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CELLULOSE: STRUCTURAL AND FUNCTIONAL ASPECTS

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Bacterial cellulose

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INTRODUCTION

The major industrial source of cellulose is vascular plants. Trees are resources for most applications in the building industry, and the cotton plant is the major source for textiles. Most paper products originate from wood pulp. If vascular plants are sufficient raw materials for industrial uses of cellulose, why is the consideration of bacterial cellulose appropriate? Most industrial cellulose operations involve harvesting of large areas of the world's great natural forests. In some cases, the harvesting of tropical equatorial forests is leading to a major loss of valued ecosystems and species, not to mention erosion of land and pollution. Thus, there is a logical need to begin considering alternative sources of cellulose. Bacterial cellulose has some unique features rather distinct from other sources: (a) it is generated in the form of a never dried membrane; (b) no lignin and few other polysaccharides are co-synthesized; (c) the cellulose is of great mechanical strength; and, (d) the cellulose can be modified during synthesis.

Except for a product, Nata de Cocoa from the Philippines, bacterial cellulose is mostly a laboratory curiosity (Lapuz, *et al,* 1969). Bacterial cellulose synthesis has provided scientists new research avenues for exploring the basics of this process, including polymerization and crystallization steps, isolation of the cellulose synthase, and production of cellulose *in vitro*. This brief review will cover the known sources of bacterial cellulose, highlight the major scientific breakthroughs in cellulose structure and biosynthesis, cover the past and present industrial exploitations of this product, and peer into the future for scientific and commercial utilization of the process and product.

SOURCES OF BACTERIAL CELLULOSE

The best known source of bacterial cellulose is the gram negative rod, *Acetobacter xylinum* (*Bergey's Manual*, 1984). Although the nomenclature of this

genus is not well settled, the generic and species elaborated is preferred since most of the historical aspects of nomenclatural coverage refer to the species epithet "xylinum" since it connotes "wood" (Greek, *xylon*).

Other bacteria are known to synthesize cellulose. These include: *Alcaligenes, Pseudomonas, Aerobacter, Rhizobium,* and *Agrobacterium* (Dienema and Zevenhuizen, 1971); and *Sarcina* (Roberts, *et al,* 1989). In addition, the prokaryotic cyanophycean alga *Nostoc*, has been reported to synthesize cellulose (Frey-Wyssling, 1976). Undoubtedly, many more prokaryotic organisms probably have cellulose synthesizing capacity, but to this author's knowledge, no one has recently undertaken a systematic approach to determine the extent and range of cellulose synthesis among prokaryotes.

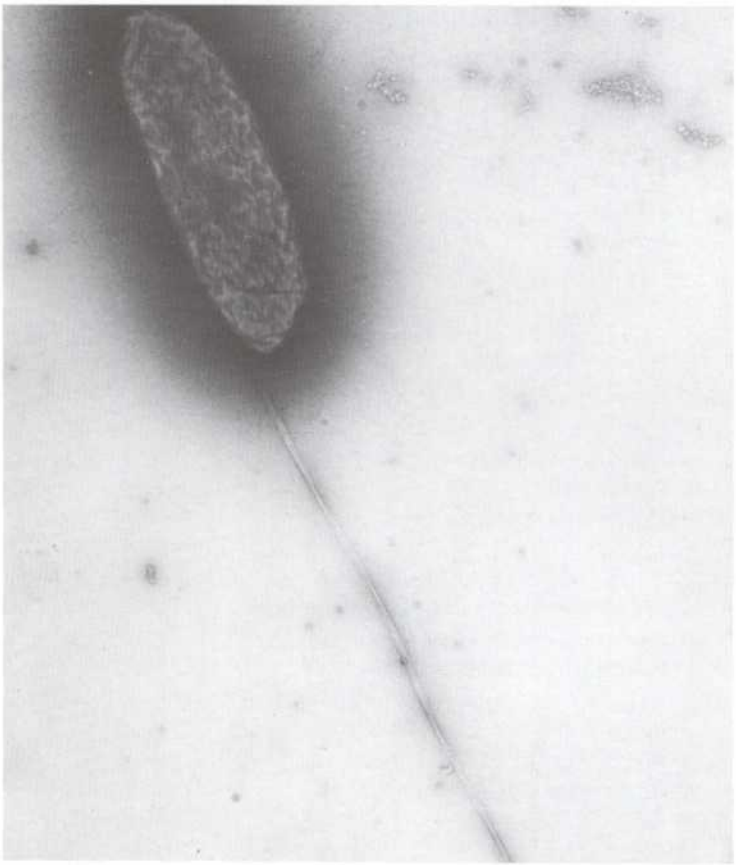

Fig. 1. A single rod of *Acetobacter xylinum* synthesizing a ribbon of pure cellulose microfibrils. Note periodic twisting of the ribbon, indicative of imperfect association of microfibrils. Specimen preparation – negative staining.

NOTABLE ADVANCES IN CELLULOSE RESEARCH USING MICROORGANISMS

Acetobacter was well known over 100 years ago. Adrian J. Brown (1886) published on the cellulose producing activities of *Bacterium xylinum*, clearly elaborating the formation of a leathery thick membrane at the air-liquid interface. During the intervening century, many researchers studied *Acetobacter xylinum*. Some of the highlights of research with *Acetobacter* are given in Table 1.

TABLE 1

Highlights of Major Advances in the Field of Bacterial Cellulose

Date	Discovery	Key Reference
1886	First description of bacterial cellulose	A.J. Brown
1949	Early electron microscopy studies of microfibril structure	K. Muhlethaler
1958	First cell-free synthesis of cellulose	L. Glaser
1969	First report of a commercial food product of bacterial cellulose	M.M. Lapuz, *et al.*
1971	First publication of diversity of microbial cellulose	M.H. Dienema and L.P.T.M. Zevenhuizen
1976	First direct visualization of bacterial cellulose synthesis	R.M. Brown, Jr., *et al.*
1980	First separation of polymerization from crystallization steps	C.R. Haigler, *et al.*
1985	Characterization of an activator of cellulose synthase	P. Ross, *et al.*
1985	First visualization of cell-free synthesis of cellulose	F.C. Lin, *et al.*
1987	Lattice imaging of glucan chains	S. Kuga and R.M. Brown, Jr.
1987	Localization of bacterial cellulose synthase in the cytoplasmic membrane	T.E. Bureau and R.M. Brown, Jr.
1987	Bacterial cellulose as a superior material for speaker diaphragms announced	*Japan Industrial Journal*
1988	Reducing end staining demonstrates that bacterial cellulose I allomorph has parallel chain structure	S. Kuga and R.M. Brown, Jr.
1989	Commercial prospects of bacterial cellulose elaborated	D.G. White and R.M. Brown, Jr.
1989	First stable cellulose II-synthesizing mutant	E. Roberts, I. Saxena and R.M. Brown, Jr.
1989	First purification and characterization of cellulose synthase	F.C. Lin and R.M. Brown, Jr.

COMMERCIAL PROSPECTS FOR MICROBIAL CELLULOSE

It is clear from Table 1 that *Acetobacter* has played a major role in our understanding of many of the primary phenomena associated with cellulose

biosynthesis. In light of biotechnology and fermentation advances, it is ironical that so few commercial ventures have been established. The major impediments lie in cost of production and lack of understanding of the unique properties of microbial cellulose.

One area of notable commercial success has been the Nata industry. Nata is a sweet confectionary enjoyed in many areas of the world but most especially in the Philippines where it is presumed to have originated (Lapuz, *et al.*, 1969). As late as the 1960s, the source of Nata formation was unknown. The detailed studies by Lapuz *et al* (1969) conclusively demonstrated that a bacterium was responsible for generating the gelatinous pellicle. This latent discovery was quite interesting inasmuch as Adrian Brown had demonstrated cellulose from *Acetobacter* nearly 100 years earlier, and the scientific literature was abundant with examples of bacterial cellulose. Geographic isolation from the mainstream of this particular area of science probably led to the late realization of the source of Nata. In spite of this, many years of practical cellulose production in the Philippines have demonstrated the potential for industrial scale up of this product. One of the most important aspects of Nata culture is that a *stable* cellulose producing strain can be maintained over many generations and hence, many years. In addition, efficient sucrose-utilizing strains have been selected over the years, thus paving the way for utilization of relatively low cost, abundant substrates. The Nata industry is world-wide, with exports to many countries. This is exemplified in that Nata products can be found in almost any oriental food store in the United States. The demonstration of a safe, pathogen-free bacterial cellulose Nata product is of potential significance, for one can more fully appreciate the potential uses of bacterial cellulose in the food, cosmetics, and health care industries.

The unique properties of microbial cellulose will lead industry to establish value-added market niches for this product. For instance, Ajinomoto Co. Inc. of Japan has recently exploited the high mechanical strength of dried bacterial cellulose and in conjunction with Sony Corporation has found that this is a superior source of diaphragm material for acoustic speakers (*Japan Industrial J.*, 1987). No doubt that other interesting products relying on the unique mechanical properties of bacterial cellulose will emerge, but it is too early to predict what products will be successful in an intensely competitive world market.

The economy of scale will eventually need to be improved, since the value-added markets will eventually become saturated. The comparatively high cost of microbial cellulose in contrast with wood-based sources is the greatest limitation to large scale production. Very little research on microbial cellulose fermentation has been reported, and even the world patents on this aspect are typically low in number and usually limited in scope. No major breakthroughs have yet emerged to suggest that costs of bacterial cellulose will come down and compete with wood products. In this author's opinion, the major limitations are directly linked with our understanding and ability to improve the efficiency of substrate conversion to product. Presently, we are relying on glucose, glycerol, sucrose, and a handful of natural substrates for microbial cellulose production. The best conversion efficiencies typically are no greater than 35-40% which means that 60-65% of the substrate is either not converted to cellulose or is lost to metabolism.

What can be done to improve the efficiency of substrate conversion? Two pathways of research must be continued. One is relatively short term and will most surely lead to improvements in cellulose yields. The other is long term, and if successful, could lead to direct competition with forest products. These will be considered briefly.

First, the research community needs to continue collecting cellulose-producing strains from many natural habitats and then study them for efficiency of conversion, substrate utilization diversity, and extended environmental range of cellulose production. Coupled with this study will be the necessity of an ongoing assessment of the quality and nature of the cellulose product. For example, it would

be of little use to have an efficiently produced cellulose if it had a low dp and crystallinity. The efficiency of production would need to surpass that of forest products in order to compete with these sources. This is possible, but only through the second pathway will progress be made.

What is needed here are long term research efforts to understand the metabolic pathways, molecular genetics, and efficiency of recombinant DNA technologies to produce stable, superior cellulose-producing strains. Obviously, the introduction of efficient cellulose synthesizing machinery into a photosynthetic bacterium or cyanophycean alga will accomplish the long term strategy. We should never give up hope on this aspect, for if successful, microbial cellulose synthesis could replace a significant portion of the cotton and forest products industries. On a global basis, these industries are staggering in scale and economy and affect human civilization in a very basic way.

THE HYPOTHETICAL "IDEAL" CELLULOSE FACTORY OF THE FUTURE

This topic is being presented largely to demonstrate what could become reality if basic and applied research and development on bacterial cellulose is given sufficient resources in the future. Many different types of "factories" are envisioned, largely to take advantage of diverse environments. For instance, a genetically stable strain may be developed to synthesize cellulose via photosynthesis and in a salty (halophilic) aqueous environment. The great inland salt lakes and estuaries of the world would become efficient culture chambers for microbial cellulose production. Other strains will be used which are tolerant for low and/or high temperatures, thus extending the range of location for cellulose synthesis and, at the same time, keeping competition (and contamination) for resources at a minimum.

The ideal factory for microbial cellulose is envisioned to be decentralized so that many people can participate in cellulose production. Thus, the cotton or tree farmer could be encouraged to continue to produce the product of their trade (cellulose) but from a different source (bacteria). Because bacteria synthesize cellulose rapidly, the overall productivity is much greater than trees or cotton. The major difference is that a substrate such as glucose or sucrose must be supplied, and while there is sufficient substrate for small value-added markets, the limitations of substrates for large scale operations would not be competitive. Possible substrates include sugar cane, beet sugar, corn-stubble residues, wood chips and similar products which can be efficiently degraded to glucose. The "ideal factory of the future" must address the ultimate source of energy for cellulose – the sun. While photosynthetic conversion of the sun's energy is relatively low (8% or less), the CO_2 substrate is of great abundance in the atmosphere and is continuing to rise (to create the "greenhouse effect"). Thus, it would be appropriate to consider tapping into this source of carbon and removing it from the global pool. Two important goals would be accomplished; (a) inexpensive cellulose would be made; and (b) atmospheric CO_2 concentration could be reduced.

BASIC RESEARCH ON CELLULOSE SYNTHESIS: ROLE OF *ACETOBACTER*

Acetobacter has been the most useful model for deciphering the basic process of the biosynthesis of cellulose. Vascular plant cell wall biogenesis models have to take into account the assembly of many other polymers in addition to cellulose. During secondary wall formation, the cotton fiber produces mostly cellulose. Even with this abundant natural source of cellulose, attempts to isolate the enzyme(s) involved in cellulose synthesis yield only ß 1,3 glucans (Delmer, 1987). Bacteria are less complex organisms than eukaryotic cells. They have a much shorter generation time. The genetic machinery is more amenable to dissection and analysis. Thus, *Acetobacter* promises to be the first organism in which the genes for cellulose will be cloned and expressed (Saxena and Brown, 1989; Brown, 1989). This basic

knowledge will be invaluable in the design of more efficient cellulose producing strains for the future. In addition, scientists will have the opportunity to initiate comparative studies for characterizing the genes for eukaryotic cellulose synthesis. Indirectly, *Acetobacter* cellulose research is predicted to greatly facilitate and advance cellulose biosynthesis research with cotton and trees. Basic knowledge in eukaryotic plant cell growth and development will emerge and have significant impact on agriculture and forestry.

On the purely scientific side, a new understanding of the genes involved with cellulose synthesis will help us to understand the phenomena of polymerization and crystallization. Does the cell have an internal "biological clock" to control or template the degree of polymerization? How does the cell control crystallization? How is the shape and size of microfibrils determined? How would the control of microfibril orientation effect mechanical properties of the cellulose? These and many other basic questions are now on the verge of being understood. Clearly, *Acetobacter* has been the organism of choice for solving the basic and fundamental mysteries of cellulose biosynthesis (see Figure 1). An exciting prospect now emerging is an understanding of the independent but linked control of polymer formation and polymer crystallization. For example, cellulose II polymorph mutants of *Acetobacter* lacked organized particles in the lipopolysaccharide envelope, yet they have a functional cellulose synthase, and when isolated and activated, it is no different from the wild type synthase. The mutation appears to involve the regulation of gene(s) which affect expression of an "export" component (Figure 2). This component could be a structural protein which has a pore or channel to efficiently "thread" the nascent glucan chain aggregate to the cell surface and properly orient it for maximum interaction with its neighbors. Loss of this control means loss of native cellulose I polymorph synthesis. So far, only the cellulose I polymorph has been synthesized *in vitro* (Lin and Brown, 1989). If we could learn more about the "export" component and form a complex with the cellulose synthase, it may be possible to synthesize cellulose I *in vitro*. The potential efficiencies of a cell-free system would mean lower costs for cellulose from conventional substrates such as sucrose. We must continue to explore the basic and fundamental principles of cellulose assembly, for it is from these experiments that new data emerge, leading to more efficient applied research and development.

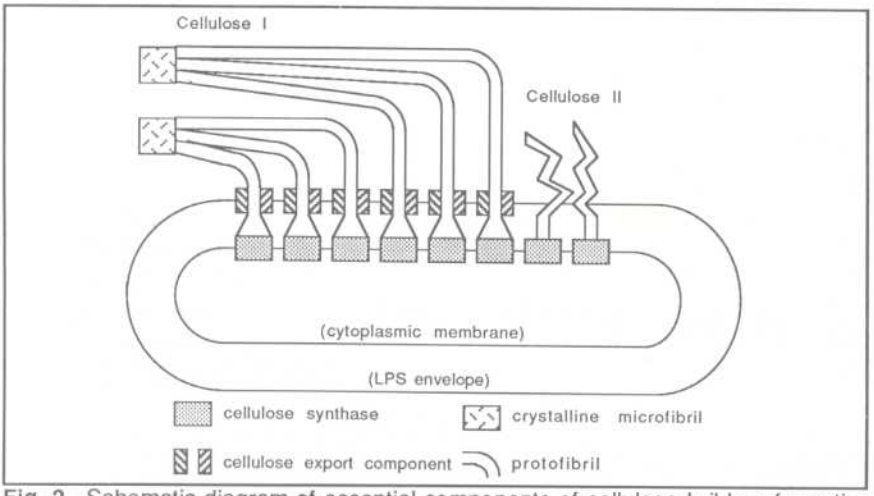

Fig. 2. Schematic diagram of essential components of cellulose I ribbon formation by *Acetobacter*. If the export component is missing or altered, cellulose II allomorph is synthesized.

CONCLUSIONS AND OUTLOOK

Cellucon '88 Japan is the first conference to provide a forum dedicated to presentations on bacterial cellulose structure and biosynthesis as well as economic aspects for its potential commercial utilization. The conference will long be remembered as the starting place for bringing together the elements of basic and applied research on microbial cellulose. There is a great potential for the production and use of microbial cellulose on a global scale. It will require intensive interactive efforts of academia and industry to develop and implement strategies for a new world-wide source of the most abundant macromolecule on earth.

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