MEMORIES OF LATE PROFESSOR HIDEAKI YAMADA, A GIANT IN ENZYME ENGINEERING, AND SUCCESSIVE ACTIVITIES STEMMED FROM HIS PHILOSOPHY

Jun Ogawa, Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Kyoto, Japan
ogawa.jun.8a@kyoto-u.ac.jp
Sakayu Shimizu, Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Kyoto, Japan

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This talk is a tribute to the late Professor Hideaki Yamada, a giant in enzyme engineering, who passed away on July 12, 2021, at the age of 92.
Prof. Yamada was born on 11 March 1929 in Toyama, Japan. He graduated Kyoto University under the supervision of Prof. Hideo Katagiri, a distinguished biochemist. This made Prof. Yamada’s science mainly related to biochemistry, especially enzyme technology. He became professor of the Department of Agricultural Chemistry, Kyoto University, in 1977. The title of his doctor thesis is “Enzymatic studies on group transfer”. He published his first paper on transglycosylation relating to riboflavin based on his doctor thesis.
Continuing to this paper, he worked on the production of coenzymes. After these works, he was passionate about making industrially useful compounds by using enzymes and microorganisms. The famous examples are amino acid production using β-tyrosinase, D-amino acid production using hydantoinase/dihydropyrimidinase, amides production using nitrile hydrates, chiral alcohol production using aldonolactonase or aldehyde/carbonyl reductase, polyunsaturated fatty acid production by fermentation, etc., as listed in the below table.
In this talk, I would like to look back on Prof. Yamada with three words that I heard from him when I was his student.

i) Do you get good microorganisms?
This means “Good microorganisms lead to good research”. He usually said “The workings of nature are healthy. We should follow the nature, and work hard to learn from nature. That is the research”.

ii) Are you ready to scoop with a spoon?
He asked the production amounts every time to let the studensts maintain the mentality of industrialization. Only highly potential microorganisms or enzymes could withstand industrialization. This represents Prof. Yamada's passion for the industrial application of microbial and enzymatic functions.

iii) Do not bother Japanese sake.
The words in drinking party mean keeping respect to who made Japanese sake. Not only in the case of alcohol production, but also every industrial production. He usually said in collaborations “keep mutual respect”.

表1　微生物酵素を用いる有用化合物の合成

生成物	酵素（起源）	収量 g/L (mol%)
Amino acids		
D-*p*-Hydroxyphenylglycine	Dihydropyrimidinase (*Bacillus* sp.)	5 (74)
D-Phenylglycine	〃 〃	6 (91)
L-Tyrosine	β-Tyrosinase (*Erwinia herbicola*)	61
L-Dopa	〃 〃	53
L-Tryptophan	Tryptophanase (*Proteus rettgeri*)	100 (95)
L-Cysteine	Cysteine desulfhydrase (*Enterobacter cloacae*)	50 (86)
〃	Cysteine synthase (*B. sphaericus*)	70 (82)
D-Cysteine	β-Chloro-D-alanine chloride-lyase (*Pseudomonas putida*)	22 (88)
L-Cystathionine	Cystathionine γ-synthase (*B. sphaericus*)	42 (92)
L-Serine	Serine hydroxymethyltransferase (*Hyphomicrobium* sp.)	52
Ethyl (*R*)-4-chloro-3-hydroxybutanoate	Aldehyde reductase (*Sporobolomyces salmonicolor*)	88 (95)
Amides and acids		
Acrylamide	Nitrile hydratase (*P. chlororaphis*)	400 (100)
〃	〃 (*Rhodococcus rhodochrous*)	650 (100)
Methacrylamide	〃 (*P. chlororaphis*)	200
Crotonamide	〃 〃	200
Nicotinamide	〃 (*R. rhodochrous*)	1465 (100)
Acrylic acid	Nitrilase 〃	380 (100)
Nicotinic acid	〃 〃	172 (100)
6-Hydroxynicotinic acid	Hydroxylase (*Comamonas acidovorans*)	120 (96)
6-Hydroxypicolinic acid	〃 (*Alcaligenes faecalis*)	116 (97)
Pyrogallol	Gallic acid decarboxylase (*Citrobacter* sp.)	23 (100)
Theobromine	Oxygenase (*P. putida*)	20 (92)
D-Pantoyl lactone	Carbonyl reductase (*Candida parapsilosis*)	100 (83)
D-Pantoic acid	Aldonolactonase (*Fusarium oxysporum*)	700 (95)
Coenzymes		
Coenzyme A	Multi-step enzyme system (*Brevibacterium ammoniagenes*)	115 (95)
Adenosylmethionine	AdoMet synthetase (*Saccharomyces sake*)	12 (45)
Adenosylhomocysteine	AdoHcy hydrolase (*A. faecalis*)	74 (97)
FAD	FAD pyrophosphorylase (*Arthrobacter globiformis*)	18 (28)
Pyridoxal 5'-phosphate	PMP oxidase (*P. fluorescens*)	0.15 (98)
NADH	Formate dehydrogenase (*Arthrobacter* sp.)	30 (90)
NADPH	Glucose dehydrogenase (*Gluconobacter suboxydans*)	73 (100)
Polyunsaturated fatty acids		
Dihomo-γ-linolenic acid	Multi-step conversion (*Mortierella alpina*)	4.1
Arachidonic acid	〃 〃	4.5
Eicosapentaenoic acid	〃 〃	1.8

Table 1. List of Prof. Yamada’s achievement -Production of useful compounds by microbial enzymes- Left column; products, center column; enzymes, right column, production amounts (yields).

In our laboratory, Lab. of Fermentation Physiology and Applied Microbiology, Kyoto University, we continue the research on microbial enzymes stemmed from his philosophy. I will introduce our current activities, too. Information obtained through detail analysis of microbial function leads to finding of unexpected enzymes, Recent omics technologies support to identify the novel genes and communities expanding the bioprocess application. Here, examples of bioprocess development by applying unique tools found through functional analysis of microbial metabolisms are introduced.