BOOK REVIEWS

Microbial Servants

The Clostridia and Biotechnology. D. R. WOODS, Ed. Butterworth-Heinemann, Stoneham, MA, 1993. xvi, 443 pp., illus. $129 or £115. Biotechnology Series, 25.

Few microorganisms have as illustrious a history as *Clostridium acetobutylicum*. Strains of this anaerobic, endospore-forming, Gram-positive bacillus showing vigorous fermentation were selected by Chaim Weizmann early in this century for the production of acetone from starchy grains. There was an urgent need for acetone during World War I for the manufacture of cordite (smokeless gunpowder) and as a general solvent. Weizmann was suitably honored for his contribution to the Allied war effort and eventually became the first president of Israel. Between the two world wars the industrial acetone-butanol (AB) fermentation of C. *acetobutylicum* became the alcohol fermentation second in importance only to that carried out by yeast. However, after World War II the chemical synthesis of these solvents from petroleum feedstocks became cheaper and gradually replaced the microbiological process.

After the sudden and steep increase in the price of crude oil in the early 1970s there was renewed interest in the use of C. *acetobutylicum* and related saccharolytic clostridia for the production of alcohol fuels and chemicals. If the process were made cost-effective, such microbiological fermentations would have several advantages, including the utilization of waste and renewable biomass and the recovery of a variety of products, including acids (butyric and acetic), alcohols and solvents, hydrogen, expensive stereospecific specialty chemicals (not discussed in the book under review here), and secreted polymer-degrading enzymes, as well as dried biomass for animal feed additives. However, to be competitive the microbiological process needs relatively cheap fermentation substrates and improved process technology, because the final concentrations of the fermentation products are low and their recovery costly. Yields of the desired products would have to be increased. Of most interest for exploitation currently are the homoacetate fermentation (C. *thermoaceticum*), the butyrate-acetate fermentation (C. *butyricum* and C. *tyrobutyricum*), ethanol production by cellulolytic thermophiles (for example, C. *thermocellum*), and, of course, the AB fermentation (C. *acetobutylicum*). Much of the current basic research on the saccharolytic clostridia is focused on strain improvement through genetic engineering of the cells to enhance the desired catabolic pathways and extend the range of substrates utilized. *The Clostridia and Biotechnology* documents the remarkable progress made in this effort in the past few years.

Biotechnology applications and strategies for improving clostridial strains are thoughtfully discussed by Morris and by Jones and mentioned in many of the other chapters as well, lending practical utility and cohesiveness to the book. Potential obstacles to the development of commercial competitive fermentations, such as the toxicity of many of the products, are also discussed. Just how little is known about regulatory mechanisms for clostridial growth and metabolism is all too clear from the chapters by Rogers and Gottschalk and by Bahl.

The critical advance was the recent development of gene transfer mechanisms for clostridia. Transfer of clostridial genes into *Escherichia coli* for cloning and analysis has been used with great success for several years. As described by Chen and by Papoutsakis and Bennett, the sequences of many of the genes encoding the enzymes that catalyze the terminal steps of the pathways have been determined, as have their regulatory regions. However, the (re)introduction of genes into the clostridial cells has been achieved only recently. Broad–host-range conjugative plasmids of enterococcal or streptococcal origin have been developed for gene transfer into clostridia, as reviewed and clearly explained by Young and by Dürre. The mobilization of nonconjugative plasmids by conjugative plasmids and electroporation are two other procedures that can be used for gene transfer. Conjugative transposons, usually transferred from the *Enterococcus* chromosome to the *Clostridium* chromosome, have proved invaluable for generating mutants in the terminal catabolic pathways and for the physical analysis of clostridial genomes. Minton and co-workers have contributed a richly detailed explanation of the construction of cloning vectors, the most useful of which are *E. coli*–C. *acetobutylicum* shuttle vectors and a variety of vectors for specialized functions. The use of such vectors is still in its infancy, but we now know that cloned copies of C. *acetobutylicum* and C. *thermocellum* genes can be expressed in C. *acetobutylicum*. This opens up the whole field of recombinant DNA technology in the saccharolytic clostridia.

Because of the high cost of agriculturally derived substrates such as molasses and starch, attention is turning toward extending the range of substrates utilized. For example, amylolytic and pectinolytic enzymes are secreted by a number of clostridia, including C. *acetobutylicum*, enabling them to degrade starch, but C. *acetobutylicum* cannot utilize the plant structural polysaccharides cellulose and hemicellulose, which are abundant and renewable biomass feedstocks. Cellulose is highly resistant to enzymatic attack because of its insolubility. Some thermophilic strains with potential for the industrial production of ethanol digest cellulose by means of ordered extracellular enzyme complexes, the cellulosomes. The best-studied system, from C. *thermocellum*, consists of a cellulose-binding glycoprotein that acts as scaffolding to which are attached the endoglucanases that act in concert to provide the soluble cellulodextrins and cellobiose. Cellulases and other polymer-degrading enzymes from a number of related clostridia, the genes encoding them, and their transcriptional regulatory elements are discussed in detail in two chapters, one by Bronnenmeier and Staudenbauer and the other by Hazlewood and Gilbert. On the other hand, as discussed by Maddox *et al.*, the commercial exploitation of whey, available from the dairy industry, must overcome the limitations of low concentrations of a substrate, lactose—a sugar that is not preferred by most clostridia. The potential use of thermophiles for production of acetate, ethanol and other products, and industrial enzymes as well as for waste management is summarized in a final, upbeat chapter by Canganella and Wiegel. This volume is the definitive text in a field poised for a bright future.

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