Japan Academy Prize to:

Sakayu Shimizu
Emeritus Professor, Kyoto University
Visiting Professor, Toyama Prefectural University

for "Novel Microbial Functions, their Discovery through Screening, and their Application in the Large-Scale Production of Useful Compounds"

Outline of the work:

Microorganisms have survived on Earth for nearly four billion years, and their diversity is immense. Their functions and products are highly varied. Dr. Sakayu Shimizu recognizes the significance of these microbial communities as crucial resources. He has dedicated years of research to exploring the wide-ranging, unknown capabilities of these microorganisms and delving deep into their discoveries. His perspective revolves around using research findings to benefit human life by unveiling the untapped potential of these microorganisms.

Dr. Shimizu first took on the challenge of researching lipid production by microorganisms, choosing arachidonic acid, a physiologically crucial fatty acid for humans, as the target. In the 1980s, when he began this research, it became clear that animals and plants cannot directly produce arachidonic acid because certain enzymes are missing the synthesis pathway. It is widely believed that microorganisms share the same biosynthetic pathways as plants. He examined numerous microorganisms for their fatty acid profiles and eventually discovered that the fungus *Mortierella alpina* accumulated oil containing arachidonic acid. The yield reached up to 600 mg/g of dried fungal biomass, with approximately 70% of the total fatty acids being arachidonic acid. This discovery was highly significant as it demonstrated for the first time the existence of microorganisms capable of directly producing arachidonic acid. Dr. Shimizu subsequently established a method for the large-scale production of arachidonic acid-containing oil using this fungus, which is now widely added as an essential component to infant formula, especially for premature babies.

Dr. Shimizu continued to examine the biosynthetic pathway of polyunsaturated fatty acids (PUFAs) in this fungus. He elucidated various biosynthetic pathways, successfully revealing them in detail. Furthermore, he achieved individual induction of these pathways, demonstrating the selective and comprehensive synthesis of 30 rare and valuable PUFAs. During the course of his research, Dr. Shimizu also discovered the saturation reaction of linoleic acid's double bonds by lactic acid bacteria, unraveling the intricacies of this complex process. Additionally, he revealed that sesamin, a component of sesame seeds, acts as a specific inhibitor of the enzyme in the final stage of arachidonic acid synthesis. Dr. Shimizu's series of pioneering studies have given rise to a new industrial field known as "oil fermentation."

Dr. Shimizu then embarked on the study of the enzymatic synthesis of optically active

compounds. Choosing the production process of D-pantothenic acid as his target, he introduced microbial reactions into the process of manufacturing its intermediate, D-pantolactone. He discovered a novel enzyme, lactonohydrolase, in a *Fusarium* fungus. This enzyme selectively hydrolyzed only the D-form of DL-pantolactone, splitting it into D-pantoic acid and L-pantolactone. The application of this fungus to the optical resolution of DL-pantolactone for the production of D-pantothenic acid offers a new manufacturing method. This method has been applied on an industrial scale, producing several thousand tons of D-pantothenic acid annually. The new method resulted in an approximately 30% reduction in energy consumption and reduced various environmental burdens compared with conventional chemical optical resolution methods. These achievements are highly appreciated from the perspective of the current emphasis on sustainable development goals.

Dr. Shimizu aimed constructed a versatile asymmetric reduction system capable of converting various ketones into optically active alcohols. He organized the gene library of the enzymes responsible for the asymmetric reduction of various ketones obtained through screening. Then, he designed recombinant *Escherichia coli* strain that expressed one of these enzymes along with glucose dehydrogenase as an NAD(P)H regenerator. By using this engineered bacterial cell as a biocatalyst, he successfully converted various prochiral ketone compounds into their corresponding optically active alcohols, which had high reaction yields and optical purity. This method has spread globally as an industrial synthesis approach for diverse optically active intermediates in pharmaceutical synthesis.

Dr. Shimizu's accomplishments have been documented in approximately 700 research papers. Moreover, he has received several prestigious awards for his work, including from the American Oil Chemists' Society, the Chemical Society of Japan, the Japan Society for Bioscience, Biotechnology, and Agrochemistry, and the Engineering Foundation, USA, among others. His contributions are highly esteemed both nationally and internationally. In addition, in 2022, he was honored as a Person of Cultural Merit in Japan.

As described above, Dr. Shimizu has made groundbreaking discoveries regarding novel microbial functions through his exploratory research. He has advanced academic studies based on these findings and has successfully translated the outcomes into high-quality societal implementations, contributing significantly to the development of the biotechnology industry. His research achievements are highly esteemed, and he is worthy of the Japan Academy Prize in recognition of his outstanding contributions.

List of Main Publications

1. S. Shimizu, Y. Shinmen, H. Kawashima, K. Akimoto, H. Yamada; Fungal mycelia as a novel source of eicosapentaenoic acid, activation enzyme(s) involved in eicosapentaenoic acid production at low temperature. *Biochem. Biophys. Res. Commun.*, 150, 335–341 (1988).
2. Y. Shinmen, S. Shimizu, K. Akimoto, H. Kawashima, H. Yamada; Production of arachidonic acid by Mortierella fungi: Selection of a potent producer and optimization of culture conditions for large-scale production. *Appl. Microbiol. Biotechnol.*, 31, 11–16 (1989).

3. S. Jareonkitmongkol, S. Shimizu, H. Yamada; Occurrence of two non-methylene-interruped Δ5 polyunsaturated fatty acids in a Δ6-desaturase-defective mutant of the ungus *Mortierella alpina* 1S-4. *Biochim. Biophys. Acta*, 1167, 137–141 (1993).
4. H. Kawashima, K. Akimoto, K. Higashiyama, S. Fujikawa, S. Shimizu; Industrial production of dihomo-γ-linolenic acid by a Δ5 desaturase-defective mutant of *Mortierella alpina* 1S-4 fungus. *J. Am. Oil Chem. Soc.*, 77, 1135–1138 (2000).
5. E. Sakuradani, M. Kobayashi, S. Shimizu; Δ9-Fatty acid desaturase from arachidonic acid-producing fungus: Unique gene sequence and its heterologous expression in a fungus, Aspergillus. *Eur. J. Biochem.*, 260, 208–216 (1999).
6. E. Sakuradani, M. Kobayashi, T. Ashikari, S. Shimizu; Identification of Δ12-fatty acid desaturase from arachidonic acid-producing *Mortierella* fungus by heterologous expression in the yeast *Saccharomyces cerevisiae* and the fungus, *Aspergillus oryzae*. *Eur. J. Biochem.*, 261, 812–820 (1999).
7. S. Takeno, E. Sakuradani, S. Murata, M. Inohara-Ochiai, H. Kawashima, T. Ashikari, S. Shimizu; Establishment of an overall transformation system for an oil-producing filamentous fungus, *Mortierella alpina* 1S-4. *Appl. Microbiol. Biotechnol.*, 65, 419–425 (2004).
8. S. Shimizu, K. Akimoto, Y. Shinmen, H. Kawashima, M. Sugano, H. Yamada; Sesamin is a potent and specific inhibitor of Δ5 desaturase in polyunsaturated fatty acid biosynthesis. *Lipids*, 26, 512–516 (1991).
9. J. Ogawa, K. Matsumura, S. Kishino, Y. Omura, S. Shimizu; Conjugated linoleic acid accumulation via 10-hydroxy-12-octadecaenoic acid during microaerobic transformation of linoleic acid by *Lactobacillus acidophilus*. *Appl. Environ. Microbiol.*, 67, 1246–1252 (2001).
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11. S. Shimizu, M. Kataoka, M.C-M. Chung, H. Yamada; Ketopantoic acid reductase of *Pseudomonas maltophilia* 845: Purification, characterization, and role in pantothenate biosynthesis. *J. Biol. Chem.*, 263, 12077–12084 (1988).
12. S. Shimizu, S. Hattori, H. Hata, H. Yamada; A novel fungal enzyme, NADPH- dependent carbonyl reductase, showing high specificity to conjugated polyketones: Purification and characterization. *Eur. J. Biochem.*, 174, 37–44 (1988).
13. M. Kataoka, S. Shimizu, H. Yamada; Purification and characterization of a novel FMN-dependent enzyme, membrane-bound L-(+)-pantoyl lactone dehydrogenase, from *Nocardia* asteroids. *Eur. J. Biochem.*, 204, 799–806 (1992).
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23. N. Kizaki, Y. Yasohara, J. Hasegawa, M. Wada, M. Kataoka, S. Shimizu; Synthesis of optically pure ethyl (S)-4-chloro-3-hydroxybutanoate by *Escherichia coli* transformant cells coexpressing the carbonyl reductase and glucose dehydrogenase genes. *Appl. Microbiol. Biotechnol.*, 55, 590–595 (2001).
24. M. Wada, A. Yoshizumi, Y. Noda, M. Kataoka, S. Shimizu, H. Takagi, S. Nakamori; Production of a doubly chiral compound, (4*R*,6*R*)-4-hydroxy-2,2,6- trimethyl cyclohexanone, by two-step enzymatic asymmetric reduction. *Appl. Environ. Microbiol.*, 69, 933–937 (2003).