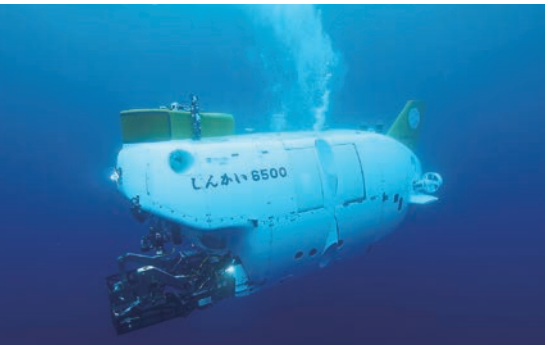
Volume 20

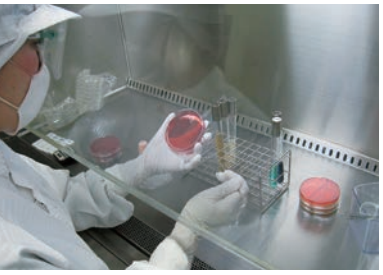
Courtesy of JAMSTEC

Enzyme Wave

2017

AMANO
Amano Enzyme





Enzyme Wave vol.20

CONTENTS

Information

Food processing enzymes that meet a diverse range of needs

Amano Enzyme Inc. features a wide range of products with food processing enzymes to meet customer expectations for further improvement in added value.

Worldwide Trends

Average life expectancy for the Japanese people continues to be among the highest worldwide for both men and women. The secret is said to lie within traditional dietary habits, including fermented foods such as natto (fermented soy beans), soy sauce, miso (fermented soy bean paste), etc. In the United States and Europe, the food market is experiencing an influx of new health-improvement foods such as gluten free (wheat protein free foods) and low-carb products. More and more products in super markets throughout the United States, Europe, and Japan are featuring labels such as “natural,” “organic,” “allergen free,” and “non-GMO” as the number of health-conscious consumers rises, bringing with them a diverse range of health and safety needs.

Although globalization continues to move forward, traditional dietary habits continue to be passed down from generation to generation in each region and nation, resulting in an infinite variety of tastes and foods desired by people throughout the world.

Amano Enzyme produces a diverse range of solid and liquid culture food processing enzyme formulas with safe microorganisms, used in the traditional Japanese brewing and fermentation industry for generations.

In addition to our non-GMO enzymes on the market produced with existing technologies, we are utilizing the latest in protein engineering to develop and commercialize GMO enzymes featuring new, never-before-seen functionality.

Right : Lipase AY “Amano” 30
Left : Kleistase SD 80

We also offer products in line with religious dietary restrictions, such as kosher and halal products, and work earnestly to meet the food additive laws and ordinances of various countries.

By fusing tradition with cutting-edge technology, we offer food processing enzymes that meet a diverse range of customer needs.

Halal Products

Report

Frontiers in Regenerative Medicine

Author

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Program director,
Regenerative Medicine Realization Highway

The branch of medicine that aims to restore the dysfunction tissues and organs in vivo by utilizing cells is termed "regenerative medicine", and its early realization is desired.

A brief overview of the current state of regenerative medicine research in Japan is presented below.

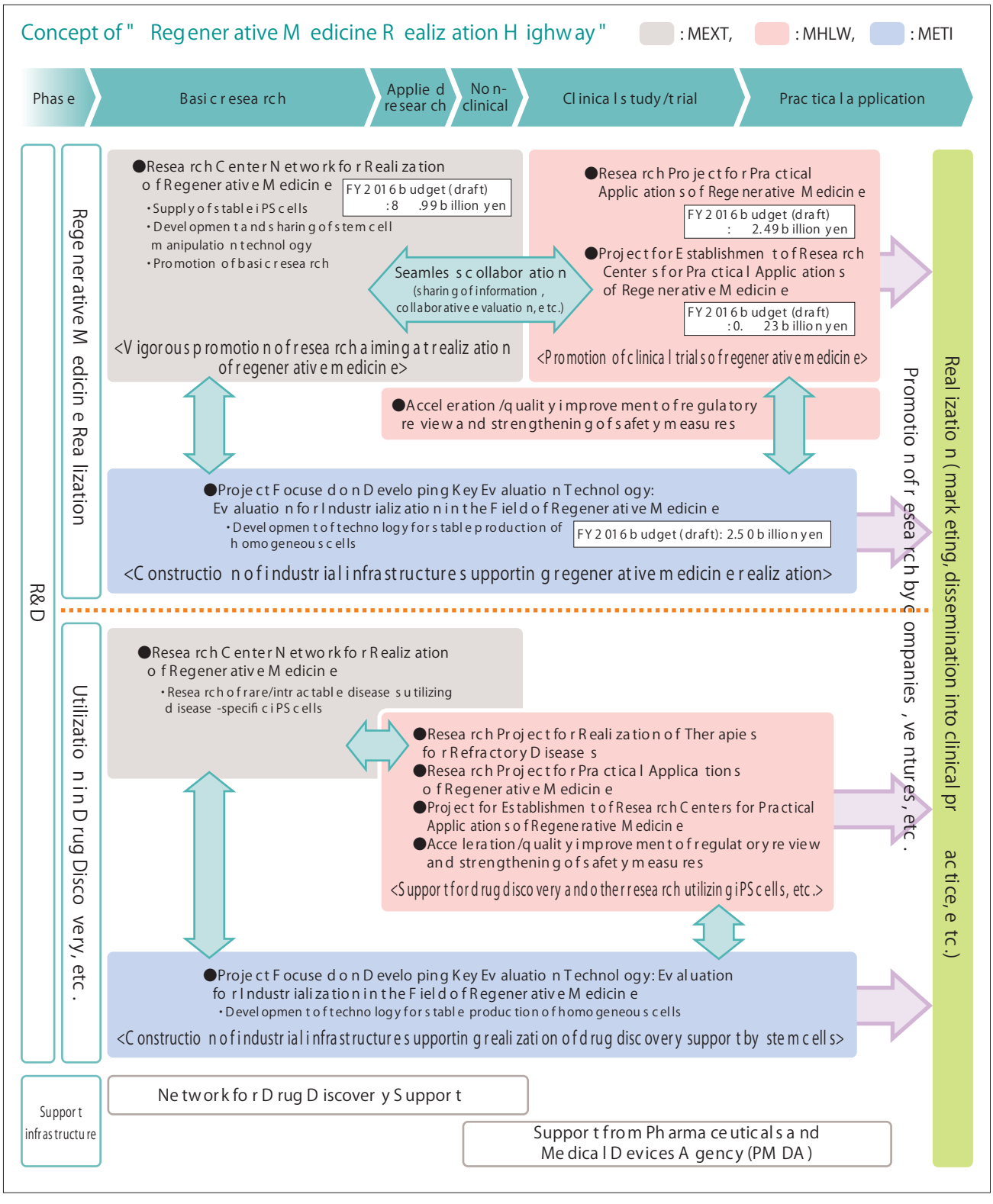
Concept of "Regenerative Medicine Realization Highway"

Regenerative medicine research in Japan had been promoted nearly independently by The Ministry of Education, Culture, Sports, Science and Technology, The Ministry of Health, Labour and Welfare, and The Ministry of Economy, Trade and Industry since approximately 2002 or 2003. The establishment of the Japan Agency for Medical Research and Development in Spring 2015 has enabled unified management of research and development budgets from these 3 ministries and led to the organization of a seamless support system, the "Regenerative Medicine Realization Highway", covering various aspects from basic research to practical application(Figure 1). Additionally, 3 acts on regenerative medicine enacted in 2013 have provided the basis for a clear policy that aims at industrialization of innovative findings in regenerative medicine involving an all-Japan system.

The FY 2015 budget (14.3 billion yen) has been promoting research and development organized under 2 major frameworks: "Regenerative Medicine Realization" and "Utilization in Drug Discovery, etc." Approximately 75% and 20% of the budget are allocated to the study of pluripotent stem cells (iPS and ES cells) and somatic stem cells, respectively. For research and development steps, approximately 80% of the funds is distributed to basic and pre-clinical research, while 20% is distributed to clinical research and clinical trials. The main program, "Research Center Network for Realization of Regenerative Medicine", plans to distribute HLA-homozygous iPS cells for clinical use prepared at the Center for iPS Cell Research and Application (CiRA), Kyoto University to individual disease-/tissue-specific centers for cardiac muscle (Osaka University), spinal injury (Keio University), Parkinson's disease (Kyoto University), eye (RIKEN), diabetes mellitus (University of Tokyo), liver (Yokohama City University), articular cartilage (Kyoto University), cancer (RIKEN and Yokohama City University), and intestine (Tokyo Medical and Dental University) for induction of differentiation into target cells and clinical application at individual centers. In parallel, 20 specific technical tasks (covering culture technologies, scaffold materials, quality control, and laboratory animals for studies of regenerative medicine, etc.) are being developed in collaboration with the industry. As a part of the "Research Project for Practical Applications of Regenerative Medicine," a project involving platelet production from iPS cells (Kyoto University), has successfully prepared 1011 platelets. The greatest challenge in the clinical application of iPS/ES cells is safety assurance, particularly the control of stem cell tumorigenicity. Our attempt at using regenerative medicine for the treatment of retinal degenerative diseases (RIKEN Kobe Institute) successfully transplanted 105 retinal pigment epithelial cells of iPS origin confirmed to be free of other cell types; to the best of our knowledge, this is the first such attempt. However, obtaining 109 retinal pigment epithelial cells free of other cell types remains beyond the current technical.

Numerous cell therapies investigated in the "Research Project for Practical Applications of Regenerative Medicine" have been evaluated in clinical trials, including liver regeneration therapy with interstitial cells isolated from autologous adipose tissue for the treatment of hepatic cirrhosis and transplantation of cardiac stem cells in pediatric heart failure. Both use somatic stem cells.

The Disease-Specific iPS Cell Project involves the establishment of iPS cells from patients with various refractory diseases, induction of differentiation to target cells in vitro to reproduce the pathology, screening of drugs to correct abnormalities, and drug discovery. For example, the effectiveness of a statin in achondroplasia has been published as a breakthrough.



Goals to be achieved by FY 2015

- Number of research projects using human stem cells proceeding to clinical studies/trials: approximately 10 (examples: age-related macular degeneration, corneal diseases, knee meniscal tear, bone/cartilage reconstruction, hematological disorders)
- Development of drug discovery technology using iPS cells

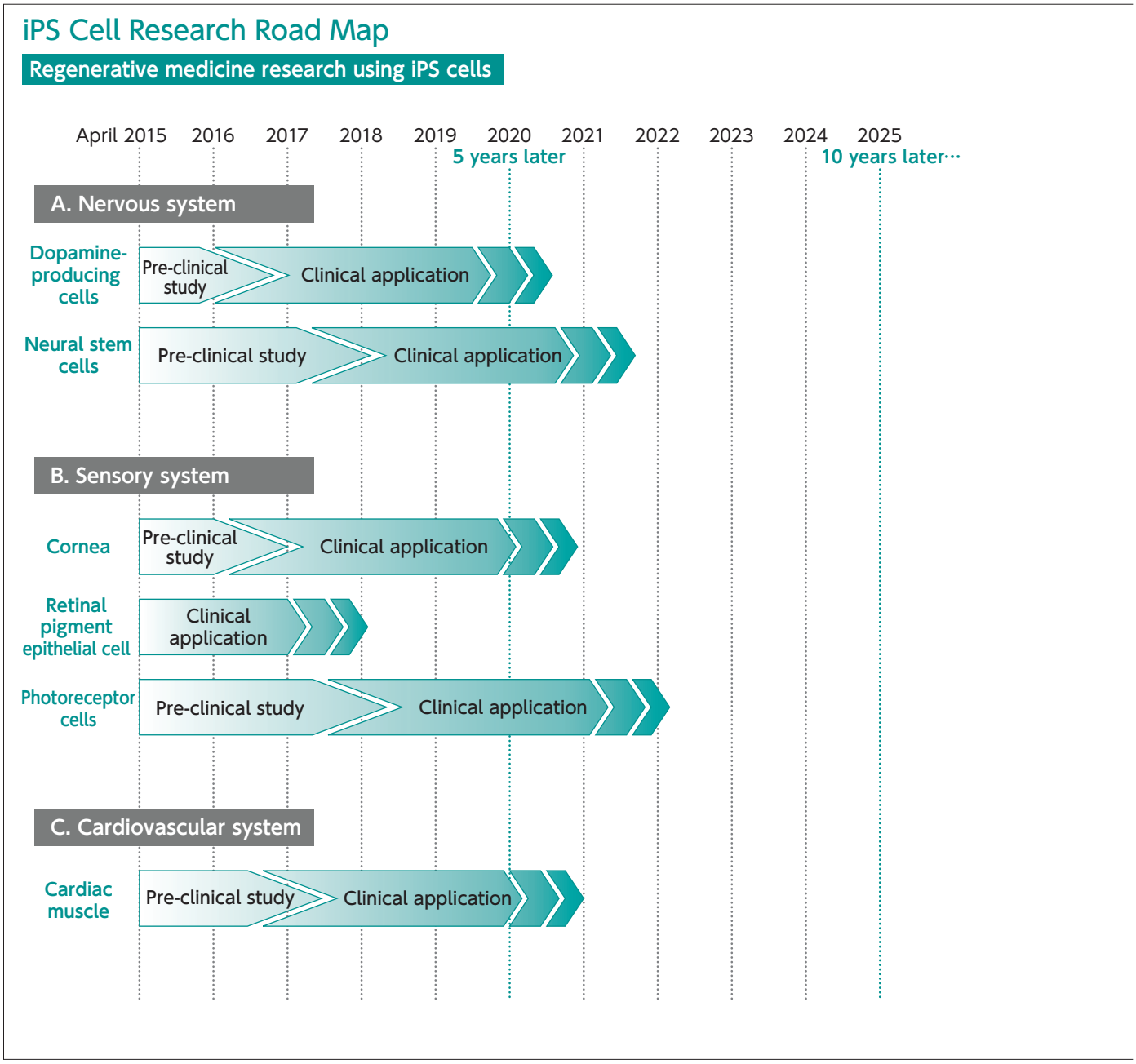
Goals to be achieved by approximately 2020

- Clinical application of new therapeutic drugs prepared by utilization of iPS cell technology
- Increase in number of regulatory approvals granted to regenerative medical products
- Extension of target diseases for regenerative medicine subjected to clinical studies or clinical trials (approximately 15 studies/trials*)
- Practical realization of peripheral devices/equipment related to regenerative medicine
- Proposal for international standardization of evaluation methods for drug cardiotoxicity applying iPS cell technology

*Include 10 studies/trials referred to in "Goals to be achieved by FY 2015"

Frontiers in Regenerative Medicine

Hidehiko Saito, National Hospital Organization Nagoya Medical Center

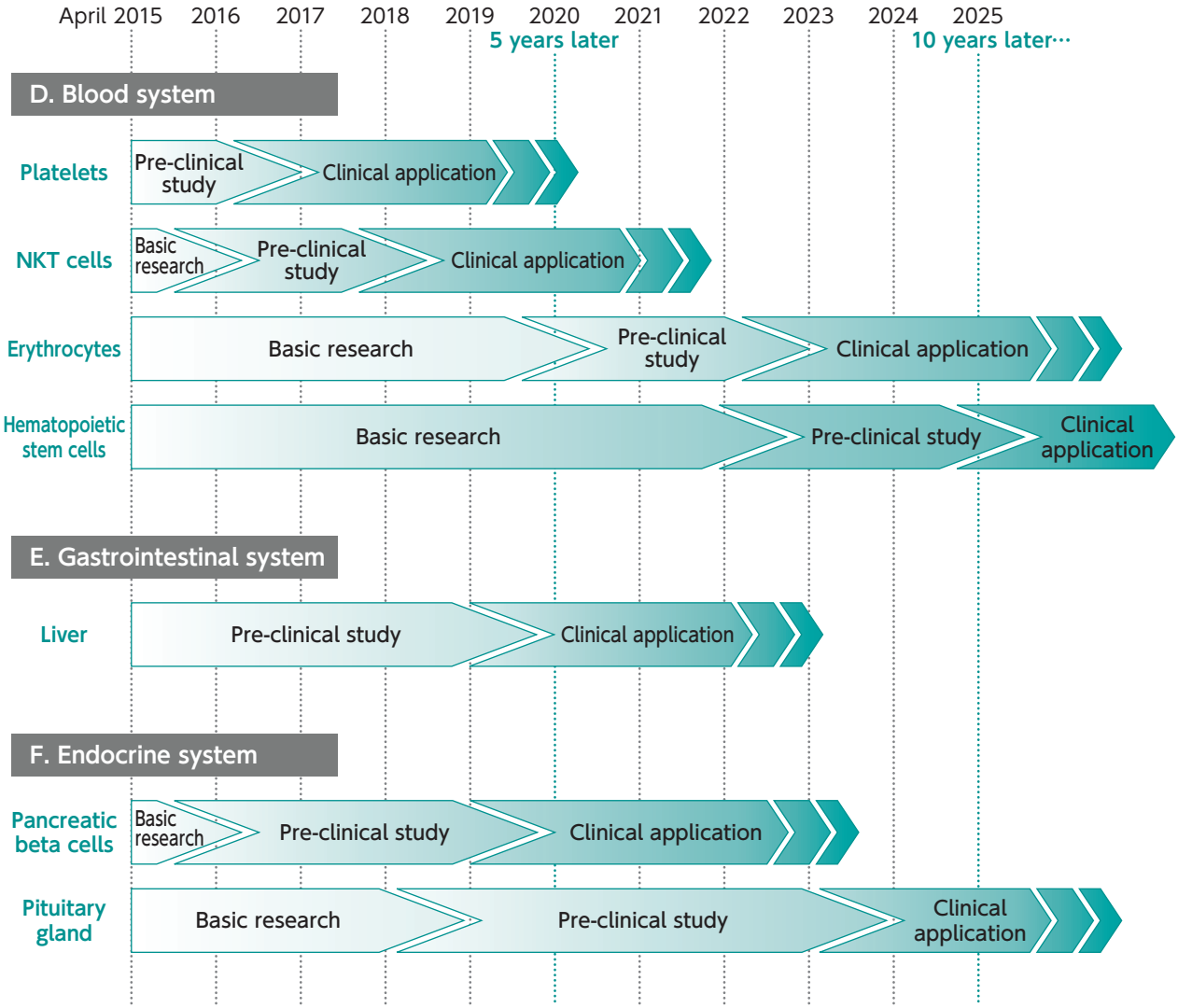


iPS Cell Research Road Map

A revised version of the iPS cell research road map published in November 2015 by the Task Force on Strategies for Stem Cells and Regenerative Medicine, The Ministry of Education, Culture, Sports, Science and Technology describes the timing of clinical application predicted for individual tissues and organs generated from iPS cells. This varies between both tissues and organs depending on the difficulty in inducing differentiation from stem cells and subsequent organ construction. Whether the first-in-man application will be a clinical study (under the Act on the Safety of Regenerative Medicine) or a clinical trial (under the Pharmaceuticals and Medical Devices Act) is currently unknown, and attention should be given to future trends to determine which type of evaluation will be conducted. The involvement of companies in projects aiming at the clinical application of regenerative medicine may be another determinant. Generally, in Japan, the number of venture companies is small and major companies are slow to act because of their policies that value prudence.

Created in November 2015

*"Clinical application" refers to a research/development stage in human subjects (clinical study or clinical trial).
The left end of each bar labeled "Clinical application" represents the targeted start time.
The length and position of the endpoint for each bar are arbitrary and have no intended meaning.



*This chart defines goals defined on the basis of expectation/prediction at the time point of publication and may contain uncertainty leading to an end practically different from that expected.

Future tasks

While production of a single type of cells (e.g., retinal pigment epithelial cells, platelets) from iPS cells has been achieved, 3-dimensional construction of organs containing multiple types of cells (e.g., liver, kidney) or preparation of tissues containing vessels, nerves, and interstitium (stroma) demands further innovation. In addition, the superiority to conventional treatment in safety, efficacy, and cost is needed. Furthermore, industrialization that enables low-cost, large-scale production is indispensable for dissemination of these methods as healthcare technology. Application to drug discovery is a unique advantage of iPS cells over ES cells, and somatic stem cells and will be investigated more extensively in the future. Application of iPS cells to drug discovery is a field involving intensive global competition.

Information

Enzyme Products from Amano Enzyme Support Regenerative Medicine

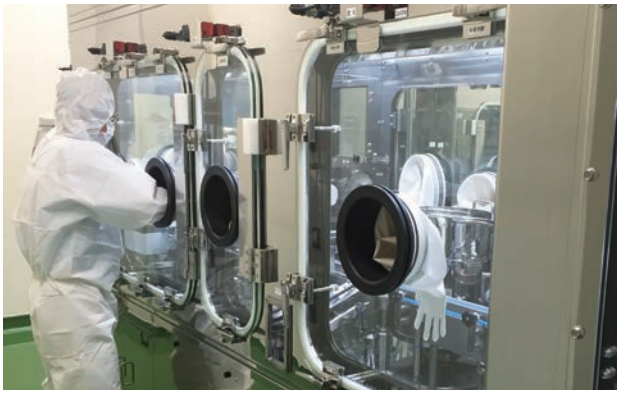
Application of Enzymes to Regenerative Medicine

Since the advent of iPS cells, there have been high expectations regarding their potential application in the field of regenerative medicine. Two and a half years have elapsed since the enforcement of the Act on the Safety of Regenerative Medicine and the Pharmaceutical and Medical Device Act on November 25, 2014, and movements toward industrialization of regenerative medicine are growing more and more active. Enzymes also play an important role in the field of regenerative medicine. Examples of applications of enzymes to regenerative medicine include cell dissociation in cell culture (for dissociation of cell aggregates and isolation of target cells from tissue) as well as removal of cumulus cells containing hyaluronic acid (a viscous substance interfering with sperm induction) in intracytoplasmic sperm injection. Since these manipulations require countermeasures for protection against bacteria and viruses at the highest level, enzyme products used for these manipulations must be of higher quality than conventional reagents.

Upgrading Production Equipment at the Yoro Plant

In response to these needs, Amano Enzyme has not only achieved animal-free (no materials of animal origin used), low-endotoxin, and GMP-compatible production, but new production equipment aimed at achieving class 100 (grade A) sterility in the filling/lyophilization process has also been introduced to ensure enzyme production in a clean environment. The inner space of a building at Yoro Plant (Ogaki City, Gifu, Japan) has been refurbished and installation of isolators for isolation of filling/lyophilization machines has been completed. To our knowledge, such efforts towards providing a complete supply of enzyme products for regenerative medicine are unprecedented in the enzyme industry.

the Yoro Plant



Filling Equipment

Product Information for Sterile Enzyme

Amano Enzyme is planning to launch a total of 5 items for regenerative medicine, including 3 "first-shot" items (**Collagenase "Amano" SF, Thermolysin "Amano" SF, and Hyaluronidase "Amano" SF**) and 2 follow-on items (clostripain and clostolysin, a neutral protease). Except for hyaluronidase for intracytoplasmic sperm injection, the remaining 4 items are intended for cell dissociation. In addition, customizable orders such as production of blended enzyme preparations will be accommodated in response to special customer needs.

Collagenase "Amano" SF

Report

Digestive enzymes to help improve nutrition for the elderly

One in every four people in Japan is over the age of 65. In order to continue living an active lifestyle, it' s important for the elderly to take in plenty of nutrition from daily meals. However, as people get older, the functionality of their stomach and intestines tend to decrease. Self-medication is recommended for these symptoms, such as oral administration of a digestive enzyme formula.

As the body ages and stomach changes, people tend to eat less at meals, in line with a decrease in overall daily activity. Because of this, a sufficient amount of nutrients are not being absorbed to live a healthy lifestyle in many cases. When looking at people other than the elderly, a change in dietary habits has led to a higher intake ratio of animal proteins and fats compared to long ago. Since foods high in proteins and fats tend to delay gastric emptying from the stomach, they are thought to be related to gastrointestinal symptoms such as stomach pain due to slow digestion.

In order to make up for gastrointestinal symptoms and reduction in gastrointestinal functionality due to the insufficient secretion of digestive enzymes caused by aging, it is useful to take digestive medicine containing digestive enzymes. Research results have indicated that administering digestive medicine to the elderly improves their digestion and nutrition absorption, providing proof that digestive enzyme formulas are indeed beneficial to improving nutrition intake. Digestive enzyme formulas have been used for over 100 years in Japan since Dr. Jokichi Takamine developed Taka-Diastase. For more than half a century, they have been used to improve symptoms of indigestion, and pancreatic enzyme replacement therapy for exocrine pancreas functional disorders. Even today, they are used widely in the treatment of digestive organs.

Amano Enzyme is participating in awareness activities to stress the benefits of digestive enzymes in digestion and absorption, as a part of self-medication for the elderly. Since 2013, institutes such as the Japan Pancreas Society, Japan Digestive Disease Week, and General Meeting of The Japanese Society of Digestion and Absorption, have been holding luncheon seminars, setting up business booths, and giving oral presentations on the topic. Information has also been published in academic journals such as “Journal of Biliary Tract & Pancreas 2016, Vol. 37, No. 2,” “Digestion & Absorption 2016 Vol. 38, No. 2,” and the Medical Tribune, with TV programs touching on the benefits as well.

By increasing awareness of the benefits digestive enzyme formulas can offer, we hope to contribute to a healthier lifestyle for everyone.

Medical Tribune, published October 27, 2016
Symposium at the Japan Pancreas Society

The 24th Japan Digestive Disease Week (JDDW2016) Luncheon Seminar

Column

The Depth and Potential of Research on Deep-sea Microorganisms

Author

Ken Takai Ph.D.

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Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Explorations to search for source microorganisms are indispensable for the development of new novel enzymes. A researcher at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC), investigating various extremophiles, including microorganisms, will present a brief overview of the current topics in deep-water research.

Introduction

For years, JAMSTEC has collected and preserved samples from the deep seafloor within the exclusive economic zone of Japan as part of our deep-water research program. In total, more than 11,000 microbial strains have been preserved when an appropriate system is fully organized. We hope that these strains will be utilized for research and product development by Japanese business enterprises. Here, I will present a brief overview of the depth and potential of our research on deep-sea microorganisms.

Deep Sea Drilling Vessel "CHIKYU"

Depth of our research on deep-sea microorganisms at JAMSTEC

Why does JAMSTEC study deep-sea microorganisms? The answer can be summarized through the following five points: (1) to define the limits (marginal conditions) for sustaining life in a particular biosphere or for all life on earth; (2) to characterize the biodiversity in the deep sea as the final frontier and to enhance our understanding of the evolutionary scenario and adaptive strategy of individual microbial species; (3) to utilize of these adaptive functions of individual microbial species, not just understand them; (4) to increase our understanding of the lifestyles of deep-sea microorganisms, which we think will be key to answering some of the most important scientific questions, such as the origin of life on Earth and the possible existence of extraterrestrial life; and (5) to contribute to global development and cultural/political diplomacy through science and technology by exploring unexplored biofrontiers and improving our understanding of the world.

Regarding point number one, our research group at JAMSTEC has established four world records related to the limits of life (microbial growth), including an upper temperature limit (122° C), upper pH limit (pH 12.4), upper pressure limit (1,300 atm), and upper gravity limit (400,000 G). Through a research project that began in 1997, we discovered a diverse microbial ecosystem in the hadal zone at the bottom of the Mariana Trench (at a depth of approximately 11,000 m), in the deepest part of the ocean on Earth, using the remotely operated vehicles "KAIKO" (ocean trench) and "ABISMO." In addition, in 2015, we reported that the Integrated Ocean Drilling Program (IODP) Expedition 337, working offshore of the Shimokita

Peninsula of Japan using the Japanese riser-drilling vessel “CHIKYU” (the Earth), identified not only the existence of deep subseafloor microbial communities, which include methanogenic bacteria, but also the limits of life in the biosphere of the world’s deepest subseafloor environment (2,500 m below the seafloor). Additionally, in 2016, we reported the findings of IODP Expedition 331 in the Mid-Okinawa Trough using CHIKYU, which showed that there was a clear boundary between habitable and uninhabitable terrains in the environment of subseafloor hydrothermal fluids beneath active hydrothermal vents and that this boundary was determined by temperature, which ranged from 120° C to 150° C. A new IODP Expedition using CHIKYU in the Nankai Trough is underway with an aim to more clearly pinpoint the temperature boundary between habitable and uninhabitable terrains. In the course of these research projects, we have discovered the environmental conditions that are indispensable for the origin and continued existence of life on Earth as well as those required for life in extraterrestrial environments based on cutting-edge theories and observations, thereby making significant contributions to research on the origin of life and the exploration for extraterrestrial life.

Potential for research findings on deep-sea microorganisms at JAMSTEC

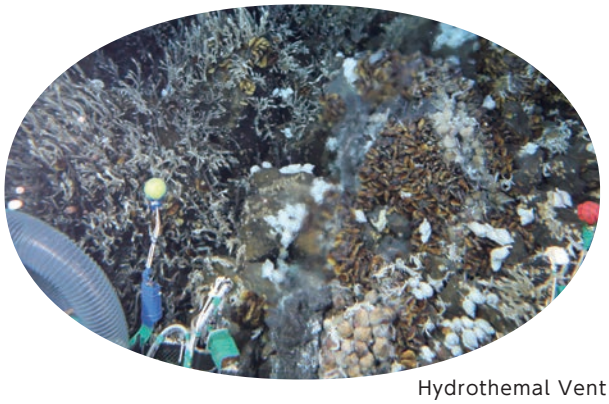
Research related to point number three in the preceding section demonstrates the potential applications for research findings on deep-sea microorganisms. At JAMSTEC, we also conduct applied research and commercialization of the physiological functions of deep-sea microorganisms, with a focus on enzymes. For example, through a collaborative research project on agarose-degrading enzymes from deep-sea microorganisms, a Japanese company, Nippon Gene Co., Ltd., commercialized a thermostable β-agarase as a reagent for research use in 2009. In addition, a new cellulose-degrading enzyme was discovered in Hirondellea gigas, a hadal amphipod living in the Challenger Deep in the Mariana Trench, the deepest point in the ocean in 2012, and this enzyme is currently being used for industrial applications. A number of other research projects aimed at industrial applications of enzymes from deep-sea microorganisms are in progress.

One for all, all for one

However, we realize that our research alone may be far from sufficient for realizing the full, overwhelming potential of hadal and subseafloor microbial communities, which are likely to be the richest on Earth in terms of genetic and species diversity. This is analogous to the management of a professional sports team that is incapable of training a youth with overwhelming physical potential to become an excellent professional player questioning its player development program (the practical application of research results).

Manned Research Submersible “SHINKAI 6500”

At JAMSTEC, we believe in the saying “one for all, all for one,” commonly used by team sports, such as rugby football, meaning that the whole team is indispensable for successfully realizing the potential of hadal and subseafloor microorganisms. Led by our motto “utilize the abilities and environment of JAMSTEC in research/survey to develop science and industry in Japan, to unite the research and development activities of academia and industry in Japan for full utilization of the potential of hadal and subseafloor microorganisms,” we wish to go enter an “open innovation” scrum for industry-academia collaborations. At JAMSTEC, we hope to help you, public organizations and private enterprises, exploit the vast microbial and genetic resources of the exclusive economic zone of Japan by making full use of our unique abilities in research and development to meet your needs. We hope to drive first-rate science that contributes to development and academic achievement in Japan. We look forward to hearing your ideas and proposals.


Hydrothemal Vent

Report

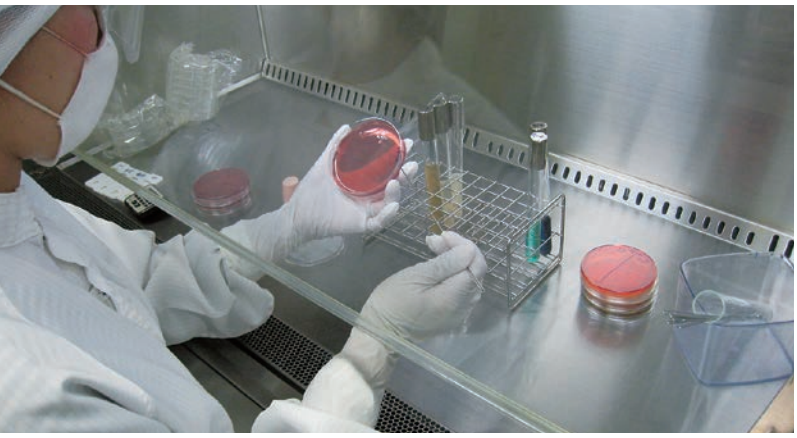
Outline for Revision of Japan's Specifications and Standards for Food Additives, 9th Edition with Related Considerations

In Japan, enzymes used in foods, either during food manufacturing or for the purpose of food processing or preservation, are categorized as food additives. Japan's Specifications and Standards for Food Additives, the official compendium defining the specifications and standards for food additives used in Japan, is currently being revised and many new enzymes will be added. An outline for the present revision and future tasks is provided below.

Japan's Specifications and Standards for Food Additives compile the specifications and standards for food additives used in Japan, based on the provisions of Article 21 of the Food Sanitation Act. Since publication of the 1st Edition in 1960, the text has been repeatedly revised until publication of the 8th Edition in 2007. The present revision is intended to compile specifications and standards for food additives newly defined after publication of the 8th Edition, to re-examine general tests and compositional specifications, and to define specifications for existing food additives.

Publication of Japan's Specifications and Standards for Food Additives, 9th Edition will greatly alter the regulation of enzymes. The current 8th Edition contains compositional standards for 5 enzymes of animal or plant origin (trypsin, papain, bromelain, pepsin, and lysozyme). Among the 89 food additives to be added to the revised 9th Edition, 62 are enzymes, which means that 67 of the 68 enzymes categorized as "existing food additives" will be formally included in Japan's Specifications and Standards for Food Additives, with the exception of isomaltodextranase.

The revision schedule for Japan's Specifications and Standards for Food Additives, 9th Edition, as well as an outline for revision of related considerations, is presented below.



①Revision schedule for Japan's Specifications and Standards for Food Additives, 9th Edition

March 1, 2013	Deliberation by Committee for Preparation of Japan's Specifications and Standards for Food Additives completed
June 14, 2016	Evaluation by Food Safety Commission completed
August 30, 2016	Deliberation and approval by Pharmaceutical Affairs and Food Sanitation Council, Food Sanitation Council, and Committee for Food Additives
December 1, 2016 to December 30, 2016	Public comment period
December 16, 2016 to February 14, 2017	WTO notification/Opinion request period
Autumn, 2017	Public notice in the official gazette

②Outline of Revision of Specifications/Standards for Enzymes Contained in Japan's Specifications and Standards for Food Additives, with Related Considerations

【Definition】

The origin, method of preparation, and definition of individual existing food additives were previously compiled in the List of Existing Food Additives, as reference information. Descriptions of the origin, method of preparation, and definition, once formally included in Japan's Specifications and Standards for Food Additives, will be legally binding. The origin, method of preparation, and definition of existing food additives to be included in Japan's Specifications and Standards for Food Additives will be defined according to the origin, method of preparation, and definition in the List of Existing Food Additives. However, concerning the origin, 1) information provided in the

aforementioned List, 2) information regarding items confirmed to be used for business during the period between publication of the aforementioned List and the survey performed for preparation of the draft specifications, and 3) information regarding self-cloning and natural occurrence will be included. In addition, the included enzymes will be defined as "enzyme ingredients," including those containing foods or food additives used for limited purposes, such as excipients and strength-controlling agents (changes to product label claims will be required).

[Description]

"White (colorless) to deep brown" will be added as a common color description, with the following exceptions: "colorless to dark green" for catalase (liquid), "white to light yellow" for glucose oxidase (solid), and "white to greenish white" for polyphenol oxidase (solid) (solid: powder, grains, paste).

[Purity Test]

(1) Lead: The specification for lead (as Pb) will not be changed from the voluntary specification (not more than 5 μg/g). The amount of test sample will be changed to 0.80 g. In cases where the residue after incineration for preparation of the test solution is insoluble in 5 mL of nitric acid (1 in 100), a chelate extraction step will be added (Method 3).

(2) Arsenic: The specification for arsenic will be changed from the voluntary specification (not more than 4 μg/g as As_2O_3) to not more than 3 μg/g as As. Regarding testing methods, Method 5 (a solution of magnesium nitrate in ethanol (95) (1 in 10)) will be added (amount of test sample, 0.50 g).

[Microbial limits]

The total bacterial count is not to exceed 50,000/g. Escherichia coli and Salmonella must be negative. Negativity for Escherichia coli and Salmonella will be added to the compositional specifications for lysozyme, already present in the 8th Edition (the specification of the total bacterial count will not be added). However, the specification of the total bacterial count will not be applied when the item, without disinfection, is used in foods to be self-consumed, and disinfection or sterilization is performed prior to completion of the final food product.

[Enzyme activity determination (Identification)]

For items already included in the 8th Edition, the specification of enzyme activity will remain in the 9th Edition. For items to be added to the 9th Edition, the enzyme activity will be determined qualitatively (Identification). If identification according to the described method is impossible, modification of substrate, sample dilution ratio, buffer, and reaction temperature may be allowed, but only when scientifically justified.

Future Tasks

Once Japan's Specifications and Standards for Food Additives, 9th Edition is in force, a business person manufacturing or processing enzymes contained therein will be obliged to acquire a food business license (as a food additive manufacturer) and employ a food sanitation supervisor, and this obligation will also impose limitations in selection of any subcontractor manufacturing or processing these enzymes. Furthermore, once compendial specifications for a particular item are defined, the item will be removed by government officials if it does not meet the specifications.

In addition, as a consequence of changes to the definition of enzymes introduced in the present revision (from "preparation" to "ingredient"), amendment of product labeling and delivery specifications will be needed. Furthermore, addition of origin after public notice of the revised version will hereafter require submission of application for a new designation, and this is expected to reduce the speed of development of new products. For enzymes of microbial origin, determining how to address changes in naming of source species resulting from advances in taxonomy and species identification methods is a task for the future.

Author

Tomonari Ogawa

Director, Quality Assurance Division

Symposium

2016 Sino-Japan Symposium on Biocatalysis and Biotransformation

In China and other parts of Asia, there is growing awareness of the problem of environmental pollution caused by the rapid development of chemical industries, and the importance of "Green Chemistry" is being stressed. Green Chemistry refers to the manufacture of chemicals that will have minimal adverse impact on humans, ecosystems, and the environment. Because enzymes are able to produce chemicals under mild conditions, they are becoming the focus of attention as a method for achieving Green Chemistry.

Program

1	Opening speech	 Prof. Yang Lirong, Zhejiang University
2	Speech on behalf of Amano Enzyme Inc.	 Dr. Shigeki Kimura, Amano Enzyme Inc.
3	Introduction of Amano Enzyme Inc., as a specialty enzyme producer.	 Dr. Shotaro Yamaguchi, Amano Enzyme Inc.
4	Screening and application of novel microbial enzymes for the production of useful compounds.	 Prof. Michihiko Kataoka, Osaka Prefecture University
5	Hierarchical Iterative Mutagenesis : Molecular Evolution of Phosphotriesterases.	 Prof. Xu Jianhe, East China University of Science and Technology
6	Identification, molecular evolution and high-level expression of novel lipase from Rhizopus chinensis.	 Prof. Xu Yan Jiangnan University  Prof. Yu Xiaowei Jiangnan University
7	Rationally Designed Lipases for Organic Synthesis.	 Prof. Tadashi Ema, Okayama University
8	Synthesis of L-phosphinothricin by biotechnology.	 Prof. Zheng Yuguo, Zhejiang University of Technology
9	Key technology of preparation of trehalose by one-step enzymatic catalytic method.	 Prof. Huang He, Nanjing Tech University
10	Tailor-made biocatalyst enzymes for industry.	 Prof. Yang Sheng, The Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences
11	Molecular mechanism: Enzymes and value-added chemicals via microbial catabolism of environmental N-heterocycles.	 Prof. Xu Ping, Shanghai Jiao Tong University
12	Biocatalysis technology applied in pesticide synthesis.	 Prof. Yang Lirong, Zhejiang University

The "Sino-Japan Symposium on Biocatalysis and Biotransformation" was held on December 10, 2016. The symposium was co-hosted by Amano Enzyme Inc., and achieved a successful turnout of nearly 100 participants.

Zhejiang University's Professor Yang Lirong held this symposium aim to bring together Chinese businesses with an interest in biotransformation using enzymes, along with professors from universities and public research institutions carrying out the latest research in this field.

Seven professors from Chinese universities and research institutions joined Professor Michihiko Kataoka of Osaka Prefecture University Graduate School and Professor Tadashi Ema of Okayama University, total of nine members, to lecture on the benefits of the enzymatic method, and discuss the latest research results. A great deal of interest was expressed by Chinese businesses recently focused on environmental problems, with lively Q&A and discussions held. It became abundantly clear that Amano can play a useful role in green chemistry through the enzymatic method.

Product introduction

Chiral Enzyme Spectrum "Amano"

The Chiral Enzyme Spectrum "Amano" is an enzyme kit used in the division and synthesis of chiral compound, a key in the production of intermediates and other useful compounds. Amano Enzyme is offering it free of charge.

The kit currently contains 17 types of enzymes, with a focus on hydrolytic enzymes such as Lipase PS, Esterase, and Protease, as well as Transaminase and Nitrilase. Additional enzymes will be added as necessary for samples under development. The kit is provided to those engaged in enzymatic R&D activities, who can use it to screen suitable enzymes for the conversion of desired chemical compounds. We are also working together with clients on research to further enhance the properties of hit enzymes.

Information

Introduction to the Amano-style aerated koji-making process

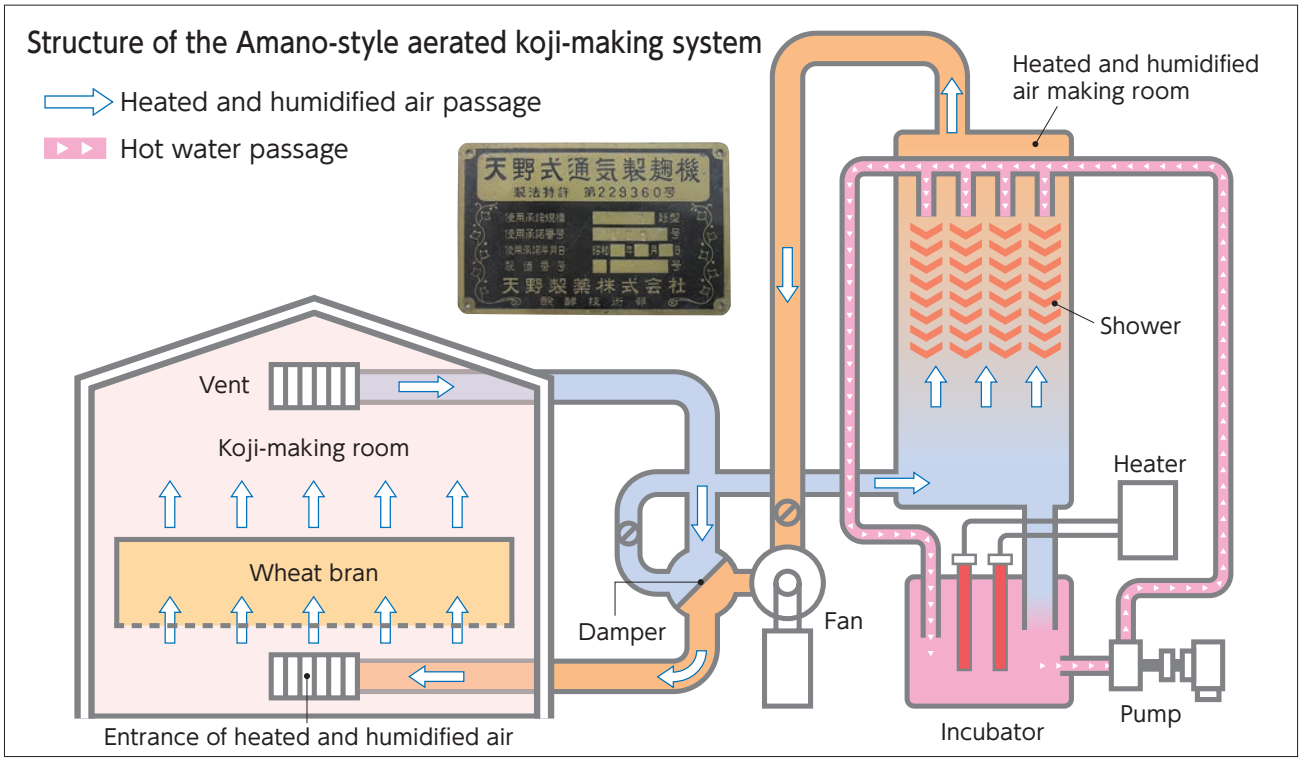
~ From our Histrical Archive of Enzymology ~

At Amano Enzyme we are continuously developing enzyme products of various origins, including microorganisms. We previously developed a koji-making process, a method for culturing microorganisms, as a key process in solid-state fermentation. Here, we provide an overview of some of the major events in our history of the development of the koji-making process.

At around 1955, Japan was entering an era of high economic growth. In the enzyme industry, many new enzymes were discovered during this time, and attempts for their practical application were actively pursued.

Since koji-making had been traditionally considered to be a difficult process, we started our investigation with the aim of developing a new method for koji-making that is labor saving and ensures high enzyme production for practical applications. Since this was an unprecedented attempt, the field was entirely new to our staff in those days, and therefore the entire process involved exploration of the unknown. Nevertheless, after overcoming many difficulties, the development project proved to be successful and the result was the practical realization of a new method for koji-making. This method, called the Amano-style aerated koji-making process, has a number of unique features, including (1) easy koji-making operation; (2) assurance of constant product quality, regardless of the experience and skill of workers; (3) high enzyme production; and (4) a reduced number of workers required for koji-making. In response to a patent application

Advertisement of the JYOKAI TIMES (July, 1960)



filed in 1955, this method was granted a patent in 1957.

The outline of this method is illustrated in the figure (Structure of the Amano-style aerated koji-making system). Heated air flow (prepared by showering air for aeration with water maintained at a constant temperature) is delivered to the koji-making room. In the koji-making room, a layer of wheat bran (approximately 20-cm thick), inoculated with an enzyme-producing mold and mixed well, is uniformly spread over a stainless-steel plate with evenly distributed small holes. Heated air flow under a constant temperature passes through the holes, which enables koji making under constant temperature and humidity conditions that are optimal for enzyme production. In contrast to the conventional koji-making method that relies on the spontaneous growth of koji mold, this method enables the artificial control of koji mold growth and enzyme production.

Since this method was subsequently proven to be applicable for the production of sake (Japanese rice wine) as well as miso (fermented soybean paste) and soy sauce, this triggered many requests for patent licensing, and thus our patent was licensed to brewing companies. Some brewing companies are still using this method to make koji for miso and soy sauce production.

In this way, from the early days in our history, we at Amano Enzyme have continued our efforts to address challenges and to provide new technologies and products that contribute to society.

Amano Enzyme Message Board

We look forward to seeing at the exhibition.

Exhibition Information

Date	Exhibition	Location
May 24~26, 2017	**Ifia Japan 2017**	Tokyo, Japan
Jun 20~22, 2017	**CPhI China 2017**	Shanghai, China
Jun 25~28, 2017	**IFT 17**	Las Vegas, USA
September 13~15, 2017	**Fi Asia 2017**	Bangkok, Thai
October 24~26, 2017	**CPhI Worldwide 2017**	Frankfurt, Germany
November 28~30, 2017	**Fi Europe 2017**	Frankfurt, Germany

Please check the latest information on the website.

Amano Enzyme participation status at 2016 exhibitions

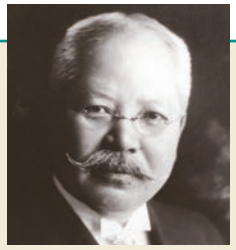
During 2016, Amano Enzyme exhibited at various pharmaceutical and food industry-related trade shows at several locations around the world. (Japan, China, Indonesia, Germany, Spain, Mexico, and the U.S.A)

Information

The Prof. Jokichi Takamine Research Foundation, Activity News

Professor Jokichi Takamine

Living in the turbulent times of the late 19th and early 20th centuries, Jokichi Takamine left his mark not only as a scientist and entrepreneur but also in international diplomacy. He is known as the father of modern biotechnology due to his research and development of microbially-derived starch degrading enzymes centered around Takadiastase.



Dr. Jokichi Takamine
(photo courtesy Great People of Kanazawa Memorial Museum)

Professor Jokichi Takamine Research Foundation

The Prof. Jokichi Takamine Research Foundation conducts mainly education-oriented activities such as issuing newsletters and holding lectures aimed at raising awareness about Professor Jokichi Takamine, who made a significant contribution to the development and commercialization of science and technology in modern Japan and to the friendship between Japan and the United States.

Main activities

In fiscal 2016 we held a total of six lectures extending from Tokyo and Kanagawa to Ishikawa, Toyama and Kyoto and attended by more than 1,000 people in all. We have also received a steadily increasing number of inquiries from newspapers, book publishers, television companies, and so on, whom we have provided with materials and information.

In its 2010 Chemical Heritage Issue 002, the Chemical Society of Japan recognized the laboratory notebook of Keizo Uenaka, a research partner of Professor Takamine, in which he recorded the world's first crystallization of the hormone adrenaline.

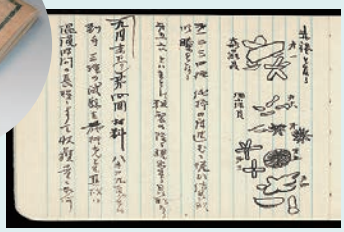
A reprint (replica) was used as material for the lecture.

Following on from last year's scientific biographic manga The Jokichi Takamine Story, we have also published a second book titled This is just the beginning, Jokichi! Story of his childhood (Not for general sale, only distributed to members, educational institutions, lecture participants, etc.)

We will continue to devote ourselves to such activities in future and kindly ask for your ongoing support and cooperation.

Takaoka City Junior High School lecture
(held annually, 9th school)

Notebook
of Keizo Uenaka



Adrenaline crystals illustrated

The Jokichi Takamine Story,
a scientific biographical manga

The Jokichi Takamine Story
of his childhood
This is just the beginning, Jokichi!

Recruitment of new members

Our research foundation widely recruits people who agree with our goals. All members receive publications related to Professor Takamine and a regularly issued journal, along with invitations to various talks and events and other newly obtained information. If you would like to join, please apply by letter including your name (in the case of corporations, your company name and department name), address with postcode, telephone number (landline only), profession, age and gender. We will reply with a payment slip for your initial membership fee and annual membership fee.

The Secretariat

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*For further details, visit our website : http://www.npo-takamine.org/ask.html

Conference presentation, Journal and Book

In 2016 Amano Enzyme has decided academic presentations such as the following.
Please look forward to the activities of the future of Amano Enzyme.

Conference/Meeting	Date	Title	Speaker
The 2016 Annual Meeting, the Institute of Electrical Engineers of Japan	Mar. 16th-18th, 2016 (Sendai, Japan)	Study of activated conditions for enzyme by high voltage pulsed electric field.	Saito, S., Minamitani, K., Sugiura, T.
70th Annual Meeting of Japan Society of Nutrition and Food Science	May. 14th, 2016 (Nishinomiya, Japan)	Novel function of digestive enzyme —Effect of protease on intestinal flora— (Luncheon seminar sponsored by Amano Enzyme)	Kato, N., Kuroda, M. Chaired by Yamaguchi, S.
Joint Technical Meeting on "Plasma Science and technology", "Pulsed Power Technology" and "Electrical Discharges", the Institute of Electrical Engineers of Japan	May. 26th-28th, 2016 (Morioka, Japan)	Investigation of the method for extracting β-galactosidase by pulsed high electric field.	Saito, S., Minamitani, K., Sugiura, T.
18th Workshop of Applied Glycoscience	May. 27th, 2016 (Osaka, Japan)	Industrial carbohydrate-related enzymes and applications thereof.	Okada, M.
47th Annual Meeting of Japan Pancreas Society 20th Meeting of International Association of Pancreatology 6th Meeting of Asian Oceanic Pancreatic Association	Aug. 5th, 2016 (Sendai, Japan)	Pancreatic enzyme and insulin replacement therapy for patients with pancreatic diseases. (Luncheon seminar sponsored by Amano Enzyme)	Tando, Y. Chaired by Nakamura, T.
		Comparison of total enzymatic activities of digestive enzyme formulas with the digestive tract model.	Kuroda, M., Ko, S.
5th International Conference on Cofactors & Active Enzyme Molecule 2016	Sep. 5th, 2016 (Toyama, Japan)	Introduction of Amano Enzyme Inc. and its biotransformation enzymes. The application of lipase in the manufacture of fine chemicals. (Luncheon seminar sponsored by Amano Enzyme)	Yan, L. Chaired by Yamaguchi, S.
The 10th Symposium on Biorelevant Chemistry, The Chemical Society of Japan	Sep. 7th-9th, 2016 (Kanazawa, Japan)	Creation of thermostable variants of *burkholderia cepacia* lipase (BCL)	Yoshida, K., Koikeda, S., Ema, T.
13th Meeting of Japanese Society of Hospital General Medicine	Sep. 16th, 2016 (Tokyo, Japan)	Digestive enzyme therapy for outpatients of endocrine pancreatic insufficiency, exocrine pancreatic insufficiency and lactose intolerance. (How to use pancreatin formulas and lactase formulas) (Luncheon seminar sponsored by Amano Enzyme)	Nakamura, T. Chaired by Takeuchi, T.
24th Annual Meeting of Japan Digestive Disease Week (JDDW2016)	Nov. 3th, 2016 (Kobe, Japan)	Digestive enzymes for medicine —Visiting old, Learn new— Proper usage of digestive enzymes for medicine based on latest research. (Satellite symposium by Amano Enzyme)	Ko, S. Chaired by Shimosegawa, T.
		Digestive activity of digestive enzyme formulas with the digestive tract model.	Kuroda, M., Ko, S.
Asian Pacific Digestive Week 2016 (APDW2016)	Nov. 4th, 2016 (Kobe, Japan)	Comparison of total enzymatic activities of digestive enzyme formulas with the digestive tract model.	Kuroda, M., Ko, S.
9th Hokuriku District Bio Symposium	Nov. 4th-5th, 2016 (Fukui, Japan)	Industrial enzyme ;learning from history	Koikeda, S.
The 3rd Myanmar-Japan Symposium	Dec. 3th-4th, 2016 (Pathein, Myanmar)	Unlimited potential of microorganisms and their enzymes.	Yuki, K.
2016 Sino-Japan Symposium on Biocatalisis and Biotransformation	Dec. 10th, 2016 (Hangzhou, China)	Introduction of Amano Enzyme Inc., as a speciality enzyme producer.	Yamaguchi, S.
The 18th Symposium on the Chemistry of Biocatalysis, The Society of Biocatalysis Japan	Dec. 21th-22th, 2016 (Tokyo, Japan)	Creation of *burkholderia cepacia* lipase (BCL) variants and their thermostability, acid resistance, and organic solvent tolerance.	Yoshida, K., Koikeda, S., Ema, T.

Journal / Book	Date	Title	Author
Journal of Biliary Tract & Pancreas 2016, Vol.37, No.2, p.163-169	Feb, 2016	Difference in measurement method for activity of digestive enzyme and digestive formulas between in Japan and in the West.	Ko, S., Kuroda, M
Digestion and Absorption 2016, Vol.38, No.2, p.118-125	May, 2016	Comparison of digestive activity of digestive enzyme formulas approved Japan	Kuroda, M., Ko, S.
Medical Tribune 10/27	Oct, 2016	Digestive enzyme formulas — How can we master old medicine in modern medical practice? (Article sponsored by Amano Enzyme)	Ishiguro, H., Tando, Y., Ko, S., Chaired by Nakamura, T.
Bioscience, Biotechnology, and Biochemistry Vol.81, p.54-58, 2017	Dec. 2016	The quest for industrial enzymes from microorganisms.	Yamaguchi, S.

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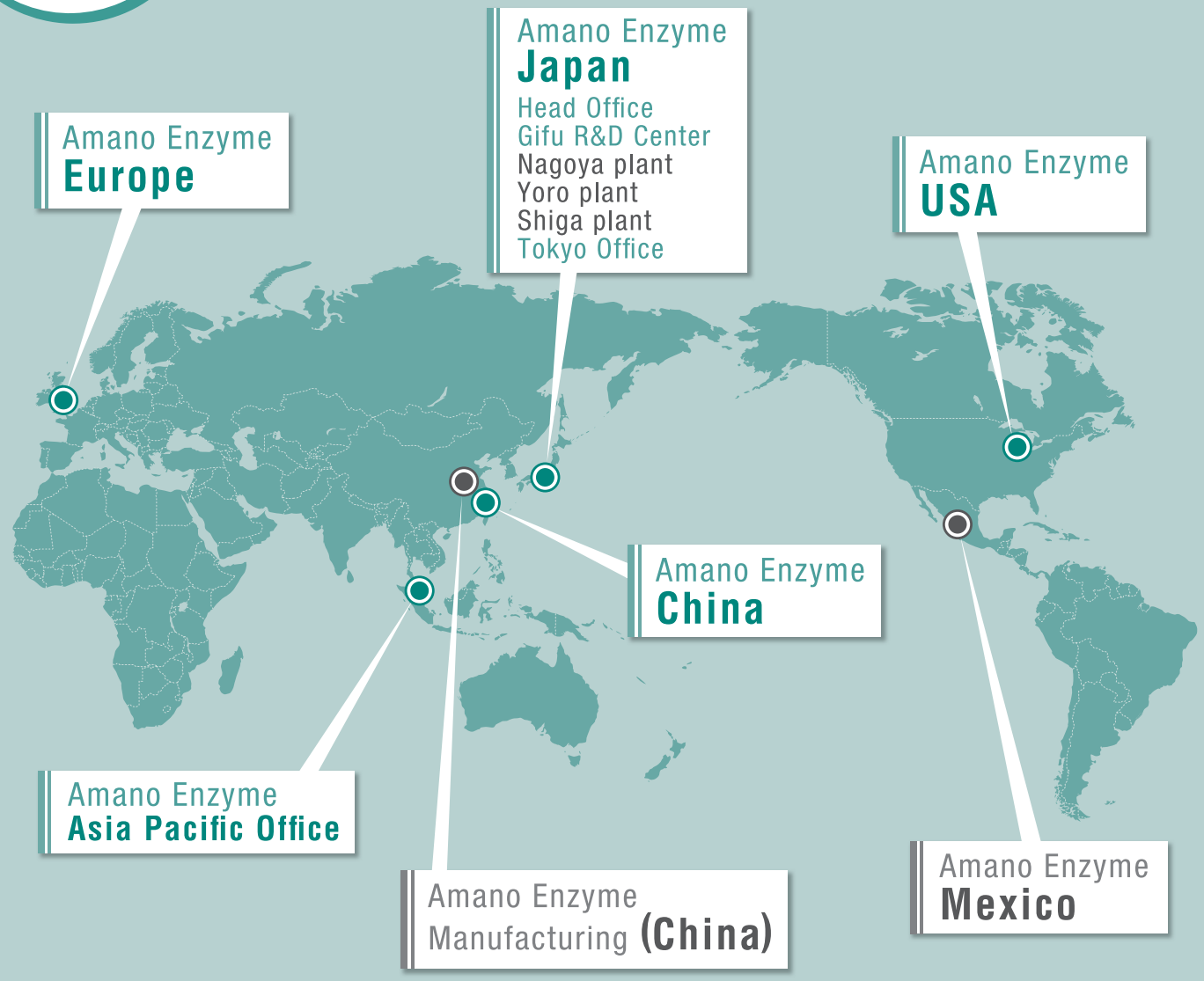
Amano Enzyme

World Network



AMANO

Enzyme-Explore Unlimited Possibilities

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

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