

## Enzyme Wave vol.27

#### Trend

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Cellulose Degradation, a World in Which Oxidoreductase Enzymes Assist Hydrolase Enzymes

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## Trend

## Cellulose Degradation, a World in Which Oxidoreductase Enzymes Assist Hydrolase Enzymes

#### Author

#### Kiyohiko Igarashi

Professor, Forest Chemistry Laboratory, Department of Biomaterial Sciences, Graduate School of Agricultural and Life Sciences, the University of Tokyo

#### [Brief background]

Igarashi became a research assistant at the Graduate School of Agricultural and Life Sciences at the University of Tokyo in 2002, an assistant professor in 2007, an associate professor in 2009, and has been in his current position



since 2021. Since 2016, he has also worked as a visiting professor at the VTT Technical Research Centre of Finland Ltd., Finland. He is a leader in research on producing energy and materials from trees and grasses. He has served on numerous committees, including review committees for major projects, promotion committees, and evaluation committees for the Ministry of the Environment, the Ministry of Agriculture, Forestry and Fisheries, the Cabinet Office of Japan, and others.

Although studies on enzymatic cellulose degradation have been conducted since the late 19th century, the mechanistic research was first reported by Reese et al. in 1950<sup>1)</sup>. Reese et al. proposed the "C1-Cx theory," in which cellulose is first hydrated by a component called C1, and then the hydrated cellulose is hydrolyzed by an enzyme called the Cx component. However, in the 20 years following the report, the enzyme corresponding to the C1 component was never discovered. As a result, the Endo-Exo theory, in which Endo-type enzymes degrade amorphous cellulose then subsequently Exo-type cellulases degrade cellulose from the ends came to be widely accepted. However, in the 1970's, a paper by Eriksson et al. found that cellulose degradation would advance faster with oxygen and proposed the theory of the "oxidative boost."<sup>2)</sup> In the 21st century, the enzymes

"lytic polysaccharide monooxygenases (LPMOs)" were discovered to be the reason for this. LPMO contains copper atoms, which could be used to oxidatively split the surface of crystalline cellulose and cause other enzymes to react. We have recently shown through high-speed atomic force microscopy that LPMO (AA9D) produced by Phanerochaete *chrysosporium*, a type of wood-rotting fungi, increases the activity of other hydrolytic enzymes while producing only a small amount of the oxidized oligosaccharides, and increases the number of hydrolytic enzyme molecules moving on the cellulose surface.<sup>3)</sup> This finding proves that the three previously discussed theories, C1-Cx, Endo-Exo, and oxidative boost, are simply different ways of looking at the same phenomenon, ending a 70-year debate. Considering the efficiency of cellulose degradation that such organisms can conduct in nature, it is thought to be possible to make cellulose degradation more efficient. Increased efficiency of cellulose degradation is expected to lead to effective use of biomass. Learning from nature will be important for human beings in its endeavor to achieve a sustainable circular society.

#### References

Reese *et al. J. Bacteriol.* 59(4), 485-497 (1950)
 Eriksson *et al. FEBS Lett.* 49(2), 282-285 (1974)
 Uchiyama *et al. Science Adv.* 8, eade5155 (2022)



Comic strip showing cellulose degradation by enzymes (© Natsumi Furuyama)

Column

## **Business Hint from Kyudo**

#### Author

#### Jérôme Chouchan

Representative Director & CEO of Orchid Inc. [Brief background] Mr. Chouchan has been the Representative Director & CEO of Godiva

Japan, Inc. since 2010, the President of the French Chamber of Commerce in Japan since 2022, and he is currently the Representative Director & CEO of Orchid Inc., the parent company of Godiva Japan, Inc., and Pierre Marcolini Group. He has a profound knowledge of Japanese culture and



holds a Kyudo Renshi (Instructor license) with a 5th Dan degree. He is currently a Board Director of the Kyudo International Federation. He has a Master of Management from the French Leading Business School: HEC Paris.

In the "Biotechnology in Japan" series, we share contributions on Japanese culture and traditions. In this fifth installment, Jérôme Chouchan, the Representative Director & CEO of Godiva Japan, Inc., who has been managing luxury brands for over 30 years in the international market, particularly in Japan and Asia, writes about business tips from the teachings of Kyudo, the Japanese martial art.

I began practicing Kyudo at the age of 29, and since then, I have strongly felt that there are commonalities between Kyudo and business. While Kyudo offers various teachings, the spirit of "Seisha Hicchū" (正射必中), meaning "the right shoot always results in a hit" has proven to be particularly valuable in my approach to business.

In Kyudo, the "target" corresponds to the business goal of sales and profits. "Seisha Hicchū" emphasizes that a correctly shot arrow will invariably hit the target. However, just as one can fail to hit target by being overly fixated on hitting the center of the target and neglect the correct "form" of shooting the arrow, a business can falter if too focused on sales and profits. Succumbing to the pressure of numbers may lead to losing sight of the essence of strategy and, consequently, missing the mark.

Simultaneously, the principle of "Seisha Hicchū" entails the indispensable elements of preparation and concentration. When releasing the bowstring, it is essential to maintain the correct posture and focus. This parallels the business world, where preparation and concentration are key to success. I believe that prioritizing the creation of products and high-quality services for customers, constructing and implementing strategies to achieve this, is crucial for a company's growth.

Our company has a mission to "We Create Memorable Occasion of Happiness" I have adopted this mission as my 'Seisha,' and I contemplated that for Godiva to provide happiness to customers, the brand should be both "aspirational" and "approachable." While the previous image of the Godiva brand was strongly associated with "special" and "luxurious," I aimed to develop products, distribution channels, and formats that maintain quality while making the brand more relatable, thereby narrowing the distance with customers.

Another important teaching I value is the concept of "Isshā Isshā" (一射一射), which translates to "One Shot at a Time." This attitude involves approaching each shot without being influenced by past successes. In business, it translates to consistently facing customers with a fresh mindset without being bound by past achievements. Despite the natural inclination to repeat what has worked before, approaching product development, marketing strategies, and engagements with business partners with a renewed dedication each time is a vital process for achieving successful outcomes.

In Kyudo practice, my instructor only observes my posture while drawing the bow. No comments are made about whether the arrow hits the target or not. Strangely, by consistently concentrating on shooting with a renewed mindset and correct posture, positive results follow. Business operates in a similar manner, where focusing on the process, valuing customer experiences, and giving one's best effort are crucial.

In two years, Godiva will celebrate its 100th anniversary. With the spirit of "Seisha" to deliver gratitude and memorable happiness to customers, I will shoot each arrow with heartfelt dedication. Please look forward to the future of Godiva. Additionally, in my life, Kyudo has provided numerous guidance and insights. I aspire to contribute, albeit modestly, to the global promotion of Kyudo as a way of expressing my gratitude.



#### Enzyme Wave 2024 AMANO

#### The 1st North America-Japan Enzyme Symposium **Technology Symposium**

On May 5, 2023, the University of Minnesota and Amano Enzyme co-hosted the 1st North America-Japan Enzyme Technology Symposium in Minneapolis, Minnesota, United States. The themes for the first symposium were biotransformation and food technology. Both of these themes address two major challenges the world faces, environmental conservation and the global food crisis. Around 70 people gathered at the University of Minnesota for a lively exchange of ideas.

Ten researchers were invited from Japan, the US, Canada, and Denmark to present their latest findings in the invited lectures (shown on the right). Much to the interest of the audience, a wide range of enzyme research, from the basic enzyme research to food applications, was presented. Some of the lectures are available on the University of Minnesota's YouTube channel (https://www.youtube.com/@biotechnologyinstitut e8954/).

At a poster session, young researchers were given the opportunity to present their findings, with the most outstanding poster receiving an award. At a panel discussion focused on career design for graduate students, a moderator led a discussion with four speakers in which they discussed their career transition points.

We would like to thank Prof. Romas J. Kazlauskas, Prof. Claudia Schmidt-Dannert, and Prof. B. Pam Ismail from the University of Minnesota for their support in organizing the symposium. Amano Enzyme will continue to regularly host symposia in the US and around the world to encourage and advance enzyme research.



Speaker	Affiliation
Jun Ogawa	Kyoto University
Claudia Schmidt-Dannert	University of Minnesota
Todd Hyster	Cornell University (presently Princeton University)
Emma Master	University of Toronto
Stefan Lutz	Codexis, Inc.
Tomoko Matsuda	Tokyo Institute of Technology
Gregg Whited	International Flavors & Fragrances Inc
B. Pam Ismail	University of Minnesota
Keita Okuda	Amano Enzyme Inc.
Anne S. Meyer	Technical University of Denmark

## We are Supporting Athlete

In September 2022, Haruna Baba, a member of the Japanese national women's fencing epee team, joined Amano Enzyme.

Baba has our full support as a member of our company and we hope to see her achieve even greater heights in fencing.

Profile

Name	Haruna Baba
Sport	Fencing epee
Birthplace	Gifu Prefecture
Affiliation	General Affairs Division

#### Notable Results

2019	World Cup Satellite Costa Rica, champion
2022	Asian Championships 2022 Team epee, Bronze Medal
	Asian Championships 2023 Team epee, Bronze Medal
2023	World Championships 2023 Individual and team
	epee, participated
2023	19th Asian Games Team epee, Bronze Medal
	Mandal Cons Catallita Constin Deserve Mandal

2023 World Cup Satellite Croatia, Bronze Medal



The Asian Championships 2023. Haruna Baba is first from the right. ©Japanese Fencing Federation : Augusto Bizzi/FIE

#### Enzyme Wave 2024 AMANO

## Report 3rd Japan-Switzerland-Germany Workshop on Biocatalysis and Bioprocess Development

The 3rd Japan-Switzerland-Germany Workshop on Biocatalysis and Bioprocess Development was held from September 10 to 13, 2023. With a long history, the workshop originally began as a bilateral meeting between Japan and Germany in 1978 and Japan and Switzerland in 1988. The two were eventually integrated in 2019. This year's workshop, the third one ever, was held with support from Amano Enzyme in Inuyama, Aichi, Japan, which itself has a long history.

A total of 96 people attended the event, including 22 from overseas. 42 talks were given with another 30 poster presentations. A wide range of topics were discussed, from the development of enzymes to new applications for them. Amano Enzyme gave one talk and two poster presentations. Many presentations reported the latest AI technology applications and provided an exciting look at examples of combining traditional and new technologies. The workshop was an opportunity for us to reaffirm the incredible potential of biocatalysts (enzymes), substances that have long attracted researchers the world over.

During the event period, an excursion was organized mainly for attendees from overseas. The tour saw the group go Ukai cormorant fishing on the Kiso River and explore the Japanese national treasure Inuyama Castle, before enjoying matcha green tea in the national treasure tea house Jo-an in the Urakuen Japanese Garden and visiting IMASEN Inuyama Karakuri Museum, for a special presentation about Karakuri dolls from Tamaya Shobei. Attendees were also invited to tour the Amano Enzyme Innovation Center.

The fourth workshop will be held in Europe in 2025 and the fifth in Japan in 2027. We will continue to support the workshop and make every effort to contribute to this historic event as one of Japan's leading enzyme manufacturers.









#### Enzyme Wave 2024 AMANO

## Symposium The 7th Sino-Japan Joint Symposium on Enzyme Technology

The 7th Sino-Japan Joint Symposium on Enzyme Technology was held on December 2, 2023. This symposium has been held every other year since 2011, co-hosted by Jiangnan University (Wuxi, Jiangsu, China) and Amano Enzyme, with the aim of "contributing to the promotion of enzyme applications in Asia through exchanges between Chinese and Japanese researchers related to enzymes." While the last symposium was held as a hybrid online event due to the pandemic, this year, it was held on-site in Jiaxing, China.

With 125 participants from universities, research institutes, and companies, the symposium was held around the theme of "the latest trends in enzymes for food and beverage applications." Eight talks were held by speakers from China and Japan, including lectures by Professor Toru Nakayama (Tohoku University) and Associate Professor Wataru Saburi (Hokkaido University) from Japan.

The talks featured a wide variety of topics, from the current state of the beverage market in China and the application of enzymes in foods to protein engineering and AI. The event ended on a high note with a lively debate being had at the panel discussion. Speakers and lecture titles are listed in the table below.

By continuing to hold this symposium, Amano Enzyme will do its best to deepen the technical exchange between China and Japan, and to develop the enzyme industry.



Group photo



Opening remarks (President Amano)





Professor Nakayama

Associate Professor Saburi

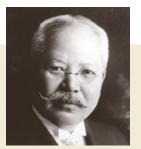
Speaker	Affiliation	Title
Yi Cheng	China Beverage Industry Association	Introduction to the current situation of China's beverage industry
Toru Nakayama	Tohoku University	Biosynthesis of flavonoids responsible for coloration and health benefits of foods
Wataru Saburi	Hokkaido University	Development of functional foodstuffs produced using carbohydrate-metabolizing enzymes
Fang Zhong	Jiangnan University	Application of lipase and protease for cheese flavoring
Jianhui Ye	Zhejiang University	Research on the application of enzymes in tea beverages
Kan Yamashiro	Amano Enzyme Inc.	Enzyme Application in Beverage Industry
Gaowei Zheng	East China University of Science and Technology	Protein engineering technologies for enzyme evolution
Yifei Zhang	Beijing University of Chemical Technology	Engineering of Enzyme Catalysts; From Nature and Going Beyond

## Information

## Jokichi Takamine Study Group, NPO

## Dr. Jokichi Takamine

Dr. Jokichi Takamine lived through the dramatic period of time from the end of the Tokugawa shogunate, Meiji era, and Taisho era. He left a great legacy as a scientist, entrepreneur, and an international goodwill ambassador. Dr. Takamine is called the father of modern biotechnology for his research and development of amylolytic enzymes derived from microorganisms, mainly Taka-Diastase.



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

#### Jokichi Takamine Study Group, NPO

The NPO, Dr. Jokichi Takamine Study Group, is engaged in educational activities, such as publishing journals and holding lectures, in order to make more people aware of Dr. Takamine, who made a great contribution to the development of science and technology in modern Japan, its commercialization, and goodwill between Japan and the United States.

#### Main Activities

The year 2023 saw the pandemic begin to subside, and with it, our lectures at educational institutions in the Hokuriku region return for the first time in four years. Furthermore, Kanazawa Institute of Technology students traveled to a junior high school in Tokyo to co-host and instruct students in the Science Experiment Classroom, creating opportunities for young people to become interested in biotechnology.

This year also marked the 100th anniversary of the opening of Unazuki Onsen, one of Toyama Prefecture's most prominent hot spring resorts. As Dr. Takamine engaged in its development, the Study Group was invited to participate in the opening ceremony. These were only some of the many events that were held in 2023. Interest in Dr. Takamine has been growing as of late, so we hope to continue to provide accurate information and support educational opportunities in 2024.

## Topic Measures to keep young people interested in science

There are 11 junior high schools in Takaoka, Toyama, Japan. The Study Group has been holding talks since 2008, but this was the first time returning to this school in 14 years. It is deeply moving to think that the children who attended the talk back then are now working adults.

This will be the ninth year giving lectures at the Kanazawa Institute of Technology. Our long-term, continuous activities have focused on Takaoka and Kanazawa, Dr. Takamine's hometowns, but we also give talks utilizing scientific and biographical cartoons and videos at educational institutions throughout Japan. Biotechnology has become ever more important in this time of drastically changing lifestyles. If you are interested in holding a talk, please contact our office.



Our lectures at the Kanazawa Institute of Technology are included as part of its course.



Nakada Junior High School in Takaoka seems to have a very active science club.



The Science Experiment Classroom is also a learning experience for the university students who teach the classes.

Scan the QR code to visit the Study Group's website.



#### Notification of the recruitment of new members

The Jokichi Takamine Study Group is accepting supporting members.

Supporting members will receive publications related to Dr. Takamine and regularly issued newsletters. They also receive information about lectures, events, and news.

If you would like to join us, please write your name (if you are a corporate body, company/organization name and department), postal code, address, phone number (no cell phone number accepted), occupation, age, and sex on a letter or a postcard and mail it to us. We will return a bank transfer form to pay the initiation fee and the annual membership fee.

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\*For more details, please visit our website. https://npo-takamine.org/membership/

## Report

## **Enzyme Use in Plant-Based Meat Alternatives**

Due to an imbalance in the supply and demand of protein caused by population growth, the world is currently facing a protein crisis. To overcome this issue and achieve a sustainable society, attention is being turned to the development of "plant-based meat alternatives" that recreate the delicious taste of meat using soybeans and other plant materials. The industry has seen remarkable technological innovations, but at the same time, food manufacturers must respond to the needs of clean-label-conscious consumers who call for the use of fewer food additives. Enzyme technology is being utilized to address the challenge of food additives and develop clean-label, high-quality plant-based meat alternatives. This section outlines a new technological approach developed by Amano Enzyme to address three technical challenges in the production of plant-based meat alternatives: Binding, color, and the off-flavor of soybeans.

First is about binding. Methylcellulose, a chemical binding agent, is used in plant-based meat alternatives. As such, a chemical-free binding solution is required. A new protein crosslinking solution developed by Amano Enzyme uses a sugar beet pectin and laccase to improve the shape retention and binding properties of meat alternatives without chemical binding agents (Figure 1).

Next, to address the issue of recreating the browning process of real meat in meat alternatives during cooking, we developed a technology where laccase reacts with food coloring (beet red) to turn brown when cooked (Figure 2). This reproduces the same color variation as meat and improves the visual appeal of plant-based meat alternatives.

Finally, in order to reduce the unpleasant off-flavor of soybeans often found in plant-based meat alternatives, we have developed a technology that uses enzymes to reduce the volatility of the causative compounds. By developing plant-based meat alternatives with fewer off-flavors, the alternatives are expected to be more accepted by a broader consumer base.

Using these enzyme-based technologies, we are taking steps to improve the quality of plant-based meat alternatives and achieve a future with a sustainable food supply.

#### Figure 1

#### Proteins bound with laccase Shows plant protein binding in the same way as with methylcellulose

Methylcellulose	Without	With	Without
Laccase + Sugar beet pectin	Without	Without	With

#### Figure 2

#### Color change in meat alternative Shows browning of the color during the cooking process due to an enzymatic reaction



Before cooking



After cooking

## **Conference presentation**

Amano Enzyme has decided academic presentations such as the following.

Conference/Meeting	Date	Title	Speaker
23 <sup>rd</sup> Kansai Glycoscience Forum	May 20th, 2023 (Osaka, Japan)	Carbohydrate-active enzymes contributing to the achievement of the SDGs	Kan Yamashiro
75 <sup>th</sup> SBJ Annual Meeting KSBB-BEST-SBJ JOINT SYMPOSIUM	Sep. 4 <sup>th</sup> , 2023 (Aichi, Japan)	Utilization of Genome Information in Enzyme Industry Atoshi Koikeda and Yasuo Ohnishi	
		Improvement of industrial enzymes by knowledge-based protein-engineering	Satoru Ishihara, Kazunori Yoshida Atsushi Ohno, Satoshi Koikeda, and Shotaro Yamaguchi
<sup>3rd</sup> Japan-Switzerland-GermanyWorkshop on Biocatalysis and Bioprocess Development	Sep. 10 <sup>th</sup> -13 <sup>th</sup> , 2023 (Aichi, Japan)	Visible light driven enzymatic CO2 fixation using the NADH regeneration system of water-soluble zinc porphyrin and homogeneous colloidal rhodium nanoparticles	Takayuki Katagiri, Yutaka Amano
		Regulation of multidrug efflux and secondary metabolism by TetR family transcriptional repressor in Streptomyces coelicolor A3(2)	Yukun Lei, Shumpei Asamizu, and Hiroyasu Onaka
NGS Expo 2023	Nov. 15 <sup>th</sup> -16 <sup>th</sup> , 2023 (Osaka, Japan, Hybrid)	Identification and application of useful mutation points for production from genome analysis of industrial enzyme-producing strains made by mutation breeding.	Hirotaka Matsubara, Satoshi Koikeda and Yasuo Ohnishi
<sup>3rd</sup> Amano Enzyme Research Grant Presentation	Nov. 17 <sup>th</sup> , 2023 (Aichi, Japan, Hybrid)	Application of microdroplet technology to industrial enzyme development Kazunori Yoshida	
The BACELL meeting 2023	Nov. 20 <sup>th</sup> -23 <sup>rd</sup> , 2023 (Kobe, Japan)	Genome analysis of Bacillus amyloliquefaciens industrial strains and its application to heterologous protein production	Hirotaka Matsubara, Satoshi Koikeda and Yasuo Ohnishi
ISISM13-ISIB10-ACM20 (13 <sup>th</sup> International Symposium of Indonesian Society for Microbiology)	Nov. 27th-29th, 2023 (Bogor, Indonesia)	Development of Microbial Enzymes for Accelerating Sustainable Bioeconomy Shotaro Yamaguchi	
7 <sup>th</sup> Sino-Japan Joint Symposium on Enzyme Technology	Dec. 2 <sup>nd</sup> , 2023 (Jiaxing, China)	Enzyme application in beverage industry Kan Yamaguchi	
9 <sup>th</sup> International Food Convention	Dec. 7th-10th, 2023 (Mysuru, India)	Amano Speciality enzymes towards a greener and safer food society Shotaro Yamaguchi	
17 <sup>th</sup> International Conference on Polymer Science and Technology	Dec. 10 <sup>th</sup> -13 <sup>th</sup> , 2023 (Guwahati, India)	Speciality Enzymes for GREENER & SAFER BIOECONOMY: Its Challenges towards Biopolymer Interventions	
The Japanese Society for Food Science and Technology, Chubu Symposium 2023	Dec. 16 <sup>th</sup> , 2023 (Aichi, Japan)	Color change in plant-based meat analogs by laccase and beet red pigments Kiyota Sakai	

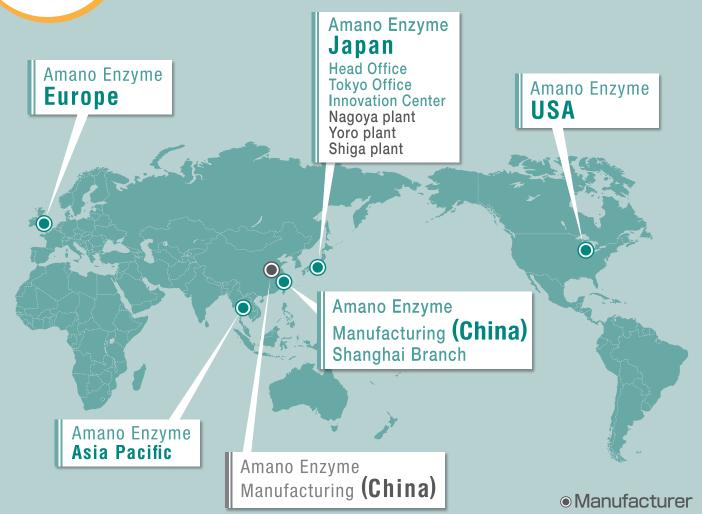
Journal/Book	Date	Title	Author
Catalysts and Catalysis Vol.65 No.5	Oct. 2023	Contribution of Industrial Enzyme to Development of Production Process with High Efficient Energy Conversion	Takayuki Katagiri, Satoshi Koikeda
PLOS ONE 2023, Vol.18 No.12 e0294637	Dec. 2023	Protein-glutaminase improves water-/oil-holding capacity and beany off-flavor profiles of plant-based meat analogs	Kiyota Sakai, Masamichı Okada, and Shotaro Yamaguchi
Bioscience & Industry 2024, Vol.82, No.1	Jan. 2024	Enzyme application for improving the functionalities of plant-based meat analogs	Kiyota Sakai

## **2024** Exhibitions

Date	Exhibition	Location
January 25	Florida Section IFT	Orlando (USA)
February 2	Functional Food & Beverage Roadmap	Nakornsawan (Thailand)
March 12	SCIFTS Suppliers Night Expo	Garden Grove (USA)
March 12-16	Natural Products Expo West	Anaheim (USA)
March 20-22	Food Ingredients China 2024	Shanghai (China)
March 27	Food Focus Thailand Roadshow 2024	Chonbri (Thailand)
April 9	NYIFT Suppliers Expo	Edison (USA)
June 19-21	CPHI China 2024	Shanghai (China)
June 25-27	FBIF 2024	Shanghai (China)
July 14-17	IFT FIRST 2024	Chicago (USA)
August 7	Beverage and Trend Roadmap	Nonthaburi (Thailand)
August 25-29	biocat2024	Hamburg (Germany)
August 30	Food Focus Thailand Roadshow 2024	Samutsakhon (Thailand)
September 4-6	Fi Asia Indonesia 2024	Jakarta (Indonesia)
September 11-12	Plant Based World Expo	New York (USA)
October 23-25	FiT Japan 2024	Tokyo (Japan)
October 30-31	Fi North America 2024	Las Vegas (USA)
November 5-7	Gulffood Manufacturing	Dubai (UAE)
November 7	Chicago Section IFT	Chicago (USA)
November 19-21	Fi Europe 2024	Frankfurt (Germany)

For details and the latest information, please refer to our website or each exhibition website.

# Amano Enzyme World Network





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