

Enzyme Wave

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Ms. Atsuko Naruse, a descendant of the Naruse clan, the former lords of Inuyama Castle, tells us that in order to understand the beauty and historic significance of the Inuyama Castle, we only need to take a moment and appreciate the plain but simple structure of the castle and see the view from the top floor which once served as a watchtower. Ms. Naruse enjoys looking upon Inuyama Castle, because for her, the castle symbolizes her parents, her ancestral roots and the Naruse family's history itself.



Atsuko Naruse
President, Inuyama Castle
Hakutei Bunko

As Ms. Naruse turns to the numerous historical records and diaries stored in the Hakutei Bunko foundation's depository, of which she is the current President, she learns about her ancestors, their ideas, their thoughts, how they lived, and about the difficult situations they had to face. In doing so, she continues to watch over and protect Inuyama Castle.

What history could Inuyama Castle entail?

Inuyama Castle stands on a hill in the plains on the left bank of the Kiso River. In spring, the cherry trees on the castle grounds come into bloom all at once, while in autumn, the approach to the castle is enveloped by arches of red maple leaves. It is also known as Hakutei Castle.

It is generally believed that Inuyama Castle was first established in the first part of the Tenbun era (1532-1555) by Nobuyasu Oda, uncle to the famous conqueror Nobunaga Oda during the Sengoku (Warring States) period. At that time, Nobuyasu Oda had the castle relocated from Kinoshita Castle (near the present-day Inuyama Municipal Library).

Later, during the Sengoku period, Inuyama Castle became a theater of fierce war on three occasions.

The first time was in 1565, when Nobunaga Oda's forces attempted to invade the castle. Nobukiyo Oda, who was the lord of the castle at that time, was forced to flee, crossing the Kiso River before Nobunaga Oda's men could enter the castle's headquarters.

The second time was the Battle of Komaki and Nagakute in 1584. At that time, Sadanari Nakagawa was the lord of the castle. The Battle of Komaki and Nagakute was fought between Hideyoshi Hashiba (later known as Hideyoshi Toyotomi) and the allied forces of Nobuo Oda and Ieyasu Tokugawa over supremacy. While Nakagawa was away from the castle, his uncle, Seijoshu, was defending it. He died in battle when Tsuneoki Ikeda, of Hideyoshi's side, made a sudden assault upon the Castle.

After this battle, the castle changed hands frequently. At one point, it was entrusted to Mitsuyoshi Ishikawa by Hideyoshi Toyotomi. As the Battle of Sekigahara began in 1600, Mitsuyoshi joined the western forces on the side of Mitsunari Ishida, but was forced to surrender the castle even before fighting, due to betrayals by his reinforcement troops, who were secretly communicating with the opposition troops on Ieyasu Tokugawa's side.

On all three occasions, the lord of Inuyama Castle was defeated, but the castle itself was never burned down.

When Ieyasu Tokugawa came to power and established the Edo shogunate, his ninth son, Yoshinao Tokugawa, became the daimyo of Owari domain. Since Yoshinao was still very young, his father appointed Masanari Naruse, Ieyasu's former page, as Yoshinao's senior retainer, to look after him. In 1617, the second shogun, Hidetada Tokugawa, endowed the Naruse clan with Inuyama Castle. Since then, the Naruse clan has remained lords of Inuyama Castle.

In 1868, the Meiji government promoted Masamitsu Naruse to the rank of daimyo and created the Inuyama domain. However, soon after, in 1869, the daimyos were obligated to return their territories to the Emperor and were made governors of their domains. The domains were then reorganized under the prefectural system, and Masamitsu Naruse was removed from the governor's post. In 1891, the ownership of Inuyama Castle, which was seriously damaged in the Nobi Earthquake, was transferred back to Naruse on condition that the Naruse clan undertook its repair. With donations from local residents, the castle was restored, and in 1935, Inuyama Castle was designated a National Treasure in recognition of its historical value.

Inuyama Castle has been damaged on several occasions by typhoons and other incidents, but has since been restored. In 2004, the Inuyama Castle Hakutei Bunko foundation was established, to which cultural properties that had been preserved by the Naruse family were donated. At present the castle is owned by the foundation and managed by Inuyama City.

Therefore, it can be said that Inuyama Castle is maintained by private individuals and its surrounding citizenry. The presence of the castle nurtures a sense of pride among the local residents, which in turn serves to fortify the castle.

References: Inuyama Castle, Inuyama City Board of Education, Inuyama Castle Hakutei Bunko, The Mainichi Newspaper



→ About Amano's Symposium on Enzyme Applications ←

Fifteen years have passed since the inauguration of Amano's Symposium on Enzyme Applications. Over the years, I have had the honor and pleasure of working with the late Professor Sueharu Horinouchi, selecting awardees, planning symposium programs and organizing the events. During the first few years, we were both inexperienced. We had many people kindly support us while causing them worry and made it through somehow.

There are many symposiums similar to "Amano's Symposium". However, "Amano's Symposium" is unique in various aspects. Firstly, it is sponsored by a private company, which is very rare for symposiums of this kind. Also, this symposium gathers together representatives from both academia and industry. Moreover, we have a multidisciplinary program that covers not only science and technology, but also subjects of the humanities. We select awardees not only on the basis of their achievements, but also in consideration of the application potential of the ideas presented. I believe that these unique characteristics account for the successful continuation of the Symposium.

In the last 15 years, some 70 scientists have been awarded. I am deeply pleased that these research results have led to products and technologies that benefit society, and that the awardees have continued to work actively in their respective fields. The commitment and enthusiasm of one company, Amano Enzyme, as translated into the organization of this unique event, encourage - and I believe will continue to encourage - young researchers in related fields. It is my hope that the Symposium will continue contributing to the



Sakayu Shimizu
Chair, Steering
Committee
Professor Emeritus,
Kyoto University/
Professor, Kyoto
Gakuen University

*The position is that at the time of the receiving an award.

The 1st Symposium

May 26, 2000

[Special Lecture] Exploration and industrial use of microorganisms as catalysts as catalysts

Hideaki Yamada (Kyoto University)

Award lecture

- The structure and function of quinoprotein glucose dehydrogenase
Mamoru Yamada (Yamaguchi University)
- Analysis of maturation activation process of thermophilic *Bacillus* TB-90 urease
Makoto Hidaka (The University of Tokyo)
- Functional analysis of chitin metabolism related enzymes in mycelium growth and morphological formation of filamentous fungi
Hiroyuki Horiuchi (The University of Tokyo)
- Structure and function studies of aspartate kinase
Makoto Nishiyama (The University of Tokyo)
- *Escherichia coli* Gamma-glutamyltranspeptidase from *Escherichia coli* and its application.
Hideyuki Suzuki (Kyoto University)

The 3rd Symposium

June 14, 2002

[Special Lecture] Acetic acid bacteria enzyme and its application

Osao Adachi (Yamaguchi University)

Award lecture

- Molecular dissection of transcriptional factor regulating genes of carbohydrate metabolism enzymes in filamentous fungi
Masashi Kato (Nagoya University)
- Modification of actinomycete chalcone synthase-type polyketide synthase
Yasuo Ohnishi (The University of Tokyo)
- Enzymological research on synthesis and modification of flavonoid in plant.
Toru Nakayama (Tohoku University)
- Improvement of protease functions by pro-sequence engineering.
Hiroshi Takagi (Fukui Prefectural University)
- Optimization of production system for biodegradable plastic by using enzyme evolution.
Seichi Taguchi (Meiji University)

[Reporting Lecture] Characteristics of beta-glycosidase from *Penicillium multicolor* and its industrial application
Kazutaka Tsuruhami (Amano Enzyme Inc.)

The 2nd Symposium

June 1, 2001

[Special Lecture] From zymology to biotechnology

Teruhiko Beppu (Nihon University)

Award lecture

- Development of the optically active alcohol production system using genetic engineering.
Michihiko Kataoka (Kyoto University)
- New actinomycete cyclic dipeptide dehydrogenase useful for the production of bioactive compound.
Hiroshi Kanzaki (Okayama University)
- Functional analysis of nitrilase for material production and development of a promoter for expression of nitrilase
Michihiko Kobayashi (University of Tsukuba)
- Development of enzymes involved in biogenesis of active-form sulfur and active-form selenium.
Hisaki Mihara (Kyoto University)
- Molecular conversion of CO₂ by microbial decarboxylase catalyzing carbon fixation
Toyokazu Yoshida (Gifu University)

[Reporting Lecture] Amano New Specialty Enzyme, protein glutaminase; possible applications in food industry
Shotaro Yamaguchi (Amano Enzyme Inc.)

The 4th Symposium

June 6, 2003

[Special Lecture] From microbial enzymes to functional proteins

Hidehiko Kumagai (Kyoto University)

Award lecture

- Improvement of quinoprotein alcohol dehydrogenase based on the three-dimensional structure.
Hirohide Toyama (Yamaguchi University)
- Elucidation of aldoxime metabolic pathway in microbe and its application to organic synthesis
Yasuo Kato (Toyama Prefectural University)
- Genome-based drug discovery using glycerophosphodiester phosphodiesterase in microbes
Noriyuki Yanaka (Hiroshima University)
- Structural and functional analysis of anti-tumor enzyme L-methionine gamma-lyase and its application to cancer treatment
Kenji Inagaki (Okayama University)
- Activity control of epidermal transglutaminase (protein cross-linking enzyme) and its application
Kiyotaka Hitomi (Nagoya University)

[Reporting Lecture] Utilization of microbial enzymes in pharmaceutical intermediate manufacturing and future prospects
Yoshihiko Hirose (Amano Enzyme Inc.)



This year marks the 15th anniversary of the inauguration of the Amano's Symposium on Enzyme Applications, which commenced in 2000. On this occasion, we would like to present the scientists who received the awards at each of the past Symposiums along with the titles of the work awarded, as well as the speakers' names and titles of the Special Lectures.

Symposium

← Congratulations →

On the occasion of the Amano's 15th Symposium on Enzyme Applications, which commemorates the 15th anniversary of this event, as one of those who have contributed to the establishment and organization of the Symposium, I would like to offer my heartfelt congratulations to Amino Enzyme Inc.

I would also like to express my deep respect to all those concerned at Amano's Symposium on Enzyme Applications for their great efforts over the past 15 years in conceiving and realizing this remarkable forum, where enzymemologist from academia and industry meet and discuss relevant subjects, from the exploration of new enzymes and enzyme functions to the possibility of new enzyme applications.

I strongly hope that the Symposium will continue developing year after year, raising and expanding expectations toward and the role of enzymological research in industry and academia, thereby playing an essential role in industrial development and significantly contributing to society.



Hideaki Yamada,
Professor Emeritus,
Kyoto University

The 5th Symposium

June 11, 2004

[Special Lecture] **New dawn of food processing, transglutaminase**

Noriki Nio (Ajinomoto Co., Inc.)
Hiromu Kusui (Ajinomoto Co., Inc.)
Kohichi Umeda (Amano Enzyme Inc.)

Award lecture

- Fundamental analysis of enzymes involved in N-substituted formamide metabolism and their application to the production of useful substances
Yoshiteru Hashimoto (University of Tsukuba)
- Application of pigment-dependent dehydrogenase from hyperthermophilic bacterium to electrode sensor
Haruhiko Sakuraba (The University of Tokushima)
- Discovery of new physiological functions of secretory phospholipase A2 and analysis of its molecular mechanism
Manabu Arioka (The University of Tokyo)
- Enzyme production system employing methanol-induced gene expression in yeast and its molecular basis
Yasuyoshi Sakai (Kyoto University)
- Constructing highly efficient system for synthesizing fine chemicals using bioprocess of asymmetric reduction
Nobuya Ito (Toyama Prefectural University)

The 6th Symposium

June 10, 2005

[Special Lecture] **Cell-free transcription/translation system**

– improvement in efficiency and its application to protein engineering –
Tsuneo Yamane (Chubu University)

Award lecture

- An investigative method to explore oxidases applicable to material conversion
Kimiyasu Isobe (Iwate University)
- Development of functional anaerobic microorganisms as catalysts for functional lipid production
Jun Ogawa (Kyoto University)
- Analysis of heat-resistant chitin metabolism system in thermophilic bacterium
Toshiaki Fukui (Tokyo Institute of Technology)
- Synthesis of new glycosides using multifunctional glycoside transferase
Hideshi Yanase (Tottori University)
- Three dimensional structure analysis enabling to use polyphosphoric acid to production system of enzymatic phosphorylate substance
Shigeyuki Kawai (Kyoto University)

[Reporting Lecture] Regulation on food enzymes
Satoru Asada (Japan Food Additives Association / Amano Enzyme Inc.)

The 7th Symposium

June 9, 2006

[Special Lecture] **Manufacture of yeast extract by using enzymes**

Yoshiki Aoyagi (Kohjin Co., Ltd.)

Award lecture

- Development of salinity-tolerant glutaminase from *Aspergillus oryzae*
Kazuaki Yoshimune (National Institute of Advanced Industrial Science and Technology)
- Functional analysis of PLP-dependent dehydratases and its application to material production
Masaru Wada (Hokkaido University)
- Elucidation of solid structure and reaction mechanism of isopullulanase
Takashi Tonozuka (Tokyo University of Agriculture and Technology)
- Generation of artificial heme enzyme containing functional synthetic heme to obtain highly active oxidizing catalyst
Takashi Hayashi (Osaka University)
- Improvement of cell-free protein synthesis system and its application to protein engineering
Hideo Nakano (Nagoya University)

[Reporting Lecture] World enzyme markets
Kuniyasu Kitoh (Amano Enzyme Inc.)

The 8th Symposium

June 15, 2007

[Special Lecture] **Manufacture of functional oils and fats by enzymatic interesterification with lipase**

Haruyasu Kida (Fuji Oil Co., Ltd.)

Award lecture

- An investigation of collagen-degrading enzymes produced by thermophilic bacteria for material recycle
Kunihiko Watanabe (Kyoto Prefectural University)
- Elucidating the structure and functions of fatty acid desaturase genes in oilseed and its application to the production of useful oils
Eiji Sakuradani (Kyoto University)
- Enzymes related to D-amino acid metabolism: structure, function and application
Tohru Yoshimura (Nagoya University)
- Analysis and functional modification of non-ribosomal peptide synthase
Masaaki Morikawa (Hokkaido University)
- Construction of artificial enzyme with organometallic complex by supramolecular assemblies of protein and metal complex catalyst
Takafumi Ueno (Nagoya University)

[Reporting Lecture] Current use of enzymes in the health food market
Satoshi Koikeda (Amano Enzyme Inc.)

The 9th Symposium

June 13, 2008

[Special Lecture] **Current situation and future prospects of enzymatic saccharification of cellulosic biomass**

Yasushi Morikawa (Nagaoka University of Technology)

Award lecture

- Structural analysis of oxidative modification protein – the molecular mechanisms of oxidative stress –
Sohei Ito (University of Shizuoka)
- Cell-free synthesis and functional analysis of cytotoxic enzyme of bacteriophage
Nobutaka Hirano (Nihon University)
- Regulation of formaldehyde-fixing enzyme gene expression and development of application of its catalytic function
Hiroya Yurimoto (Kyoto University)
- Development of heat-tolerant yeast producing thermostable cellulase
Hisanori Tamaki (Kagoshima University)
- Development of enzymatic biofuel cells
Selya Tsujimura (Kyoto University)

[Reporting Lecture] Moving Japanese Enzymes to the US Dietary Supplement Market – Importance of New Clinical Research and Scientific Data –
Setsubo Omata Jolly (Amano Enzyme USA Co., Ltd.)



← Congratulations →

Sixteen years ago, President Motoyuki Amano of Amano Enzyme Inc. told me about his plan to start a symposium focusing on applied research on enzymes. I expressed my support and asked him to continue the symposium, once it had begun, for a long time, even though it had to be on a small scale. Recalling this exchange and now seeing the symposium reaching its 15th year, I feel very happy and would like to offer my congratulations to Amano Enzyme Inc. on reaching this milestone. I would also like to express my gratitude to the company for its generous support for the research community. Research and development concerning the basics and application of enzymes is now entering a new phase. I sincerely hope that the Symposium will continue making progress in the future as an expression of our will to lead the world in this field.

Teruhiko Beppu, Professor Emeritus, The University of Tokyo



The 10th Symposium

June 12, 2009

[Special Lecture] **Jokichi Takamine, Father of Biotechnology**
Yutaka Yamamoto, (The Jokichi Takamine Research Foundation)

[Special Lecture] **Lively and freely, Nagoya culture**
Bunkichi Yasuda (Nanzan University)

[Special Lecture] **Production of useful proteins using koji-mold**
- fluorescent bio-imaging analysis to development of production hosts -
Katsuhiko Kitamoto (The University of Tokyo)

Award lecture

- Sophisticated control of methanol metabolism by alcohol oxidase (AOD) isozymes and its application to mass-production systems of useful enzyme
Tomoyuki Nakagawa (Gifu University)
- Elucidation of the molecular structure of flavin enzyme involved in biosynthesis of lipid in archaea membrane and its application
Hisashi Hemmi (Nagoya University)
- Functional modification of phospholipase D from actinomycete
Yugo Iwasaki (Nagoya University)
- Strain improvement of filamentous fungi *Trichoderma reesei* toward the creation of bio-refinery industry
Wataru Ogasawara (Nagaoka University of Technology)
- Characteristics of chimeric enzyme of hemicellulose side chain-degrading enzyme and arabinose bonding module from koji-mold
Takuya Koseki (Yamagata University)

The 11th Symposium

June 11, 2010

[Special Lecture] **Biodiversity Convention COP 10 AICHI-NAGOYA**
Toshiro Kojima (Aoyama Gakuin University/Institute for Global Environmental Strategies)

[Special Lecture] **Applied glycoscience and its related industry**
Keiji Kainuma (Kyushu University)

Award lecture

- Functional regulation of plant polyketide synthase and development of new supranatural biocatalysts
Hiroyuki Morita (The University of Tokyo)
 - Functional analysis of aminoglycoside antibiotic biosynthesis and its application
Fumitaka Kudo (Tokyo Institute of Technology)
 - Functional modification of sphingolipid for its advanced use
Nozomu Okino (Kyushu University)
 - Elucidation of highly reactive peptide sequence of transglutaminase (protein cross-linking enzyme) and its utilization
Kiyotaka Hitomi (Nagoya University)
 - Fundamental and applied research for the structure, function and activity regulation of amino acid biosynthese
Makoto Nishiyama (The University of Tokyo)
- [Reporting Lecture] Development of microbial beta-amylase
Masamichi Okada (Amano Enzyme Inc.)

The 12th Symposium

June 10, 2011

[Special Lecture] **Enzymes in the theories of food functions and taste perception**

Keiko Abe (The University of Tokyo)

[Special Lecture] **Current situation and future prospects of enzyme Industry in China**

Chen Jian (Jiangnan University, China)

Award lecture

- Elucidation of the substrate specificity of arabinogalactan protein degrading enzyme
Satoshi Kaneko (National Agriculture and Food Research Organization)
- Development of highly versatile dipeptide synthesis method using peptidase containing aminolysis catalytic activity
Jiro Arima (Tottori University)
- Construction of fatty acid conversion technology using novel microbial enzymes
Shigenobu Kishino (Kyoto University)
- Genomic data-based explorative research on polyketide synthase and its application to material production
Nobutaka Funa (University of Shizuoka)
- Re-design of redox metabolism in amino acid-producing bacterium by using unique glycolysis enzyme of *Streptococcus mutans*
Masato Ikeda (Shinshu University)

The 13th Symposium

June 8, 2012

[Special Lecture] **A novel application world by enzymes**
- health maintenance with oligosaccharide producing enzymes -
Makoto Sasaki (Aichi Medical University)

[Special Lecture] **Exploration of microorganisms as resources in Asia and its utilization**

- Part I: Utilization of overseas bio-resources and the Convention on Biological Diversity
Katsuhiko Ando (National Institute of Technology and Evaluation)
- Part II: Before and after the International Collaboration in Inventory and Sustainable Use of Microbes in Indonesia
Puspita Lisdiyanti (Biotechnology, Indonesian Institute of Science (LIPI))
- Part III: Exploring microorganisms from fermented milk in Mongolia, and the action toward its utilization
Gentaro Yasuda (Calpis Co., Ltd.)
- Part IV: Panel discussion
Moderator: **Katsuhiko Ando**

Award lecture

- Functional analysis of unique oxidizing enzymes from *Mycobacterium* and its application.
Toshiki Furuya (Waseda University)
- Construction of a functional oligosaccharide library using novel phosphorylase
Hiroyuki Nakai (Niigata University)
- Analysis of novel enzymes involved in coenzyme biosynthesis and its application
Tohru Dairi (Hokkaido University)
- Dynamic analysis of crystalline cellulose degrading enzyme and improvement in efficiency of cellulose biomass conversion
Kiyohiko Igarashi (The University of Tokyo)
- Construction of a microbial biosynthesis system for biodegradable plastic using untapped resources in combination with exploration of novel enzyme
Miwa Yamada (Iwate University)



← Congratulations →

I would like to offer Amino Enzyme Inc. my sincere congratulations on the holding of the 15th Amano's Symposium on Enzyme Applications. I am very grateful for the Symposium and the great encouragement it has given me for my research: an award for my research at its second symposium and again with a special commendation at its 10th symposium. The Amano's Symposium on Enzyme Applications is highly significant for researchers, not only for commendation based on achievements, but also for the opportunity to obtain research funding. I hope that the Symposium will continue for many years to come.

Michihiko Kataoka
Professor, Graduate School of Life and Environmental Sciences, Osaka Prefecture University



The 14th Symposium

June 14, 2013

[Special Lecture] Role of Spring8 and SACLA in the research on protein structure

Masaki Yamamoto (RIKEN)

[Special Lecture] Ise Shrine, the sacred place of tokowaka (eternal youth)
Kiyomi Chikusa (Kogakkan University)

[Special Lecture] Digestive enzyme replacement therapy – theory and practice –

Teruo Nakamura (Hirosaki University)

[Special Lecture] Biodiversity of Microbes – the potential for Bioindustry Development in Vietnam

Duong Van Hop (Vietnam National University, Hanoi)

Award lecture

- Functional analysis of oligosaccharide isomerase and its developed application to efficient synthesis of useful oligosaccharides
Wataru Saburi (Hokkaido University)
- Conversion of inverting glycosidases to glycosynthases
Yuji Honda (Ishikawa Prefectural University)
- Development of amino acid dioxygenase useful as asymmetric conversion catalyst
Makoto Hibi (Kyoto University)
- Artificial cytochrome P450 complex with several electron pathways
Hidehiko Hirakawa (The University of Tokyo)
- Development of an on-demand bioprocess using thermostable enzyme modules
Kousuke Honda (Osaka University)

The 15th Symposium

June 13, 2014

[Special Lecture] The birth of the oldest ecosystem on earth and challenge for an experiment regarding artificial metabolism evolution (project collaborators wanted)

Ken Takai (Japan Agency for Marine-Earth Science and Technology)

[Special Lecture] Opportunities in Halal Economy

Dato' Seri Jamil Bidin (Halal Industry Development Corporation)

[Special Lecture] Power of imagination

Tetsuro Matsuzawa (Kyoto University/Japan Monkey Centre)

Award lecture

- Design of thermostable enzyme based on evolutionary information
Satoshi Akanuma (Tokyo University of Pharmacy and Life Sciences)
 - Functional analysis of chitin degradation-related enzyme producing functional oligosaccharide in bacteria
Takako Hirano (Nihon University)
 - A study of drug development using natural resources based on target enzyme guided synthesis using triazole formation
Tomoyasu Hirose (Kitasato University)
 - Addition of pyrophosphoric acid utilization to acetate kinase to increase productivity of acetyl phosphoric acid for construction of ATP regeneration system
Shigeyuki Kawai (Kyoto University)
 - Elucidation of diverse catalytic functions of cytochrome P450 and their application
Kenji Watanabe (University of Shizuoka)
 - A study of optimal use of pancreatic enzyme medicine
Atsufumi Matsumoto (Hirosaki University/Hirosaki Municipal Hospital)
- [Reporting Lecture] Transglucosidase: functional modifications and future development**
Satoru Ishihara (Amano Enzyme Inc.)



The Symposium on Enzyme Applications, which we of Amano Enzyme Inc. inaugurated in 2000 to commemorate the company's centennial, has reached its 15th year. I would like to take this opportunity to express my deepest gratitude to all who have participated in the Symposiums over the years, for their generous support, which has made these Symposiums possible.

In 2000, the inaugural year of the Symposium, Japan's economy was about to enter the low-growth phase following the long period of prosperity that had continued since the post-war rapid economic development. Worldwide problems of food supply, energy depletion and environmental destruction also began to draw serious attention, and human society has since embarked on a quest for more sustainable and recycling-oriented development. Under such circumstances, expectations for biotechnology continue to grow, year after year.

Since old times, Japan has had the fortunate tradition of using koji mold for making sake, soy sauce and miso (soy bean paste), and of using diverse microbe resources, thanks to the country's climate of high temperature and humidity, and seasonal changes.

For this year's Symposium, we have received many reports of outstanding research achievements. Following fair and impartial selection, five scientists have been chosen, whose authors will receive awards and deliver their lectures at the Symposium.

To commemorate the 15th Symposium, a milestone, we are also presenting an award to a researcher from the medical field. For this selection, we have received cooperation from Dr. Tadashi Takeuchi, Professor Emeritus of Tokyo Women's Medical University. As you know, in the medical field, enzyme replacement therapy is being used to promote digestion and absorption in patients with pancreatic diseases. We sincerely hope that enzymological research will make further progress in this field as well.

It has been over 60 years now, that Amano Enzyme Inc. has been in the enzyme business. During this period, as we have pursued our enzyme-related activities, we have cherished, and been enriched by, the Japanese cultural tradition of living in harmony with nature. We sincerely hope that the Symposium will greatly help spread the use of enzymes and eventually contribute to finding solutions to some of the common problems confronting human society.

Motoyuki Amano, President, Amano Enzyme Inc.



■ Most advanced medicine in the 21st century

Medicine using conventional pharmaceutical products almost only focus on alleviating the symptoms and keeping the symptoms not recovering bodily functions compromised by diseases. Nowadays, regenerative medicine defined as the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects is paid attention.

Regenerative medicine is the cutting-edge 21st technology and is expected to lead to new drug development to improve quality of life (QOL) of patients.

In this article, the recent progress, challenge of in regenerative medicine, enzymatic application in generative medicine is reported.

■ A growth strategy of Regenerative medicine

Creating and expanding new market of the medical and healthcare industry is set as a target for the growth strategy, one of the "three arrows" in the economic policy "Abenomics" proposed by the Prime minister, Shinzo Abe.

Market size of the medical and healthcare industry, which includes pharmaceuticals, medical devices and regenerative medicine, is aimed to increase from the current ¥12 trillion to ¥16 trillion by 2020.

Regarding regenerative medicine, the most recent and memorable news would be the awarding of the Nobel Prize in Physiology or Medicine to Professor Shinya Yamanaka of Kyoto University on October 8, 2012, for his research achievement on development of induced pluripotent stem cells (iPS cells) that has the potential to grow into any type of body tissue. Development of iPS cells is expected to establish regenerative medicine as innovative made-in-Japan technology in addition of existing health-care system. Japanese government is also trying to promote establishment of regenerative medicine.

One of the noteworthy movements resulting from this initiative includes the plan to establish a Japanese version of the National Institute of Health (NIH) as headquarters of all medical research institutions. This would put in place a centralized system of transverse and comprehensive research budget management, a task thus far assigned to the respective ministries concerned. Concrete policies have also been presented, with an eye to practical and industrial application of innovative made-in-Japan technology at the earliest possible time, so as to establish Japan's superiority in this field and embark on an "All Japan" campaign of industrial creation.

■ Current situation and challenges

Regenerative medicine, as mentioned above, involves replacing organs and tissues or stimulating self-repair mechanisms to heal previously irreparable organs and tissues, whose functions have been lost or compromised due to disease or injury. Regenerated tissues is also used for drug discovery. Regenerative medicine is expected to lead to etiological treatment of diseases

that are hard to treat with surgical intervention, drug administration or other conventional methods, thereby fulfilling currently unmet medical needs.

Since regenerative medicine is based on a new concept of treatment that radically differs from that of conventional medicine using drugs, it requires completely new ideas and legal systems accordant with the use of cells and tissues for treatment. Moreover, since in regenerative medicine is the process of creating living, functional tissues using stem cells, legislation of new guidelines regarding safety and production of cell use for medical purposes is currently under way. Regenerative medicine is thought to be an innovative medicine in 21st-century, diligent research has been carried out to grow tissues and organs using stem cells in the laboratory.

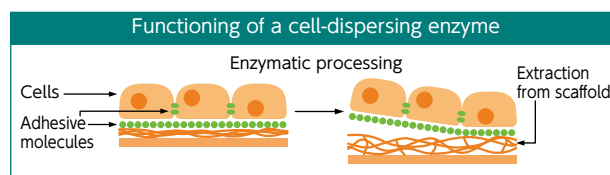
Treatment using stem cells taken from patients does not provoke autoimmune system. For this reason, regenerative medicine using autologous stem cell is expected to greatly improve patient QOL, particularly in terms of side effects.

Stem cell research is currently under way on three types of cells: ES (embryonic stem) cells, derived from fertilized eggs; iPS cells, derived by introducing a specific set of genes into a given cell type; and somatic stem cells, which animals and humans originally have. Stem cell research on somatic stem cells is the most advanced at the moment.

In such a situation, the Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labor and Welfare; and the Ministry of Economy, Trade and Industry have jointly established a basic framework of integrated support research from fundamental stage and clinical stage ("Regenerative Medicine Realization Highway Concept") toward prompt realization of regenerative medicine. Within this framework, RIKEN announced to commence the first clinical research using iPS cells for age-related macular degeneration (ARMD).

Regenerative medicine is supported by various related industrial sectors. According to a report issued by the Ministry of Economy, Trade and Industry in February 2013, regenerative medicine is estimated to yield huge economic benefits: a domestic market of regenerative treatment itself is estimated to reach 95 billion yen in 2020 and 1 trillion yen in 2030; a domestic market of regenerative medicine related industry including equipment, consumables, services, etc., 95 billion yen in 2020 and 550 billion in 2030.

There is still a number of challenges including establishment of ethical standard soundness, safety and efficiency, securing of further research funds and the formulation of a new legal framework.



Source: *Mirai Iryo-eno Chosenshatachi (Challengers of Future Medicine)*
Published by the Institute of Advanced Biomedical Engineering and Science,
Tokyo Women's Medical University

■Need for a new legal system

In regenerative medicine, Japan leads the world in terms of research on iPS cells and ES cells. However, the country largely lags behind in terms of practical application. According to data from the Ministry of Economy, Trade and Industry, as of December 2012, Europe led the world with 20 approved products and 42 products in clinical studies, followed by the United States (9 approved products, 88 products in clinical studies) and South Korea (14 approved products, 31 in clinical studies). Japan had only 2 approved products and 4 in clinical studies. The only two regenerative medical products that have been approved in Japan are JACE (autologous cultured epidermis) and JACC (autologous cultured cartilage). It is necessary to review the existing approval system under the Pharmaceutical Affairs Act and consider introducing a new approval and licensing system, in consideration of the characteristics of regenerative medical products, so as to better respond to practical application needs.

Regenerative medicine for treatment is currently practiced in various forms, ranging from clinical studies at university hospitals to non-insurance-covered consultations in private clinics, in compliance with the Medical Practitioners Act and the Medical Service Act. The practice is generally extremely inefficient, since cell-processing equipment is installed at individual medical institutions and cell culture is left to physicians themselves, who perform the task in the absence of criteria. For greater efficiency and safety, it is necessary to establish new standards for medical institutions providing regenerative medical treatment, and facilities and equipment for cell culturing and processing.

Under such circumstances, legislation introduced by a Diet member, the Regenerative Medicine Promotion Act, was passed on May 10, 2013 for the purpose of comprehensively promoting and regulating regenerative medicine at all stages, from research and development to practical application. On November 20, 2013, the revised Pharmaceutical Affairs Act and the new Regenerative Medicine Promotion Act were established (the latter was renamed the Act for Safety Assurance in Regenerative Medicine). The new law supporting safety, promptness and efficiency in regenerative medicine is scheduled to take effect in the autumn of 2014.

The table below summarizes the important points of the new legal framework.

Important points	Promptness/efficiency	Safety
Revised Pharmaceutical Affairs Act	Early introduction of an approval system corresponding to characteristics of regenerative medical products	Information to patients, informed consent, records of relevant matters regarding product users, record keeping, and other post-marketing safety measures
Act for Safety Assurance in Regenerative Medicine	Medical institution commissioning of cell culture and processing to external commercial service providers	Three levels of criteria for medical institutions, corresponding to risks in regenerative medicine, submission of plans to authorities and other formalities; criteria for cell culture and processing facilities, licensing and other formalities

■Enzymes in regenerative medicine

Regenerative medicine is expected to positively impact not only businesses involved in cells for treatment and drug development, but also related businesses, including cell-processing facilities, cell culture equipment, cell-assessment devices and consumables such as culture media, scaffolds, cell growth factors and other reagents, and culture apparatuses.

In general, there are three methods for retrieving and collecting cells from scaffolds after stem cell culture: the mechanical method, in which cells are physically removed; the chemical method, which uses a chelating agent or other chemical; and the enzymatic method, with a cell-dispersing enzyme.

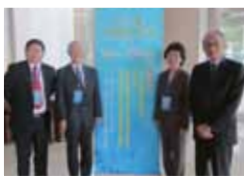
Cell-dispersing enzymes used to remove cells from scaffolding in the enzymatic method play an extremely important role with regard to cells and tissues, which are main ingredients of regenerative medical treatment. In this method, enzymes used and their quantities must be adjusted according to cell type. For this purpose, a variety of enzymes and adjusting agents for different cell types have been marketed by several companies as reagents. The domestic market of consumables for regenerative medicine is estimated to be worth 30 billion yen in 2020, 223.5 billion yen in 2030, and 536.8 billion yen in 2050.

In compliance with the new law, cell-dispersing enzymes will be required to conform to a battery of standards, such as GMP standards, asepsis, and low endotoxin and mycoplasma levels. Enzyme manufacture will most likely be subjected to the GMP standards.

In any case, enzymes have an important role to play in regenerative medicine, and as the "All Japan" campaign progresses, a variety of enzymes and related products will be introduced into the market and used in the clinical field, as well as research.

On Saturday, November 9, 2013, the Second Sino-Japan Joint Symposium on Enzyme Technology was held on the campus of Jiangnan University, situated in Wuxi in the province of Jiangsu, under the joint sponsorship of Amano Enzyme Inc. and Jiangnan University. The purpose of the Symposium was to encourage exchange between Japanese and Chinese enzymological researchers, and contribute to the advancement of enzyme applications in Asia. The event drew about 100 participants, including representatives of Chinese universities, enzyme-related institutions and companies.

A total of six lectures were delivered by Japanese and Chinese speakers. Speakers invited from Japan included Dr. Sakayu Shimizu, Professor Emeritus, Kyoto University and Professor on the Faculty of Bioenvironmental Science, Kyoto Gakuen University; and Dr. Keiko Abe, Professor Emeritus and Specially Appointed Professor at the Graduate School of Agricultural and Life Sciences, The University of Tokyo. The titles of the lectures and their speakers are indicated on the right.



Drs. Chen, Shimizu and Abe and Amano Enzyme President Amano (L-R)

- ①Dr. Sakayu Shimizu
Utilization of microbial reactions in the production of useful chemicals
- ②Dr. Keiko Abe
Japanese tradition of "food and health" science and new trends
- ③Mr. Qi Qingzhong (Chairman, China Food Additives & Ingredients Association)
Development of enzymes and evolution of legal and regulatory control in China
- ④Dr. Yang Li-rong (Professor, Zhejiang University)
Application of enzymatic catalyst technology in synthesis of applied chiral chemicals
- ⑤Dr. Wei Dongzhi (Professor, East China University of Science and Technology)
Preparation and application of biocatalyst polyol-converted through molecular evolution engineering
- ⑥Dr. Lu Zhao-xin (Professor, Nanjing Agricultural University)
Heterologous expression, molecular evolution and application of Anabaena-derived lipoxygenase in wheat flour-based products

In the final part of the Symposium, a panel discussion was held, in which Drs. Shimizu and Abe participated as panelists. The panel exchanged opinions on the great future potential of enzymes.

Dr. Chen Jian, President of Jiangnan University, highly evaluates the Symposium for its positive impact on the development of enzyme-related industries in the two countries. Amano Enzyme Inc. is committed to continuously enriching technological exchange between Japan and China, and contributing to the development of enzyme-related business in the two countries by holding the Symposium.

Amano Newsletter

Amano News Letter

Amano Enzyme USA has a new building

On November 26, 2013, the new office and blending factory building for Amano Enzyme USA was completed in Elgin in the state of Illinois, around a five-minute drive from the former one. The cost to finish the new building is estimated at about 1 billion yen.

Amano Enzyme USA commenced its business operation in 1981 in the state of Virginia. In 1995, the company relocated to Lombard, just outside Chicago. In 2003, in order to expand the R&D laboratories, the company moved again to Elgin, also outside Chicago. After about 30 years of existence, Amano Enzyme USA finally has a company owned building.

The new building, on a site of 12,700m², has a total floor area of 4,550m². It comprises of office space, a blending factory (clean room), a QC laboratory, a technical sales laboratory and a warehouse. Situated in the Greater Chicago metropolitan area, an area highly unlikely to experience earthquakes and other natural disasters, the new building enables us to assure a stable supply to our customers.

Manufacturing products near our customers also means we can provide speedy services, as well as products closely matching the local needs and requirements of our customers.

Since the state of Illinois is roughly at the center of the United States, our shipments can reach the state of New York on the east coast in only two days and the state of California on the west coast in roughly four days. As an added benefit, Chicago O'Hare International Airport, the second busiest airport in the US and the fifth busiest in the world, is only a 30 minute drive from the new building. Similarly, the location also provides us good access to other parts of the US, Mexico, and the rest of Latin America for prompt service.



From Historical Archive of Enzymology in Nagoya, Japan

Tidbit — Enzymes and Microorganisms —

Enzymes in the Western countries have been discovered in plants (enzymes those are papain, bromelain, ficin, malt diastase and etc.) and in animal organs (those are pancreatin, pepsin and etc.), and utilized for a long time.

In Asia including Japan, a variety of Koji (where a variety of fungi are grown on cereals) have been utilized for the traditional foods in each country.

A scientist who brought the mold from Koji into the United States and developed an enzyme product ahead of the rest of the world was Dr. Jokichi Takamine.

On the other hand, the microbiologists in France where the Father of Applied Microbiology Louis Pasteur was born, have been collected molds from the colonies in South-Eastern Asia, and developed Amylo-Method, by which cereals were saccharified and used for fermentation.

Both events were done in the late 19th century.

In the beginning of 20th century when enzymology and microbiology have been progressed, the resources of the most enzymes have been searched in the microbes, and the most of the industrial enzymes have been produced along with the development of fermentation technologies by fermentation with microorganisms.

Japan which had traditional technologies in utilization of Koji mold, that is *Aspergillus*, has led the world in developing the utilization technologies for a variety of enzymes ahead of the world, starting with the conversion of excess sweet potato starch after World War II into a sweetener by an enzymatic process.

Books in relation to microbiology have been collected in the *Historical Archive of Enzymology in Nagoya* in addition to enzymology. The classification book of *Aspergillus*, including the production strain of *Takadiastase* which was developed by Jokichi Takamine, has been essential for the universities and industries who were involved in

the research of enzymes. The *Historical Archive of Enzymology in Nagoya* bought the following 2 books through used book shops in the United States.

The Aspergilli, Tom and Church, The Williams & Wilkins Company, 1926

The Genus Aspergillus, Raper and Fennell, The Williams & Wilkins Company, 1965

The former book was issued in the same year 1926 when James Sumner crystallized the enzyme Urease.

In the acknowledgment of the preface of the book, the name of Jun Hanzawa at Hokkaido Imperial University can be seen along with Carl Wehmer of Hanover Institute of Technology where Hanzawa had studied, Selman Waksman who was the discoverer of Streptomycin, and Teizo Takahashi and Kendo Saito of Japan.

While on the back cover of the latter, *The Genus Aspergillus*, the corporate seals of Parke Davis & Co. and Warner-Lambert Company can be seen. Parke Davis & Co. is the company to which Jokichi Takamine transferred the exclusive sales right of *Takadiastase* excluding Japan, and Warner-Lambert is a pharmaceutical company which acquired Parke Davis & Co. in the 1970s.

Aspergillus from Japanese Koji moved to the United States had created an enzyme product by Jokichi Takamine, and the classification book of *Aspergillus* possessed by the companies who had been sold the enzyme came back to Japan serendipitously.

Amano Enzyme Message Board (May - December, 2014)

- **Information on exhibitions** We are looking forward to meeting you across the world. Come visit our booths at the following events:

May 21-23, 2014	Tokyo, Japan	ifia JAPAN 2014
June 22-24, 2014	New Orleans, USA	IFT 2014
June 26-28, 2014	Shanghai, China	CPhI China
July 29-31, 2014	Chicago, USA	AACC 2014
October 7-9, 2014	Paris, France	CPhI Worldwide 2014

- **Amano Enzyme participation status at 2013 exhibitions**

During 2013, Amano Enzyme exhibited at various pharmaceutical and food industry-related trade shows at several locations around the world (Japan, China, Thailand, USA, Mexico, Brazil, and Germany). We intend to continue our steady sales activities in the future.

Hello Irapuato, Mexico: 400th Anniversary of Japan-Mexico Relations



One of Amano Enzyme's overseas manufacturing bases, Amano Enzyme de Mexico S. A. de C. V., is located in Irapuato City in the state of Guanajuato, Mexico. In recent years, the state of Guanajuato has become country's largest automobile industry cluster. Currently, about 40 foreign-affiliated auto manufacturers from 11 countries are located there, including Honda and Toyota, whose factories have been in operation for some time. Also, as of January 2014, Mazda has opened a new factory (total area: 256ha) in Salamanca City, adjacent to Irapuato.



Such relations between Mexico and Japan are not new however. In fact, this year marks the 400th anniversary of the Hasekura diplomatic mission's arrival in Mexico. To commemorate this, a variety of events are being held this year throughout Mexico.

In October 1613, by the order of Masamune Date (1567-1636), the lord of the Sendai domain, a diplomatic mission headed by Tsunenaga Hasekura (1571-1622) left Sendai for Mexico, and by March 1614 it reached what is now Mexico City. It is believed that the three main objectives of this mission were to establish a direct trade relationship between Mexico and Japan, solicit the dispatching of missionaries to Sendai to spread Christianity in the domain, and to acquire silver production techniques from Mexico.

To mark this historic milestone, and in order to further promote exchange through tourism, and a variety of economic and cultural activities the two countries have named 2013-2014 the Year of Mexico-Japan Cultural Exchange. The commemorative events are scheduled to

include exhibitions of photography, paintings, manga and anime, video games and comic books, film projections, tea ceremony, flower arrangement shows, opera performances, sho flute concerts, debates, and aikido and karate competitions. Also, in consideration of the Cultural Exchange, Japan is invited as guest country to participate in this year's *Festival Internacional Cervantino*, the most prestigious annual arts festival in Latin America, which is to be held in October 2014 in Guanajuato (40-50 min. drive from AEM). Japanese artists of traditional performing arts, as well as active avant-garde artists from all over the world, are expected to give various performances at the festival.

If you happen to visit the area in the near future, we hope that you will take the opportunity to enjoy these events. Information on them is available on the following websites:

Year of Mexico-Japan Exchange, Hasekura Mission's 400th Anniversary:

<http://www.mx.emb-japan.go.jp/hasekura/historiajp.html>

Festival Internacional Cervantino:

<http://www.festivalcervantino.gob.mx>



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<http://www.amano-enzyme.co.jp/>

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