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Microbial Conversion of Corn Stalks to Riches

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If all of the corn stover (mature cured stalks with the ears removed) in the United States could be converted annually to an utilizable form of energy such as ethanol, the United States would not have to import as much foreign oil (2; David Johnston, personal communication). This would have a profound effect on energy supply and utilization, on cleaner air and environment, and on worldwide economics and politics. To achieve this goal and to compete with relatively low-cost petroleum, a more-efficient conversion of corn stover to sugars is required. At this time, a conversion method 10-fold more efficient is necessary to achieve an economically competitive situation with petroleum. In the current volume of the Journal of Bacteriology, two articles provide important insights on the mechanisms of cellulose degradation and its future potential for biotechnological applications (8, 9).

There has been much activity in the field of enzymatic degradation of plant cell wall materials, since plants contain cellulose (the most abundant biological material in the world), hemi-celluloses, pectin, and lignin (3). There has been a significant increase in the study of enzymes and microorganisms that can degrade these plant polymers, since there are several potential ways of degrading large quantities of biomass. One way is to engineer microorganisms to degrade these biopolymers efficiently and still yield the sugars for conversion to ethanol by other microorganisms; another way is to genetically or metabolically engineer microorganisms so that they can both degrade biomasses to sugars and ferment the sugars to alcohol; and finally, one can genetically engineer more-efficient enzymes and produce large quantities of these enzymes to degrade plant biopolymers and convert the resultant sugars to alcohol.

However, to obtain large quantities of efficient enzymes such as cellulases and hemi-cellulases and to maximize their utility, one must understand their structure, function, activity, regulation, in vitro and in vivo manipulation, and biochemical properties. Two general classes of cellulase have been studied. In one class, the cellulases are produced as individual enzymes that can work synergistically to degrade plant biopolymers. In the other class, the enzymes are produced as a complex enzyme called the cellulosome (1, 10), which has the capability of degrading plant cell walls quite efficiently. The cellulosome usually produced by anaerobic microorganisms is comprised of a nonenzymatic scaffolding protein called CbpA (11) or CipA (4) that contains cohesin domains to which is attached a number of enzymatic subunits through their dockerin domains (1, 10).

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Two papers in this issue are concerned with characterizing the basic properties of cellulosomes and with the in vivo construction of minicellulosomes (8, 9). Although the general structure of the cellulosome is known (1, 10), the findings presented in the paper by Rincon et al. (8) demonstrate that there is diversity in the structure and function of scaffolding proteins from various microorganisms and that the cohesins present in scaffolding protein ScaA of *Ruminococcus flavefaciens* show a distinct specificity in binding to the dockerins of some of the cellulosomal proteins but not to others. These findings suggest that more than one scaffolding protein may exist in this microorganism and that different cohesin-dockerin interactions can occur between scaffolding proteins and cellulosomal enzymes within a single organism. This specificity may be exploited for practical purposes, e.g., for constructing "designer" cellulosomes with specific and high activities. These significant observations about the properties and diversity of cellulosomes should lead to further investigations of cellulosomal properties from many other microorganisms and to novel and significant practical uses.

The paper by Sabathe and Soucaille reports the exciting expression in vivo of designer minicellulosomes by *Clostridium acetobutylicum* (9). This organism, whose genome has been completely sequenced (7), is very important for the industrial production of solvents such as butanol and acetone. Ironically, *C. acetobutylicum* produces a cellulosome (>665 kDa) that does not degrade crystalline cellulose, although it contains many of the genes for cellulosome synthesis (F. Sabathe, A. Belaich, and P. Soucaille, submitted for publication). If the cellulosome genes could be activated to produce a cellulose-degrading cellulosome or if cellulosome genes could be transformed into *C. acetobutylicum* and expressed to form an active cellulosome, then the organism might be able not only to degrade cellulose but also to produce solvents as a single-organism system.

Sabathe and Soucaille constructed a mini-CipA gene containing a cellulose-binding domain, four hydrophilic domains, and two cohesins and transformed it into *C. acetobutylicum* (9). After growth of this transformant on cellobiose and crystalline cellulose, they found a cellulose-bound complex (250 kDa) consisting of mini-CipA, Cel48A, and an unidentified third protein. This in vivo-constructed minicellulosome did not degrade Avicel or bacterial cellulose but did have low activity on carboxymethyl-cellulose (CMC) and phosphoric acid-swollen cellulose. Although the absence of activity of this minicellulosome on crystalline cellulose is disappointing, this in vivo minicellulosome construct is an exciting advance on the final goal of converting *C. acetobutylicum* into an efficient organism that can convert biomass to important solvents.

In this regard, recent studies on *Clostridium cellulovorans*

cellulases and minicellulosomes constructed in vitro indicate that the construction of designer cellulosomes with specific functions may also lead to synergistic combinations of cellulases and hemi-cellulases with more-efficient biomass-degrading capabilities (5, 6; A. Kosugi, K. Murashima, and R. H. Doi, unpublished data).

Thus, the current studies on cellulosome function and activity should lead to significant advances for the ultimate goal of converting corn stover and other agricultural biomasses to ethanol, butanol, and other valuable compounds.

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