Application of Biotechnology to the Perfumery Industry

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The importance of biotechnology has become more evident as we enter the 1980s. Some earlier studies were carried out on the formation of perfumery related compounds using microorganisms, enzymes and tissue cultures as well. However, the above studies have not always provided satisfactory results, since there have been so many fermented foods and dairy foods developed from the utilization of microorganisms. Studies of the flavors of these foods have since resulted in products now employed as flavor materials.

With further progress in the near future in biotechnology, deeper research and the consequent provision of better flavoring materials seems probable. Furthermore, the culturing of plants used for perfumery are seen likely to make tremendous advances through application of biotechnology to agriculture.

Here, I wish to suggest the future contributions of biotechnology to the perfumery industry by introducing the studies done in the past and also some newer techniques, such as recombinant DNA, cell fusion, bioreactor and tissue culturing.

Formation of Aroma by Microorganisms

Up to this time, we have noticed examples of aroma formation by microorganisms, e.g., marronlike aroma by koji mold, fruitlike aroma by yeast.

There have been already some detailed reports on the formation of terpenes, lactones and esters from a carbohydrate such as glucose by microorganisms.[1]

In 1930, Dorx et al. reported the isolation of a yeast which yielded a peachlike aroma,[2] after which, Tahara et al. examined aroma components from same yeast and found that various alcohols, aldehydes and lactones were formed.[2,3]

Lanza et al., on the other hand, conducted studies concerning aroma from the mold *Ceratocystis moniliformis* and reported that same mold produced many kinds of fruitlike aromas by using certain combinations of carbon sources and nitrogen sources in its medium.[4] Subsequently, they confirmed the formation of terpenes such as geraniol and citronellol by analyzing these aroma components. They also studied the metabolic pathway of the mold concerning these components and reported that they were formed via a MVA pathway similar to that in plants.[5]

There are also many reports on the aroma produced by *Ceratocystis viriospora*. Over fifty components including monoterpenes have been reported to be produced from this mold.[1]

Though many studies have thus appeared, including the three mentioned above, there remained the problem of lack of strength of the aromas developed for use as perfumery materials. As a practical matter, commercial manufacturing has thus not been carried out.

Recently, Schindler conducted studies on the formation and accumulation of monoterpenes through the use of *Ceratocystis variospora*, leading him to presume that metabolites such as

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monoterpenes inhibit the growth of microorganisms.[6] As a result, he found he was able to increase the amount of geraniol synthesized by *Ceratocystis variospora* by about ten times by adding a lipophilic absorbent to the growing culture.

The production of essential oils or specific aroma materials from carbohydrates by microorganisms will be studied more actively by introducing the new methods resulting from developments in biotechnology.

On the other hand, microbial specific conversions, such as oxidation, reduction and others by microbial enzymes have been utilized in the field of steroid chemistry, and have been industrialized for the manufacturing of steroids. In perfumery-related fields, similar attempts to produce synthetic perfumery materials or their intermediates have already been started.

There are many examples such as the conversion of terpenes,[1] optical division of dl-menthol,[7] and the formation of ω-hydroxy fatty acid[8] or dicarboxylic acid[9] which are useful as raw materials for synthetic musk, the conversion of patchoulol[10] and others. Recently, as a newer attempt, the conversion of a synthetic aroma material to aroma compounds by the utilization of microorganisms was reported. For example, Mikami[11] reported that a mixture of aroma components, useful as tobacco flavor, was produced by oxidation of β-ionone to various derivatives by *Aspergillus niger*. Furthermore, there is another patent[12] on the formation of aroma bases having the flavor of raspberry, apricot and tobacco from α-ionone, β-ionone, α-iron and β-iron by microorganisms, *Botrysphaeria* species.

The application of new biotechnology, such as the recombinant DNA and immobilizing techniques, is considered useful for these studies. Increased production of conversion enzyme systems within microorganisms, or the creation of mutant blocked conversion pathways will be obtained more easily than by previous methods because this recombinant DNA technique will make possible the creating of more stable enzymes. Formerly, the studies were done mainly by isolating microorganisms from nature which has the enzyme systems sought. In the future, we shall be able to use the technique which converts natural enzyme systems into the desired systems artificially.

The technique of reusing and improving the stability of an enzyme by immobilized enzymes or microorganisms will be applied in the perfumery industry. Enzyme reactions on aroma substances in water systems are not always efficient from the viewpoint of solubility of such substances. There are some reports of efficient conversion with water-insoluble substances in water-organic solvent systems, using immobilized enzymes or microorganisms. Recently, Fukui et al. reviewed this technique in detail.[13] And, in the perfumery area, some studies using this technique, such as the optical division of dl-menthol[14] and the formation of tobacco flavorants,[15] are reported.

Reports on bioconversion by microbial researchers have been predominant. But, recently, there have been some reports on this subject also by organic chemical researchers.[16] Therefore, bioconversion to perfumery materials will be studied also more actively in the near future.

When will perfumery materials resulting from application of biotechnology be prepared commercially? According to the Technology Assessment Reports "Impact of Applied Genetics" compiled by the office of Technology Assessment in America, the production of perfumery related substances such as adipic acid and propionic acid is predicted to be commercialized ten years hence, and production by fermentative methods of citronellal, citronellol, geraniol, linallol, linallyl-acetate, nerol α-terpinol, α-terpinyl acetate, cinnamaldehyde and others are seen to be twenty years in the future. Sumida has reviewed this report in detail.[17]

Tissue Culturing in the Perfumery Industry

It is interesting from not only the technological but also the commercial viewpoint that the production of natural perfumery materials by utilizing living things may be carried out by tissue culturing techniques. Because of problems that our population is increasing and that cultivating areas will be further limited in the near future, tissue culturing production not influenced by weather conditions is predicted to come to the forefront.

The formation of essential oil components in perfumery plants by tissue culturing has been studied for a long time. The second metabolite in the culture however is difficult to produce. It is also difficult to develop the same component as the mother plant, because the essential oil obtained from it consists of specific complex balances of components of many kinds. In fact, there are many reported studies on tissue culturing of perfumery plants, mostly unfortunately without success in producing essential oils. Of these studies, I wish to introduce some that report of the formation of essential oils by tissue culturing.

Reinhard et al. reported the formation of essential oils and the effect of light on the formation of *Ruta graveolens*.[18] Sugisawa et al. extracted essential oils from the tissue cultures of the *Perilla* plant and found limonene, α-pinene, linallol and others in this oil.[19] Tomoda et al. studied the cullus aroma by culturing the tissue of a perfumery plant and reported that the aroma produced thereby differed organoleptically to the aroma as provided by the mother plant, despite his finding that the components in the mother plant were the same as those in the tissue cultured product.[20]

It is difficult for tissue cultures to produce a second metabolite at present. But there is also some new knowledge indicating that the desired materials may be accumulated by off-differentiating of tissue cultures under various differing conditions. It is necessary for the further development of this technique to learn how to bring about the second metabolic activity in tissue culture. The production of aroma components with tissue culture techniques will greatly proceed through ascertaining this basic mechanism.

Some attempts on the conversion into projected materials' formation or accumulation are made by adding their precursors in tissue culture media. Uchida et al. reviewed these studies.[21] In the perfumery area, Suga et al. and Itokawa et al. reported on the conversion of terpenes.[22,23] And also, breeding with tissue culturing has been developed practically. Recently, cell fusion technique, protoplast formation technique and the technique on reconstructing plants from protoplasts have progressed. Breeding techniques by combining tissue culture have been spreading in application. Studies on such techniques on perfumery plants have not however as yet been reported. But it is anticipated that studies on hybrids for producing greater quantities and new types of essential oils and for improving strains for adaptation to many cultivation areas will be done in the future.

Flavors from Application of Microorganisms

Flavors are related with not only the aroma of foods, but also with the taste. They are naturally produced from living things. Most of the bioconversions done by animals and plants are conducted by enzyme reactions within their cells. So most of the natural flavor components are formed by enzyme reactions in whatever form they be. If these mechanisms for such bioconversions are clarified, many flavors may be produced through the application of such enzyme techniques. Such enzymes would be extracted from plants and animals, but it is desirable that they are produced by microorganisms and developments in biotechnology for economic reasons.

Many foods are produced mainly by microorganisms. The foods, sake, miso, soya sauces and others, have been traditional fermented foods of Japan and are considered as flavor materials as well. Since they are too weak in intensity to be commercialized as flavor materials, cheaper products with strong flavors have been sought out and studied. Fermented food flavors originating from the components of the raw materials, metabolites by microorganisms and chemical reaction are considered to be produced by complex mutual interreacting of these resultant components. Many fermented foods are produced by the cooperation of various kinds of microorganisms, there being, of course, a few produced by a single microorganisms. The flavor is formed by such complicated processes.

On the other hand, analysis for unknown natural flavor components has been progressing. If the formation mechanisms of such flavor components are clarified, fermented food flavors made by microorganisms would be commercialized in the future more actively.

Another example of use of microorganisms is dairy foods. The flavor components of dairy foods are also formed by the actions and reactions of various kinds of microorganisms, enzymes and chemicals. Since the components degraded from milk lipids have an important role as flavor, use of milk lipids degradates made by enzymes as flavor materials has been studied. First, the products by application of esterase which originated in animals were developed. After that, similar products by application of microorganism enzymes were made.

Recently, various kinds of natural cheeses have become common in Japan. Some cheese products are ripened by fungi, which is useful in the formation of characteristic flavors. A represenative example is blue cheese ripened by *Penicillium roqueforti*, having highly contributive flavor components such as methyl ketone derived from fatty acids. Methods for producing a strong blue cheese flavor in a short time from milk fat by application of such fungi and lipase has been therefore researched.

Lactic acid bacteria also play an important part in producing dairy products such as cheese, fermented butter, yoghurt, lactic acid drink and others. As the Japanese diet customs become westernized, various kinds of processed foods having dairy flavors have been actively developed, requiring the development of many kinds of the flavors. The lactic acid bacteria which play a part in producing such foods are also not a single microorganism in most cases. These dairy foods are produced by a mixed action of two kinds of lactic acid bacteria, one producing lactic acid mainly, and the other producing mainly aroma materials.[24] The characteristic flavors of these foods are formed through such actions. In fact, the development of dairy flavor materials has been done through studies on the selection of lactic acid bacteria, raw materials, culture conditions and application of enzymes, with the resulting products being applied to margarines, confections, drinks and others.

Development will be accelerated by the introduction into this field of new biotechnologies such as recombinant DNA technique, strain improvement by cell fusion technique and immobilizing technique in enzyme reaction.

In other cases the flavor substrate exists as a flavor precursor which has no aroma because of being combined at the time with other components in the plant cells. For example, when the precursors in onion and garlic are degraded with the enzyme being liberated by the crushing of the tissues, the characteristic aroma appears for the first time. It is well known that many flavor precursors like these exist in plant materials. Flavor components generally are unstable because they are easily degraded and lost by heating. However, a precursor on the other hand is relatively stable to heat. In order therefore to obtain a fresh appearing aroma, it would be advantageous to cause enzymic reactions on the precursor existing in the food immediately before processing. Such enzymes may be extracted from plant materials, but this may cause economic and quantitative problems. Production of these kinds of enzymes has been attempted by using microorganisms isolated from nature.

Plant enzymes will be produced with the microorganisms prepared by the application of the recombinant DNA technique.

Conclusion

A description has been made of the present status of application of biotechnology to perfumery related areas. Although a future dream at present, great practical possibilities exist that essential oils will be produced industrially by microorganisms into which is introduced genetic information in perfumery plants.

In general, perfumery products used are numerous in variety, but the quantities involved are small. New technologies such as the recombinant DNA technique will probably not be applied in the perfumery industry sooner than in other fields, but through a continuation of research and accumulation of much basic information in biotechnologies, successful application in the pharmaceutical and food industries of such techniques is seen as highly probable.

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