Enzyme Wave

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"Karakuri Ningyo (Mechanical Puppet or Automaton)"

"Karakuri ningyo" is a traditional Japanese automaton (mechanical puppet) that was first made around the seventeenth century. There are various kinds of karakuri ningyo, ranging from puppets controlled by strings to mechanical ones worked by mainsprings, springs and gears. They are often seen on the festival floats of the Hida region and Aichi Prefecture. The clever device and performance of karakuri ningyo adds a splendor to the festival and provides entertainment to its audience. Karakuri ningyo is an irreplaceable legacy from our ancestors that imparts the sophisticated style and culture established during the Edo period in Japan.

Report／New Products Introduction Thermostable Microbial β-Amylase: The First Successful Industrial-scale Production in the World

Topics／Biodiversity: COP10 and Corporate Activity

Topics／Established Amano Enzyme Manufacturing (China) Ltd. in Suqian City, Jiangsu Province

AMANO

New Products Introduction Thermostable Microbial β-Amylase: The First Successful Industrial-scale Production in the World

β-Amylase is used in the production of maltose syrup and preventing retrogradation of rice cake confectionery. However, until now the supply source for β-amylase has been limited to edible plants such as barley, wheat and soybean. Considering the current worldwide food crisis, it is the responsibility of enzyme producers to explore alternative sources for a stable and constant enzyme supply going forward. It was not long ago that the trend toward bioethanol as a petroleum fuel alternative caused a steep rise in grain prices and made it more difficult to obtain food grain.

Microbial β-amylase was first described in the 1970s and despite many subsequent studies performed, proved difficult to produce at a large scale. For this reason, Amano Enzyme Inc. initiated a new screening procedure to find a more thermostable β-amylase compared to the enzyme from barley or wheat, and recently succeeded in its commercialization efforts through a joint development project with Daiwa Kasei K.K., one of the Amano Enzyme Group companies.

History of β-amylase and the status of industrial production

β-Amylase was initially found among amylases in malt as an enzyme producing β-maltose and was named accordingly. Detailed studies started when a crystalline enzyme was prepared from sweet potatoes in 1946. More recently, the enzyme was found in cereal crops such as barley and wheat and leguminous crops such as soybean, and is now known as an enzyme widely distributed in higher plants. Studies with these plant enzymes have elucidated their functional mode and activation mechanisms: for instance, the X-ray crystal structure for the enzyme derived from soybeans was elucidated in 1993.

Plant-derived β-amylase has been the source of choice for industrial production because the enzyme is abundant in plant seeds, which allows for relatively low-cost production. The barley-derived enzyme produced in Europe presently accounts for the majority of the global market. In Japan, the production of a soybean-derived enzyme began in the 1960s; however, because of a change in the extraction process for soybean oil, the supply of the enzyme has been uncertain. The production and sales of a competing wheat-derived enzyme started in the late 1980s.

Despite the fact that microorganisms were not believed to contain β-amylase, a microbial β-amylase was found in Japan in 1974. Since then, a number of microbial enzymes have been discovered. Nevertheless, the industrial production of β-amylase from microorganisms has not been successful until the present time because of the insufficient thermostability of the enzyme, poor productivity on an industrial level, and the unsuitability of the microorganism as a production strain for food enzymes.

Industrial applications of β-amylase

Enzymes are used in a broad range of industrial applications and the starch processing industry in particular is one of the industries most actively using enzymes.

The enzymes used for starch processing are mainly bacterial α-amylase (such as KLEISTASE L), used as a starch liquefaction enzyme, fungal glucoamylase (such Gluczyme), used as a saccharification enzyme, and bacterial pullulanase (such as Pullulanase "Amano"), used as a debranching enzyme. Among saccharification enzymes, glucoamylase is used in the production of glucose and isomerized sugar, whereas β-amylase is used in the production of maltose syrup. Although α-amylase from *Aspergillus oryzae* (which accumulates relatively high amounts of maltose) is also used for the same purpose, its application is limited because it also produces a significant amount of glucose. Maltose is used as a sweetener in candy, confectionery, "tsukudani" (food boiled in soy sauce), ice cream and other food because maltose, compared to glucose, has a full-bodied taste, has a lower Maillard reaction rate, and is resistant to crystallization. Since maltose has a refined and mild type of sweetness and a low level of coloring, it is particularly indispensable as a sweetener for Japanese confectionery.

β-amylase can also be used to inhibit the retrogradation of starch. It is believed that β-amylase shortens the α-1,4-linkage in the straight chain starch molecule utilizing its exo-type activity and thereby reduces the intermolecular association of the straight-chain portion of amylose, which is known as the main cause for starch retrogradation. In addition, the moisturizing effect of maltose produced by the enzyme is also thought to contribute to the softness of starch-containing foods. Thus, β-amylase is mainly used in Japan for preventing the retrogradation of rice cake. Also in baking, the β-amylase naturally present in wheat flour is considered to be effective in the inhibition of retrogradation; although the barley-derived enzyme may be used on some occasions, its use is limited because of the issue of thermostability.

Discovery of microbial β-amylase and elucidation of its features

In order to develop a stable supply of β-amylase for industrial applications, Amano Enzyme undertook to screen for β-amylase activity in microorganisms; as a result we found a thermostable β-amylase produced by a strain belonging to the genus *Bacillus.* The purification of the enzyme and the cloning of the gene revealed that the enzyme is produced and secreted as a monomer comprising 515 amino acids with a molecular weight of 57.6 kDa. The primary structure demonstrated 43%–80% homology to the previously reported β-amylases derived from the genus *Bacillus.*

The optimum reaction temperature and thermostability of this enzyme was found to be 10° C higher compared to the barley- or wheat-derived enzymes, which account for the majority of β-amylase industrial use in the world, and equivalent to the thermostable soybean-derived enzyme.

■Application of the microbial β-amylase

1) Production of maltose syrup

Figure 1 and Table 1 show the results of experiments producing maltose from liquefied starch. The microbial enzyme was demonstrated to be superior to the barley- or wheat-derived enzymes, which are currently used in the production of maltose syrup. Not only is more maltose produced using a smaller amount of enzyme, but also there is less concern about possible microbial contamination during the reaction because of the higher reaction temperature. Moreover, this enzyme has a neutral optimum pH (around pH 6.0), which is more suitable for the starch liquefaction process that takes place prior to maltose saccharification by β-amylase; in contrast, the barley- or wheat-derived enzymes have more acidic optimum reaction pHs. Glucose formation was very low in the sugar composition of the produced syrup, which is desirable for maltose syrup products.

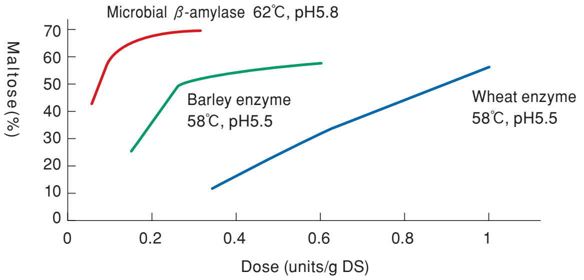


Figure 1. Maltose production from liquefied strach

Table 1. Sugar composition of maltose syrup

Reaction time : 42hr

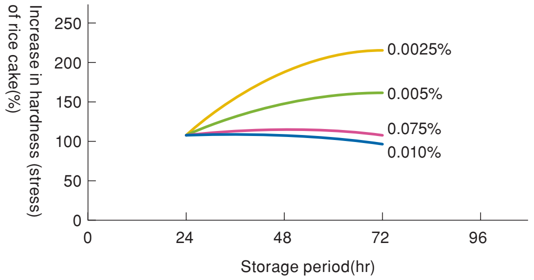
origin	Temperature	pH	Dose (units/g-DS)	Sugar composition (%)				
				G1	G2	G3	G4	G5≦
Microbial	62℃	5.8	0.3	0.2	59.1	7.2	0.7	32.8
Barley	58℃	5.5	0.7	0.2	56.1	7.2	0.8	35.9
Wheat	58℃	5.5	1.0	0.2	54.2	7.2	1.1	37.4

2) Retrogradation in starch based food

Figures 2 and 3 show the inhibitory effect of the enzyme on retrogradation in rice cake. Rice cake containing this enzyme remained soft even after storage at 15° C for 3 days. In this application, even a very small quantity of α-amylase contamination in the enzyme preparation can cause problems – for instance, the rice cake can become sticky during storage. Fortunately, the bacterial strain used for production of β-amylase does not contain α-amylase, which is one of the main advantages of this strain as an industrial production strain for β-amylase.

β-amylase added　　　　Not added

Figure 2. Inhibitory effect on retrogradation of rice cake



%: Proportion of the microbial β-amylase preparation added to rice flour

Figure 3. Inhibitory effect on retrogradation of rice cake

In addition, this enzyme also possesses the unique ability to work on raw starch (Table 2). Plant β-amylases, including the enzyme derived from soybeans, cannot act on raw starch and therefore can not work at a temperature below the gelatinization temperature of starch (up to 60° C–70° C). However, the microbial enzyme is active at a lower temperature than gelatinization, and thus has the potential for a broader range of applications, including those in baking.

One of the advantages of the microbial enzyme compared to plant-derived enzymes is that it can bypass labeling requirements on regulated foods. Allergen-related labeling is mandatory for foods that use soybean-derived enzymes. The need for gluten-free food has also recently risen especially in the West. The microbial enzyme has an advantage in comparison to cereal-derived enzymes in that it can be used in the production of gluten-free food.

Table2. Composition of activity on raw starch

origin	Hydrolysis activity on raw starch (units/g)
Microbial	800.3
Barley	1.6
Wheat	0.1
Soybean	0.0

Amano Enzyme Group has successfully commercialized the microbial enzyme by applying efforts to improve productivity through a traditional mutation, optimization of cultural conditions, and to develop optimal downstream process procedures in order to produce the enzyme at an industrial level.

Finally, the conceptual features of this product are summarized below (Table 3).

Table 3. Conceptual features of microbial β-amylase as a product

- ◆ The world's first microbial β-amylase produced at an industrial scale.
- ◆ The microbial enzyme can be produced to meet the industrial demand, unlike the plant-derived enzymes.
- ◆ Thermostability is greater than for the barley and wheat enzymes and equivalent to the soybean enzyme.
- ◆ Capable of hydrolyzing raw starch which opens the potential for a wide range of food applications.
- ◆ Does not require allergen-related labeling and can be utilized for gluten-free food applications.
- ◆ The microbial enzyme is a kosher- and halal-compatible, non-GMO enzyme.

The year 2010 is the United Nations International Year of Biodiversity, and in October, Nagoya will hold the two-week tenth meeting of the Conference of the Parties (COP10) to the Convention on Biological Diversity, preceded by the one-week fifth Meeting of the Parties (MOP5) to the Cartagena Protocol on Biosafety. Roughly 8,000 people from around the world – not only government officials and ministers, but also representatives from academic groups, companies, NGO/NPO, and the media – will converge on Nagoya. Although the word "biodiversity" tends to conjure up images of pandas, elephants, and other animals, the term applies to all living organisms and microbes, spanning three main categories: "biological diversity," "species diversity," and "genetic diversity."

It has only been several thousand years since human civilization has laid its foundations and become cognizant of its own history. For much of that time, relatively few areas were inhabitable to humans, population was limited, and humans were essentially one part of the greater natural ecosystem. Humanity's respect for nature, I think, was a matter of course. However, after humanity made the transition from wood energy to fossil fuel energy and began to develop scientific technology, Earth's inhabitable area has grown dramatically; and to support an explosive population boom, humans have replaced forests with fields, farms, and cities, and overexploited fishing resources. These developments gave rise to two important global environmental issues: biodiversity and climate change.

With the recent progress in genetic engineering, researchers have developed numerous theories about ways to create life forms – all they would need to know is the life form's genetic structure. Science fiction movies like "Jurassic Park" and "Resident Evil" have applied these concepts, using genetic engineering as a central theme. At the current stage, however, it is impossible to create genes from scratch: for genetic engineering to operate, a virus, bacteria, plant or animal must provide the basic genetic material. And yet, these genes are disappearing at an alarming rate.

It is often said that roughly 1,750,000 species have been formally described and given names, while the total of all species, known and unknown, amounts to 30 million. It may seem a bit odd that we can estimate the number of as-yet-unknown life forms, but humans are only aware of slightly more than 6% of all life on earth, and no matter how advanced our intelligence investigative capabilities may become, in my view, our knowledge of nature will be no more than a tiny fraction of the whole. Right now, as new books enter library catalogs, others are lost - valuable books might be disappearing without us noticing. Similarly, organisms that could serve as effective treatments against new infection diseases might be swept away with all of the other organisms on the path to extinction. It is very possible that organisms of any kind, from microbes to large animals, could be the raw material for the food production that supports growing populations and the development of medical products that keep us healthy. How are we to ensure their genetic safety?

One way is to define goals in numerical terms. In climate change, for example, "Measurable, Reportable, and Verifiable" (MRV) goals and actions are becoming increasingly important. The current biodiversity target is "to achieve by 2010 a significant reduction of the current loss rate of biodiversity," but the "post-2010 target," expected to be adopted at the Nagoya COP10, will likely be a more MRV-oriented goal. In addition, people also need to accept that "our knowledge of nature will be no more than a tiny fraction of the whole," think about ways to cope with that fact, and try to make the transition from the traditional perspective in which people "use materials for further development unless the resource has been proven valuable" to one in which we "preserve the things we don't yet fully understand." To put it simply, we need to treat nature with a "sense of reverence."

What does this mean in concrete terms? As stated above, it is very possible that organisms of any kind, from microbes to large animals, could be the raw material for the food production that supports growing populations and the development of medical products against new infectious diseases - and progressive, innovative structures are required to ensure that those organisms are safe and used well. At COP10, several related structures and systems will be opened to discussion.




The first structure is related to gene trading. The British Royal Botanical Gardens at Kew preserves a wide variety of plants from all over the world, even plants collected by Darwin that are now extinct. European plant hunters scoured the globe for various plants, eventually securing a food supply. Potatoes, corn, and other plants that now serve as staple foods and sources for animal feed, in fact, were not originally European commodities. Quinine, the widely-used remedy for malaria, was made from plants found in local areas. Evidently, the freedom to harvest animals and plants allowed the major industrial powers to reap historically mammoth benefits.

However, looking back now on the history of developing countries that were exploited by developed countries for their animals, plants, and genes, it is clear to me that at COP10, those developed countries have a responsibility to distribute their benefits to developing countries and indigenous peoples and create an international access and benefit sharing system (ABS) for genetic resources. The freedom of gene trading is already becoming a thing of the past, as developing countries rich in biological resources are restricting the removal of genetic resources. The Food and Agriculture Organization has also set up treaties on genetic resources for food and is emphasizing the rights of farmers against major grain companies. With an ABS system, corporations that deal in food, medical supplies, cosmetics, and other fields that make use of genetic material would be required to pay compensation for the protection of genetic resources and divide profits appropriately as the genetic resources are put to use.

Second, considerations for biodiversity are growing more important in corporate activity. Conventional CSR would normally detail areas under company control, like “going green” in a company factory, or forays into cleanup activities that have little bearing on actual company business. This format must take another step forward. Financial institutions, for instance, are beginning to investigate whether their loan customers pose a threat to biodiversity. As exemplified by the Washington Convention, which regulates the supply chain of international trading to protect endangered species, COP10 will likely be a forum for further debate on guidelines on how the raw materials acquired by companies affect biodiversity. Just as supermarkets that sell produce have to pay careful attention to whether their products harm biodiversity, pharmacies that sell medical products will have to ensure that their medicines do not do damage. CSR would then be a key element of actual business.

Come October, COP10 participants will be debating many matters. Hopefully the discussion will be met not with a passive response, but be taken as an opportunity to demonstrate Japan’s abilities to define clear rules and establish principles of action.

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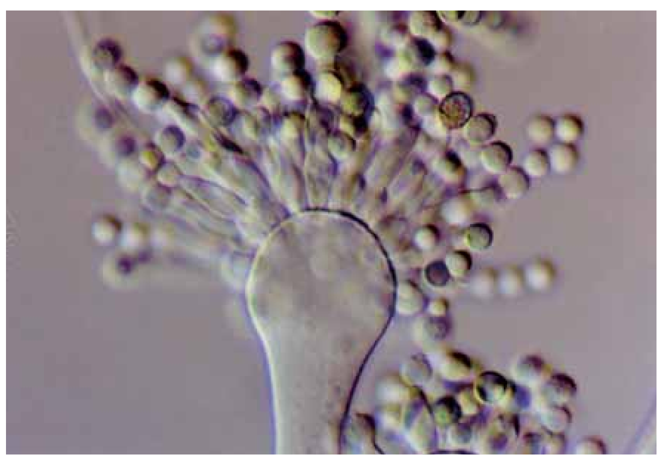

Established Amano Enzyme Manufacturing (China) Ltd. in Suqian City, Jiangsu Province

On July 27, 2009, Amano Enzyme Manufacturing (China) Ltd. (abbreviated AEMC) was established as Amano Enzyme Group's new overseas production base in Suqian City, Jiangsu Province. AEMC is a joint venture established by Amano Enzyme Inc. (investment ratio: 60%) and Siyang Syder Enzyme Co., Ltd. Motoyuki Amano, the president of Amano Enzyme Inc., became its chairman of the board.
AEMC is located in Siyang County of Suqian City in the northern part of Jiangsu Province. It takes four hours from Shanghai and two hours from Nanjing by highway. The facility occupies 17,800 m^2 and employs about 100 employees to manufacture industrial enzyme preparations by liquid culture of microorganisms.
Hongze Lake, the fourth largest lake in China, lies to south of Siyang. Forestry and wood processing industries, mainly of poplar, prosper in Siyang and are the source of its affectionate nickname, "County of the Poplar Tree."
Siyang was recently designated as a provincial economic development zone, and is currently undergoing an urban development that features green vegetation in consideration of the natural environment.

China recently celebrated its 60th anniversary of the founding of the new republic, has promptly recovered from the financial crisis and maintains an annual GDP growth rate of over 8%. As a result, AEMC plans to construct a system for providing safe and reliable products to meet the needs of its customers not only in China but worldwide. AEMC also strives to become an enterprise that is familiar with and loved by the local community. We sincerely ask for your kind support.

Opening Ceremony

Factory of AEMC

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