EnzymeWave

Volume 3

Natto - Traditional Japanese Fermented Soy Beans with Recently Discovered Health Benefits and Novel Industrial Applications

Enzymes for Chiral Resolution and Synthesis

Modification of Beer Character Using Transglucosidase

JBA: Japan Bioindustry Association

AMANO

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From the Editor,

Enzymes have unique features that make them ideal for selecting optical isomers.

Compounds that have identical physical and chemical properties but differ in configuration- mirror images, like our right hand and left hand-are optical isomers and exist in two forms, D-isomer and L-isomer. Chemical synthesis yields equal amounts of each isomer (called a racemic mixture) because chemical synthesis is not capable of distinguishing between two optical isomers. Enzymes, because of their intricate structure, are capable of distinguishing optical isomers; because of this the chemical reactions that occur in the human body catalyzed by enzymes are capable of distinguishing optical isomers-indeed, it is not uncommon that one optical isomer of a drug is beneficial to the human body, while the other optical isomer is inactive or even possibly harmful. The manufacture of pharmaceutical drugs and agricultural chemicals must now be carried out by methods that are selective for optical purity; and enzymes, because of their ability to distinguish between optical isomers have become a very important tool in the production of modern drugs and optically pure chemicals.

Recent research by Dr. Ryoji Noyori, Nagoya University, has been instrumental in developing artificial catalysts that mimic enzymes in their ability to distinguish optical isomers. Dr. Noyori was awarded the 2001 Nobel Prize for this ground breaking work and is the tenth Japanese to be awarded a Nobel Prize in science. Amano Enzyme Inc. has great respect for his accomplishments and takes great pride in the fact that Dr. Noyori's research and enzyme research at Amano Enzyme Inc., with headquarters in Nagoya, is being carried out in the same Aichi prefecture in Japan.

The understanding of the catalytic function of enzymes has unlimited potential in the industrial applications of not only enzymes but of future novel enzyme mimic catalysts useful for the large scale production of optically pure substances as a result of Dr. Noyori's research.

Natto - Traditional Japanese Fermented Soy Beans with Recently Discovered Health Benefits and Novel Industrial Applications

An early form of natto was first produced in China over one thousand years ago by soaking steamed soy grain containing *Aspergillus oryzae*; however, this prototype natto did not have the sticky like texture of traditional Japanese natto. According to legend the first person to develop traditional Japanese natto was the famous warrior Yoshiie Minamoto during the Heian era of Japanese history (794-1192). As in western feudal warfare, the horse was extremely important to the Japanese samurai warrior of the period. Great care was taken to provide suitable provisions for horses when armies were on the move. Usually boiled soy beans were cooled down, dried in the sun and packed in straw bags for transport with the army; if the army had to move quickly, the boiled soy beans were packed immediately into straw bags without cooling and drying. If the straw happened to contain certain microorganisms the soy beans would undergo fermentation and produce the characteristic sticky texture now associated with natto. This was thought to mean that the soy beans were spoiled until Yoshiie Minamoto found that the fermented soy beans were not only edible but had a distinct Umami flavor. Minamoto was then responsible for introducing natto to the northwestern section of Japan where he ruled. To this day natto is especially popular in that region of Japan.

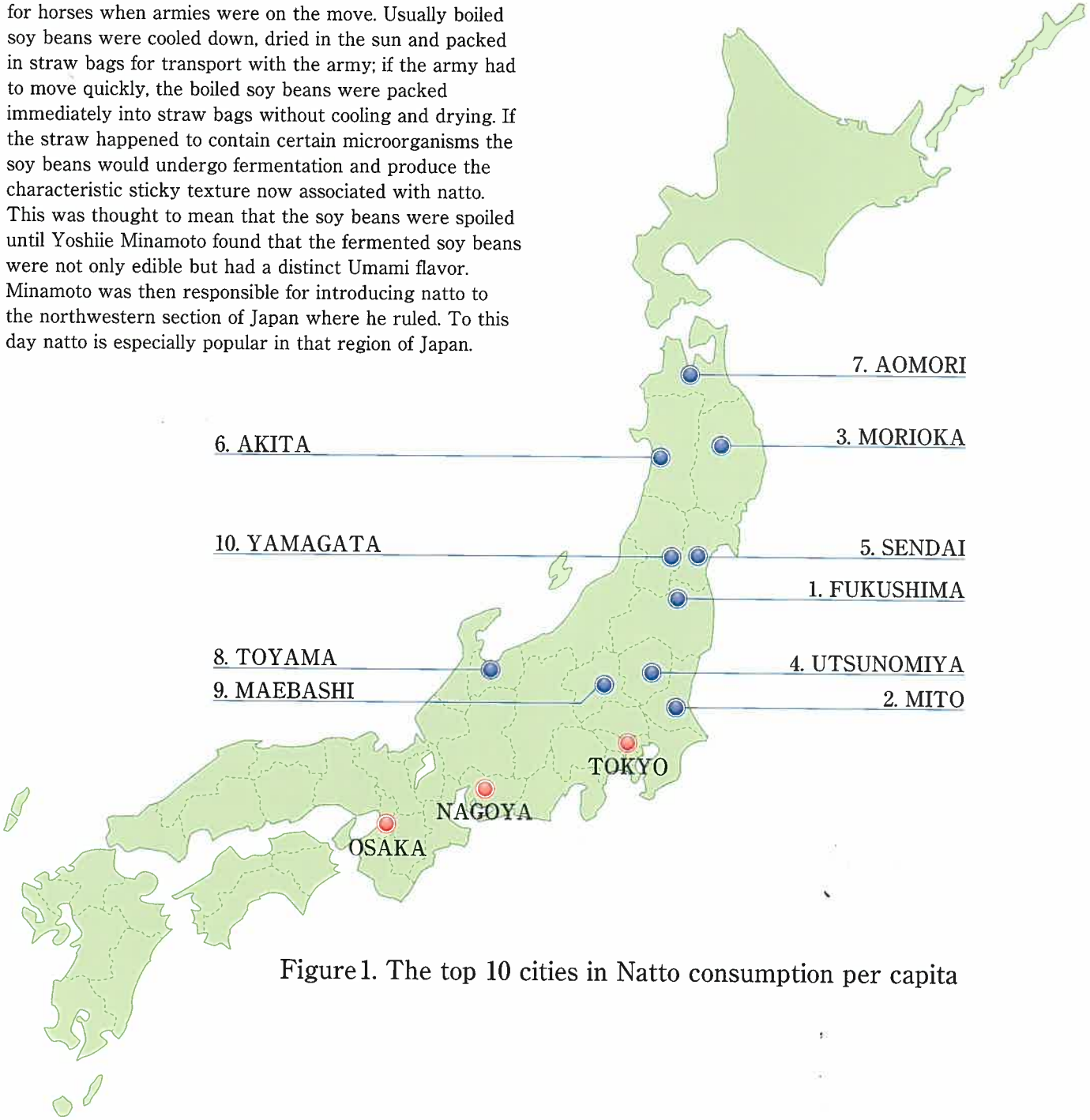
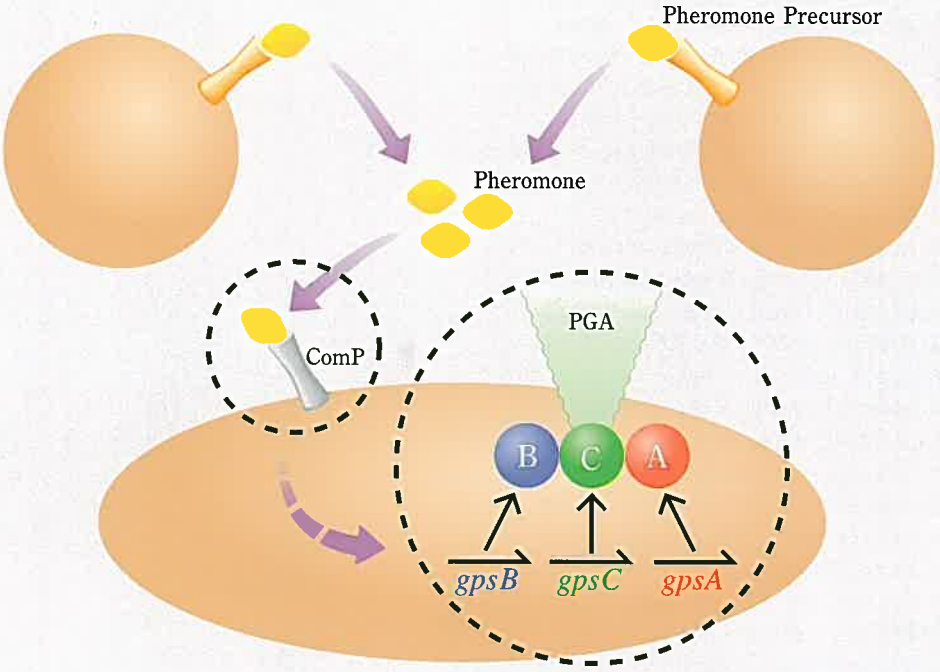


Figure 1. The top 10 cities in Natto consumption per capita

Figure2. Stimulation of PGA production by Pheromone



It has been known for some time that natto has significant nutritional benefits. Natto is very rich in protein and amino acids; especially glutamic acid, an essential amino acid, which is present in rice at very low levels. Therefore the traditional Japanese breakfast of rice and natto is a very good combination. Natto also contains ten times the amount of vitamin B2 as soy beans because the fermentation process synthesizes vitamin B2. In addition, natto is rich in vitamin B1, B6, nicotinic acid, calcium phosphate and potassium. Despite increasing western influence on the Japanese diet, a number of diseases prevalent in the West, including coronary heart disease, breast cancer and prostate cancer remain much less prevalent in Japan. Recently, the presence of isoflavone and angiotensin I converting enzyme (ACE) inhibitor have been found in natto. These compounds are felt to play an important role in protection against these diseases and natto and soy products are thought to be excellent sources of these compounds. Natto-kinase present in natto was first described in 1987 and was shown to decrease blood clots in blood vessels of dogs. The results of human clinical studies indicate that natto-kinase may help increase thrombolytic activity and guard against myocardial infarction and senile dementia.

The sticky like texture of natto is a very important characteristic and has recently been the focus of molecular analysis. The material responsible for the sticky texture of natto is polyglutamic acid (PGA), a polymer containing both D-glutamic acid and L-glutamic acid. The production of PGA is highly sensitive to fermentation conditions and the ability of *Bacillus subtilis* to produce PGA is often unstable. It is now understood that two types of enzymes, glutamate racemase and glutamyltransferase, and three genes, *gpsA*, *gpsB* and *gpsC* are responsible for the synthesis, regulation and secretion of PGA. Glutamate racemase is responsible for the conversion of L-glutamic acid to D-glutamic acid; since *Bacillus subtilis* lacking glutamate racemase has a reduced level of PGA, it appears that both D- and L- glutamic acid are required for the sticky like texture of natto. Glutamyltransferase is the enzyme responsible for the polymerization of both D- and L- glutamic acid. The gene products of *gpsA*, *gpsB* and *gpsC* area thought to form a protein cluster on the surface of *Bacillus subtilis* cell membranes and to be involved in the regulation or secretion of PGA.

PGA synthesis during natto production is observed after 16 h of fermentation (40℃) during the stationary growth phase. The growth phase regulation of PGA synthesis is controlled by a 10- amino acid peptide pheromone containing modified tryptophan residues that is processed from the C-terminus of a precursor protein (comX) located in the *Bacillus subtilis* membrane. The secreted pheromone accumulates in the growth medium and reaches a concentration during stationary growth phase that is sufficient to activate the regulatory system. The genes *gpsA*, *gpsB* and *gpsC* are regulated by the pheromone activated signal pathway (Fig. 2) One explanation for the instability of PGA production by *Bacillus subtilis* is thought to be the inactivation of the pheromone receptor gene (*comP*) by insertion of a small IS element; the *comP* gene appears to be a hot spot for IS element insertion.

A number of very interesting industrial applications have emerged utilizing PGA. Irradiation of PGA with gamma rays cross-links PGA molecules producing a resin that can very efficiently trap water by forming a transparent hydrogel. Under optimal irradiation conditions the resulting PGA resin can hold 5000 times its own weight in water. This is five times the absorption capacity of commercial diapers and sanitary napkins. Because of its high capacity to hold water, PGA resin is being evaluated as an aid in reclaiming desert areas. Currently desert regions constitute 16% of the earth's land area and growing at a rate of 60,000 km^2 per year. Technology that will regenerate agricultural land from desert will play a very important part in improving mankind's future. PGA resin has also been used to clean waste water. The addition of PGA resin to waste water coagulates solid waste that can easily be removed as flock; indeed, PGA resin was recently used to clean water in the moat of Osaka castle. Another interesting application is the addition of PGA resin to concrete which lowers the density of concrete without a loss in strength.

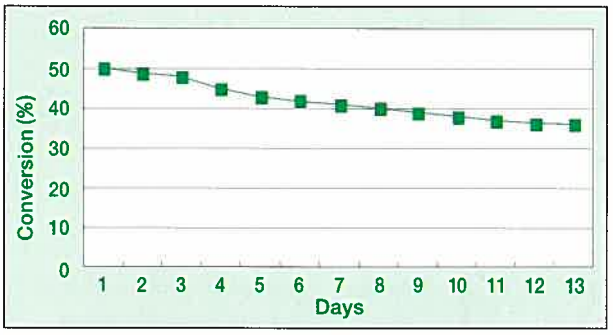
It is truly astounding that such varied industrial applications and the identification of compounds with such significant health benefits can come from basic research of a traditional Japanese fermentation food.

During the last 15 years enzymes have become important tools for the chiral resolution of racemic mixtures and also as reagents for the synthesis of chiral compounds. This is especially true when the compound of interest has multiple chiral centers - enzymes often have the required specificity while chemical methods do not. The importance of chiral resolution and synthesis to the pharmaceutical industry has dramatically increased with the realization that the chiral isomers of drugs often have profoundly different effects *in vivo*. It is now recommended by the government agencies regulating the pharmaceutical industry in the U.S., Japan and Europe that the chiral isomers of all drugs be tested for side effects and if necessary the drug must be prepared optically pure by utilizing chiral resolution or chiral synthesis methods.

An interesting example of enzymatic chiral resolution is the use of lipase AH from *Burkholderia cepacia* to give either optical isomer of a carboxylic acid by a change in solvent; if isopropyl ether is used as solvent the (*S*) isomer is prepared, while if cyclohexane is used as solvent the (*R*) isomer is produced (Fig. 1).

Another interesting method used to select which optical isomer is recovered is to change by genetic engineering the structure of the enzyme used for the optical resolution. Through intensive research into the protein structure of lipase PS from *Burkholderia cepacia* it was found that 3 amino acids are critical to the chiral selectivity of the enzyme. By engineering mutants at these three sites, a mutant lipase PS was found that had the opposite chiral specificity compared to the native enzyme (Fig. 2).

Figure 3.



An additional advantage of using enzymes for chiral resolution or synthesis is that an immobilized enzyme can be reused numerous times and does not produce waste products that are harmful to the environment. For instance, immobilized lipase PS is very stable and robust under actual industrial conditions - 3,150g of optically pure product was produced over a period of more than 600 hours with just 1 ml of immobilized enzyme (Fig. 3).

We at Amano Enzyme Inc. are very excited about the role enzymes are playing in the development of new drugs and we are dedicated to the discovery of new and useful enzymes that will provide the drugs of the future to help all mankind.

Figure 1. Complete inversion of enantioselectivity by solvent effect

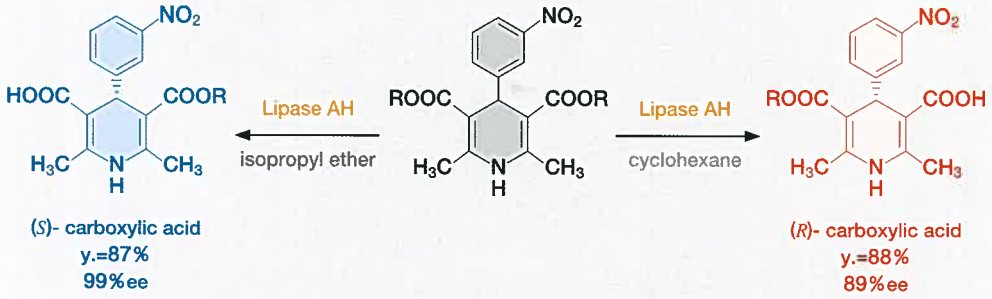
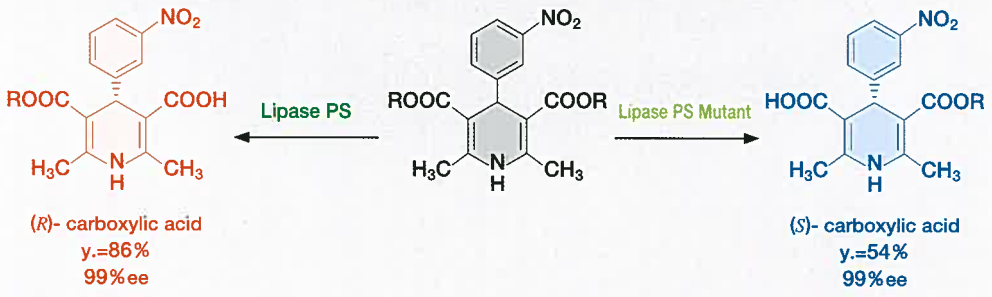


Figure 2. Complete inversion of enantioselectivity by site-directed mutagenesis



Modification of Beer Character Using Transglucosidase

Transglucosidase (EC3.2.1.20, α-D-glucoside glucohydrolase, or commonly α-glucosidase) is an exo- type carbohydrolase found widely in nature. Fungal transglucosidase catalyzes two reactions: a transglucosylation reaction that forms panose and isomaltooligosaccharide with α-1,6 glucosidic linkage and a hydrolysis reaction with maltooligosaccharides containing specifically α-1,4 glycosidic linkages, panos and isomaltooligosaccharide as substrate and producing glucose. Both reactions can be utilized in brewing and produce beer with different characteristics.

EXAMPLE 1

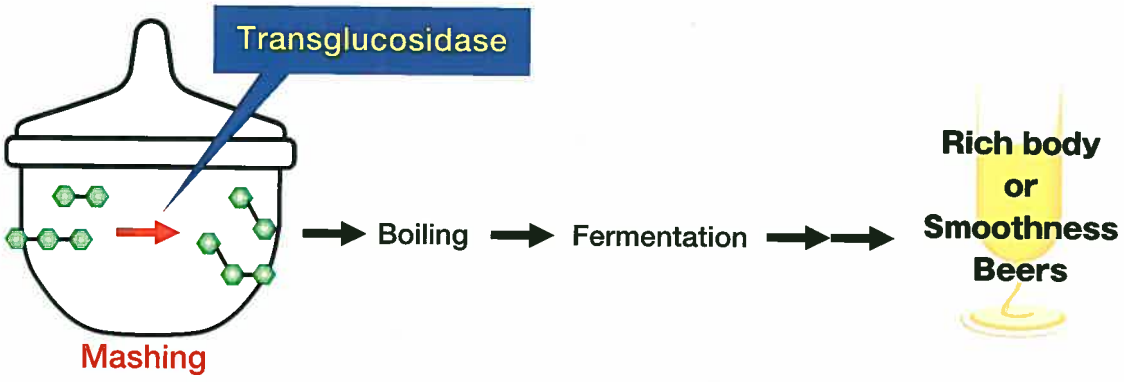
Transglucosidase when added to normal brewery wort produces predominantly isomaltose, panose and small amounts of isomaltotriose. The isomaltooligosaccharides produced cannot undergo fermentation by brewer's yeast and the resulting reduction in fermentation decreases the level of alcohol produced; the body, smoothness and general taste perception of the beer is also altered by the presence of the unfermented isomaltooligosaccharides. Alternatively, adding transglucosidase to the mash conversion vessel would have the same effect but have the advantage that the enzyme would be heat inactivated during wort boiling.

EXAMPLE 2

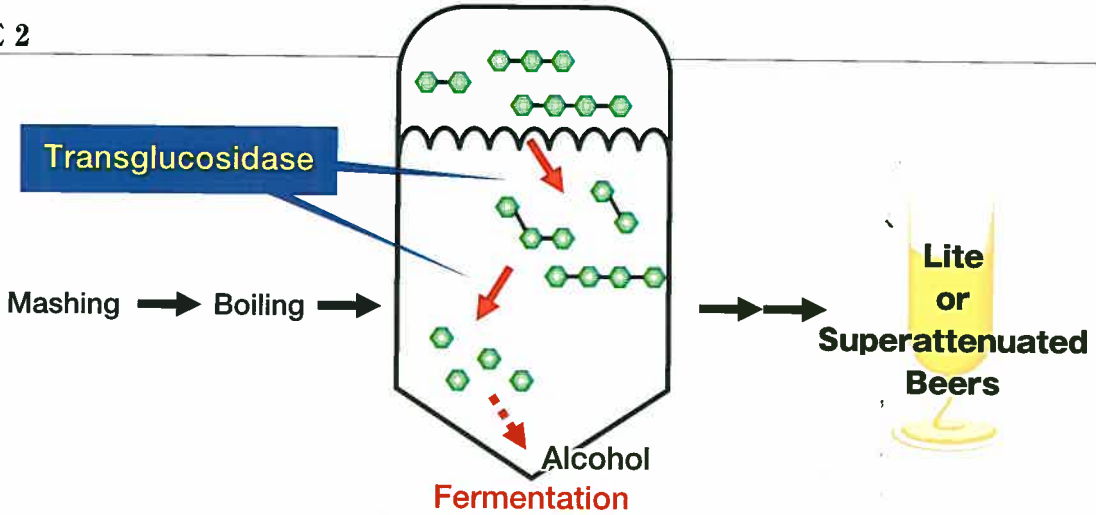
Transglucosidase can also be added during fermentation, but the results are very different from Example 1. Under the conditions of fermentation the hydrolysis reaction of transglucosidase predominates and oligosaccharides are broken down resulting in increased attenuation and alcohol production. For this reason transglucosidase can be used to produce "Lite" (low carbohydrate) or superattenuated beers. The addition of transglucosidase during fermentation has also been used to reduce the production of acetic acid during very high gravity brewing.

Amano Enzyme Inc. currently has food grade fungal transglucosidase available in bulk quantity.

EXAMPLE 1



EXAMPLE 2



JBA: Japan Bioindustry Association

The Japan Bioindustry Association (JBA) is a non-profit organization dedicated to the promotion of bioscience, biotechnology and bioindustry in Japan and throughout the world. The JBA was established and is supported by cooperation among industry, academia and government. The JBA provides a platform for communication between scientists, technologists, policymakers and managers. Amano Enzyme Inc. is proud to be a member.

JBA Activities

Science and Technology

- Promote science and technology communication
- Conduct scientific seminars: Fermentation and Metabolism, Bioengineering, Bioconversion of New Resources, and Alcohol Biomass
- Protection of intellectual property rights
- Exploration of new R&D project themes
- International Standardization
- Facilitate contract research projects with the government

JBA Research Institute

Survey and conduct research on conservation and sustainable use of biological resources and biodiversity issues.

International Exchanges and Cooperation

- Promote international communication on issues related to bioscience, biotechnology and bioindustry
- Provide training courses on bioindustry topics for third world participants.
- Cooperation with the OECD on biotechnology issues

Public Science Education

- Educate the public on basic bioscience and bioindustry issues by promoting courses of general interest
- Human resource development

Safety and the Environment

Investigation of environmental safety issues

Collection and Dissemination of Information

- Collection of relevant information from domestic and international sources and dissemination to JBA members
- Publication of a monthly journal, "Bioscience & Industry" and a quarterly newsletter, "Japan Bioindustry Letters" (in English)
- Lectures and seminars to promote communication

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