Opportunities for microbial lipids

The following article on microbial lipid technology was written by Bernard C. Sekula, principal microbial chemist at Best Foods' Research and Engineering Center, a division of CPC International, Union, New Jersey. It was prepared on the request of W. David Nes of the U.S. Department of Agriculture's Richard B. Russell Agricultural Research Center, who serves as Associate Editor for JAOCS *News for Plant Lipids.*

Efforts to bring biotechnology to the fats and oils industry generally focus on the utilization of microorganisms or their component parts, and plant breeding to improve seed-oil yields or to alter their fatty acid composition. Microorganisms, particularly bacteria and yeast, are attractive as experimental systems to both the lipid researcher and biotechnologist due to their relative simplicity and prodigious growth rates, their amenability to genetic transformation, and their ability to use a variety of substrates and synthesize an array of products. These attributes historically have made microorganisms well-suited candidates for studying lipids and their biosynthetic pathways.

Identified microbial lipid products include hydrocarbons, fatty acids and alcohols, partial and triglyceride esters, wax esters, free and esterified sterols and triterpenoids, ether-linked isopentenoids, terpenes, carotenoids, dicarboxylic acids and biosurfactants. Microbial lipid research knowledge and experience serve as a base from which to identify and explore biotechnological opportunities. Such opportunities include the development of economically competitive products, functionally superior products, product applications, new processes and new products. An examination of some of these opportunities will show that they are not without their challenges.

Economically competitive products

Yeast lipid accumulation/production is the most actively investigated area of microbial lipid technology. About 10 genera of yeast are considered to be oleaginous, i.e., capable of accumulating 25% or more of their biomass as lipids. The lipids of these yeasts are generally triglyceride-rich and the fatty acid compositions are not unlike commodity vegetable oils. When used as substrates for oleaginous yeast, carbohydrates exert little effect on the cellular fatty acid composition and their conversion rates rarely exceed 20 g lipid per 100 g substrate. Although technical feasibility has been demonstrated for yeast oil production from carbohydrates, these oils cannot compete economically with commodity vegetable oils. Accordingly, economic feasibility appears to depend on the use of inexpensive or waste materials as substrates, the production of high-value specialty fats and oils, the co-production of oil with another value-added product, and/or the extracellular elaboration of oil as a means of reducing process costs.

The microbiological production of high-value specialty fats and oils has been addressed with some success on two fronts: the identification of naturally occurring products such as gamma-linolenic acid-rich oils, and the induced accumulation of products such as cocoa butter. With respect to the former, two commercialized processes have been reported for the fungal production of gamma-linolenic acid-rich oils. A Japanese process utilizes a *Mortierella* sp., while a United Kingdom process uses *Mucor javanicus*.

One approach to the production of a cocoa butter-like product in yeast uses lipids as substrates. Although less soluble than carbohydrates, lipids offer two advantages as substrates: a higher carbon content on a per-mole basis, and the ability to influence the fatty acid composition of the product formed. Substrates used in these investigations include hydrocarbons, free and derivatized fatty acids, and fatty alcohols. A second approach uses sterculic acid, a naturally occurring inhibitor of fatty acid desaturase. Both approaches yield yeast products with fatty acid compositions similar to cocoa butter, although a need exists for further optimization to reduce linoleic acid levels.

The ability to obtain similar fatty acid compositions is somewhat remarkable considering yeasts generally do not possess stearic acid contents exceeding 10%, whereas cocoa butter has a stearic acid content ranging from 32 to 35%. An unexplored opportunity exists for the development of delta-9 and delta-12 desaturase mutants to restrict the flow of stearic acid to oleic and linoleic acids.

Investigations on the use of inexpensive or waste materials have involved a number of substrates, including hydrolyzed waste cellulose, molasses, whey, animal fat waste, and other processing and waste streams. Consequently, a commercial yeast operation reportedly has been developed in New Zealand for the production of a cocoa butter substitute from whey.

Besides triglyceride-rich fats and oils, microorganisms can produce dicarboxylic acids via the oxidation of hydrocarbons and fatty acids. Dicarboxylic acids are important as starting materials for the synthesis of polymers, plasticizers and lubricants. Since the chemical synthesis of long-chained dicarboxylic acids is difficult, the potential for their production via fermentation is being examined. Mutants of *Candida tropicalis* and *Candida cloacae* have been developed to produce dicarboxylic acids from hydrocarbons in quantities up to 64 g/l.

Functionally superior products

Ergosterol isolated from spent brewer's yeast is used to produce ergocalciferol, an analog of cholecalciferol (vitamin D_3), via ultraviolet light irradiation. Recently 25-azacholesterol and 24(*RS*),25-epiminolanosterol (inhibitors of the sterol methyltransferase) have been used to induce the accumulation of cholesta-5,7,24-trienol in an *erg5* yeast mutant (incapable of desaturating the 22 position of the sterol side chain) and pathogenic fungus *Gibberella fujikuroi*. This compound can be converted through a series of chemical reactions and ultraviolet irradiation to 25-hydroxycholecalciferol, the main circulating form of vitamin D in animals and biologically more active than either cholecalciferol (vitamin D_3) or ergocalciferol (vitamin D_2). Prior to its exploitation, the cellular regulatory mechanisms restricting sterol accumulation must be overcome.

Product applications

Although originally developed as agents for the clean-up of oil spills and chemically enhanced oil recovery, microbial biosurfactants now are being examined for their potential application as emulsifiers in food systems. This interest stems from the fact that these biosurfactants represent "natural," biodegradable products and may offer some functional advantages over currently available emulsifiers.

Microbial biosurfactants vary in structure from simple free fatty acids and esters to glycolipids (e.g., sophorolipids from *Torulopsis bombicola*), lipopolysaccharides (e.g., emulsan from *Acinetobacter* sp.), and lipoproteins (e.g., surfactin in *Bacillus subtilis*). Similarly, poly-beta-hydroxybutyrate, a polymeric storage product of certain bacteria including *Alcaligenes eutrophus*, and related polyester variants are being evaluated as biodegradable plastics and as slow-release encapsulation media.

New processes

The ability of microorganisms to metabolize hormones and related steroids has been used by the pharmaceutical industry to reduce processing steps and costs in the manufacture of therapeutically active steroids. Bacteria and fungi carry out steroid biotransformations via a number of dehydrogenation and hydroxylation reactions. For example, certain *Corynebacterium* species dehydrogenate cortisone to prednisone, whereas *Rhizopus nigricans* hydroxylates progesterone at the 11-alpha-position.

The discovery of biohydrogenation by ruminant microflora has spurred interest in its application as a potential means to reduce the degree of unsaturation in oils without the formation of *trans* isomers. There have been a number of barriers to its exploitation. First, strict anaerobic microorganisms are difficult to isolate and cultivate. Secondly, the known microorganisms utilize free fatty acids, not triacylglycerol esters, as substrates for biohydrogenation. Thirdly, the major fatty acid products of biohydrogenation are vaccenic acid (11-*trans*-C18:1) and stearic acid. No microorganisms have yet been identified that convert polyunsaturated fatty acids to oleic acids.

Along similar lines, a *Eubacterium* sp. is currently being used to develop a means of enzymatically reducing cholesterol levels in foods. Reportedly, the addition of this microorganism to foods such as eggs reduces cholesterol by more than 80% via its conversion to coprostanol (5-beta-cholestanol). Unlike cholesterol, coprostanol is poorly absorbed in humans.

New products

While their cellular functions are not always apparent, structurally novel microbial lipids are still being isolated. For example, the phytophthorols are a series of wax esters recently isolated from the mycelial fungus *Phytophthora cactorum* in which the fatty alcohol components reportedly contain two additional hydroxyl moieties (at carbon position 2 and 3). It is the biotechnologist's challenge to develop potential uses for these lipid products.

Future prospects

Although only a few commercialized processes and products currently exist, future prospects for microbial lipid technology remain optimistic. This positive outlook is based on the diversity of microorganisms, the broad potential applications of genetic engineering, and the interdisciplinary power of biotechnology. Scientific advances in one area of biotechnology can be used to overcome barriers in other areas or to open up totally new areas for exploration.

This point is exemplified by the discovery by Klibanov and Zaks that, in the presence of virtually anhydrous solvents, lipases not only retain enzymatic activity but catalyze transesterification reactions. Improved thermostability and altered substrate specificity also are exhibited. The ability to retain enzymatic activity in anhydrous solvents has led to the development of a new area of chemistry—enzyme-catalyzed organic synthesis. In addition to trans- and interesterifications, lipases can be used in ester synthesis, biosurfactant production, peptide synthesis and the resolution of racemic mixtures of compounds.

With respect to microbial systems, CPC International recently filed a patent application on the production of wax esters by yeast, which are not usual components of yeast lipids. Thus, opportunities in microbial lipid biotechnology are not limited by what nature seems to dictate, but rather by the skill and the imagination of the researchers. The greatest challenge facing the microbial lipid technologist is to identify economically feasible ways of applying this technology in the food industry.