 1C). Another strain synthesizes only fibrillar cellulose at all concentrations of brighteners tested.

In the presence of high concentrations of brightener, the strain forming thick pellicles can synthesize a single broad sheet (Fig. 1B) or several narrow discontinuous sheets. The sheets appear very thin, even on ultrathin carbon support films, and can be distinguished from the background only by their folds and edges (*5*). Some observations suggest that the sheets may be collapsed tubes or scrolled membranes. The nonfibrillar sheets of cellulose can be converted to fibrils by briefly washing them with distilled water (Fig. 1D) (*6*). Presumably, the brightener is at least partially removed from the glucan chains during washing, thus allowing more extensive interchain association. The bent and twisted, secondarily assembled fibers are reminiscent of those produced after mercerization of native cellulose (*7*).

Extensive research has shown that the fibrillar product is cellulose with altered crystallinity (*3*). The nonfibrillar sheet material described in this report is degraded by purified cellulases (*8*) and is therefore presumed to be cellulosic. The sheets are positively birefringent, suggesting that the glucan chains are aligned along the long axis of the extended sheets (*9*).

Normal cellulose I microfibrils are assembled from stacked sheets of glucan chains formed by interchain hydrogen bonding. Only dispersion forces exist between the stacked sheets in the microfibrils (*10*). High concentrations of brightener must associate so completely with the glucan chains in the plane normally participating in intersheet dispersion forces (*11*) that the chains can only

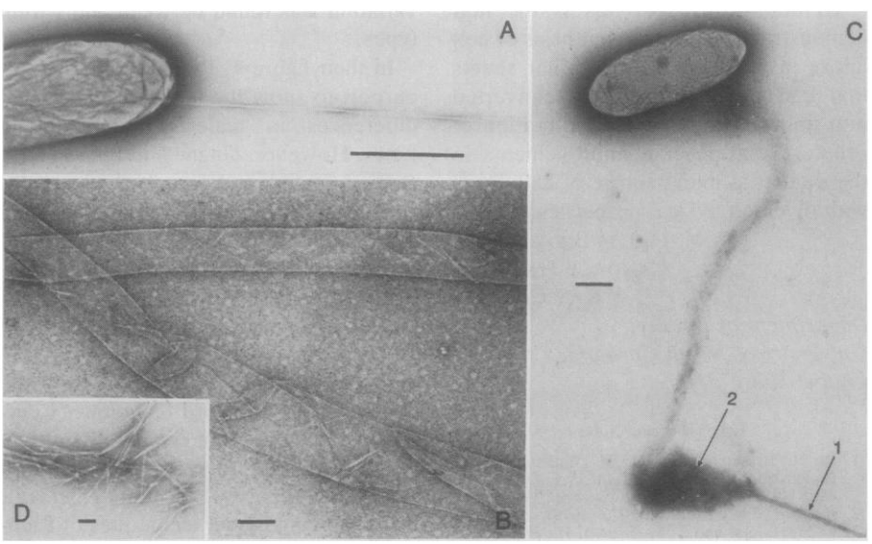

Fig. 1. (A) Unaltered ribbon of crystalline cellulose I synthesized by *Acetobacter xylinum* (scale bar, 1 μm). (B) Two extended sheets of nonfibrillar cellulose. These sheets are typical of those synthesized by thick pellicle-producing cells in brightener at concentrations greater than 8 μM (scale bar, 100 nm). (C) Cell incubated first in brightener-free glucose then in increasing concentrations of brightener. The first cellulose synthesized is a normal ribbon (arrow 1). Then a fibrillar band of cellulose is synthesized (arrow 2). This is followed by synthesis of nonfibrillar sheet cellulose in higher concentrations of brightener (scale bar, 600 nm). (D) Fibrils formed from sheet cellulose by brief washing in distilled water before negative staining (scale bar, 100 nm).

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associate laterally into sheets and are not able to stack into microfibrils. When the brightener is removed by washing, the insoluble glucan chains collapse into fibrils (Fig. 1D).

It has been proposed that, during normal cellulose synthesis in the absence of fluorescence brightening agents, (i) ordered aggregates of parallel glucan chains about 1.5 nm wide and having no detectable crystallinity are extruded through the outer membrane pores in *A. xylinum* and (ii) the aggregates cocrystallize to form microfibrils and the ribbon (*3*, *12*). In the presence of brightener at low concentrations, these aggregates must become coated with brightener while retaining their coherent structure, so that the altered cellulose appears as a band of fine fibrils. With higher concentrations of brightener, the aggregates synthesized by thick pellicle-producing bacteria must be dissociated into their composite chains by binding of the brighteners as they are extruded. The composite chains then form sheets. Most of the chains in a 1.5-nm aggregate would be exposed to the surface, resulting in a fibril with low crystallinity (*13*). As with direct cotton dyes, fluorescent brightening agents have a high affinity for noncrystalline regions of cellulose (*14*), and an aggregate of low crystallinity would probably dissociate if the surface glucan chains became extensively associated with brightener. The separated glucan chains would then interact laterally to produce a sheet rather than multidirectionally to produce a fibril.

To our knowledge, this is the first demonstration of the biosynthesis of cellulose in the form of nonfibrillar sheets that can be experimentally converted into fibrils. The controlled alteration of cellulose may lead to improvements in the industrial manufacture of celluloses with diverse physical properties.

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Mating Types in Screwworm Populations?

Richardson *et al.* (*1*) describe several different "types" of screwworm flies that occur in Mexico (*2*), and use quantitative criteria (especially chromosome length and arm ratios) as the basis for typing the flies. However, they present no numerical data and do not describe in detail the methods used to assign a sample to chromosome types A through J. Furthermore, they do not state how many samples were taken from each area and, more important, what degree of variation was found between and within types.

In their figure 7, Richardson *et al.* (*1*) purport to show the intrinsic anatomical differences in male genitalia among types. However, Gagné and Peterson (*3*) studied mated and unmated flies from single egg masses and found that such differences are due to relative wear and tear associated with mating.

The preparation of specimens for cytological examination (fixation, staining) and analysis (stage of cell division) may vary considerably even when the same person uses a standard technique. Coefficients of variation of measurements can run as high as 20 percent even within a single individual (*4*). McInnis *et al.* (*5*), using the cytological techniques of Richardson *et al.* (*1*) and a sample size of five cells per screwworm larval brain, observed coefficients of variation for standardized chromosome length and arm ratios of about 10 and 20 percent, respectively.

Chromosome polymorphism (especially of sex chromosomes) without genetic isolation is found in human, housefly, and mosquito populations. Thus chromosome polymorphism is not invariably accompanied by genetic isolation (*6*). Richardson *et al.* (*1*) emphasize that the absence of heterozygotes for various chromosome types in screwworm populations is indicative of reproductive isolation. Using the same cytological techniques and screwworm flies from the same populations as Richardson *et al.* (*1*), McInnis *et al.* (*5*) and McInnis (*7*) did find chromosomal heterozygotes, but at a reduced frequency. However, even a reduced frequency of heterozygotes does not necessarily imply reproductive isolation. In addition to the Wahlund effect mentioned by Richardson *et al.* (*1*), the limited resolution of the technique, inbreeding, heterozygote inviability, and other sampling errors might account for the low frequencies, as might the greater difficulty in measuring chromosomal variation *within* nuclei than *between* individuals in native screwworm populations. A more critical test of reproductive isolation is whether the same polymorphic nonhomologous chromosomes (a specific Y, X, II . . . VI) are consistently associated in diagnostic sets in certain populations to the exclusion of other combinations of nonhomologous polymorphic chromosomes. A mixing or recombination of chromosome types would indicate a lack of distinct karyotypes. McInnis *et al.* (*5*) found no statistically significant associations among specific nonhomologous chromosome types.

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