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Abstracts

Selected abstracts from the 8th Japanese Symposium on the Chemistry of Biocatalysis

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Available online 16 August 2005

Introduction

The 8th Symposium on Biocatalytic Chemistry Japan was held on December 16 and 17 at Keio University, Yokohama, Japan. The multimedia room of the Faculty of Science and Technology was used for both lectures and poster presentation. The active attendees to the symposium were about 60 persons from academia, 50 from industries and 60 students, i.e., 170 persons in total, including 2 foreign invited guests speakers, Prof. Franz Effenberger from University of Stuttgart (Germany) and Prof. Mahon-Joo Kim from Pohang University of Science and Technology (Korea).

The symposium consisted of 5 invited lectures, 5 contributed oral presentations and 67 poster presentations. Each poster presenter introduced his or her research by 1 min oral presentation in English before the poster session. Seven posters were selected for poster award. The next symposium will be organized by Prof. Hideo Hirohara of Shiga Prefecture University and held on January 26 and 27, 2006 at Ohtsu, Japan.

Symposium organizer: Hiromichi Ohta

Yasuhisa Asano, Editor

Invited Lectures

Enzymatic reactions and bioinformatics

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The contribution of bioinformatics has been becoming more and more important in the studies and understanding of enzymatic reactions, and we have been developing the EzCaltDB, a catalytic reaction database based on novel enzyme categorization as well as utilizing quantum chemistry simulation to reveal curious mechanism of an enzyme, P 450 NOR, for example.

Synthesis of optically active compounds with cloned enzyme library—How to get desired enzymes?

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We have constructed a powerful and unique cloned enzyme library and promoted its use to improve the productivity and speed up the time for development of bio-routes to useful optically active compounds, such as secondary alcohols, β-hydroxy esters, α-hydroxy acids and unnatural amino acids (Fig. 1).

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1381-1177/$ – see front matter
doi:10.1016/j.molcatb.2005.05.004

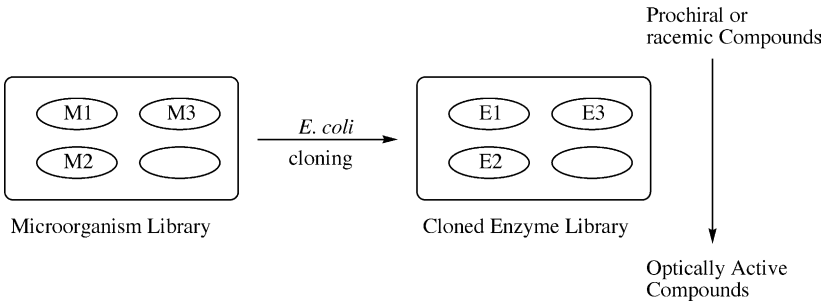

Fig. 1. Construction of cloned enzyme library for speedy development.

Crystal structures of enzymes

Masayoshi Nakasako

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One of the most powerful tools to investigate the enzymatic reaction is X-ray crystallography, which becomes easier than those in 10 years ago, owing to the innovations of several experimental techniques and software, such as X-ray diffraction data collections at cryogenic temperatures, synchrotron X-rays, high-performance data collection systems with CCD detectors, sophisticated software for structure analyses, and graphical manipulations in model building procedures (Fig. 2).

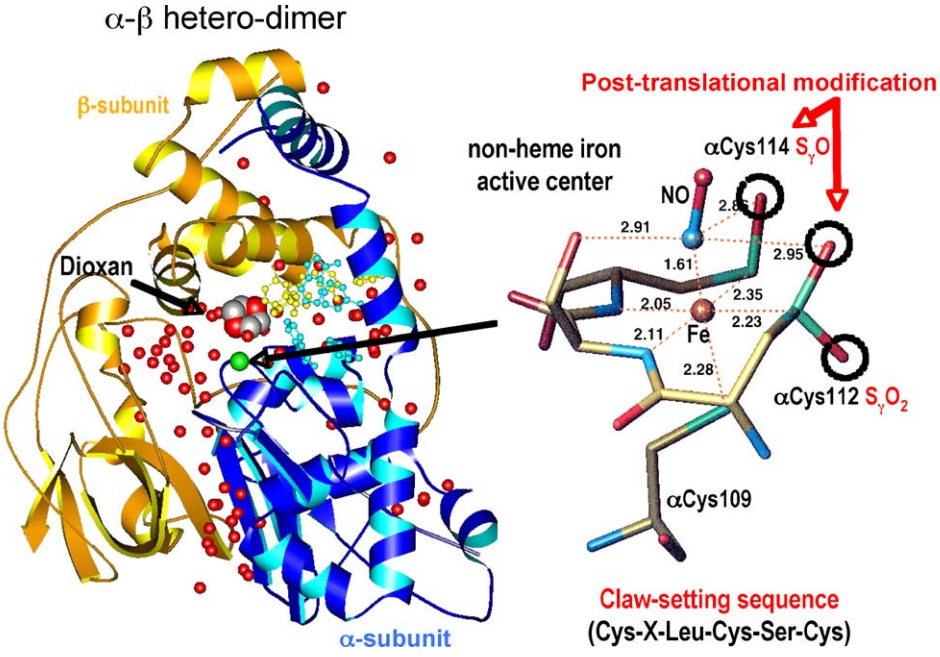

Fig. 2. 3D structure of photo-reactive nitrile hydratase at a resolution of 1.7 Å.

Asymmetric catalysis by enzyme–metal combinations

Mahn-Joo Kim

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One of the most powerful tools to investigate the enzymatic reaction is X-ray crystallography, which becomes easier than those in 10 years ago, owing to the innovations of several experimental techniques and software, such as X-ray diffraction data collections at cryogenic temperatures, synchrotron X-rays, high-performance data collection systems with CD detectors, sophisticated software for structure analyses, and graphical manipulations in model building procedures (Fig. 3).

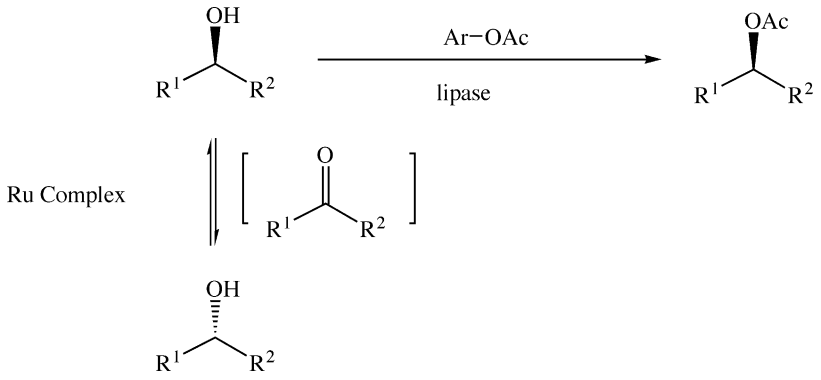

Fig. 3. DKR utilizing lipase and metal complex.

The application of hydroxynitrile lyases in stereoselective organic synthesis

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Both enantiomers of cyanohydrins are available utilizing hydroxynitrile lyases (HNL) of different origin ((*R*)-PaHNL from bitter almonds for (*R*)-cyanohydrine and recombinant HNLs from cassava and rubber tree for (*S*)-enantiomer), carrying out the reaction in organic solvents or two-phase system to suppress the chemical addition of HCN (Fig. 4).

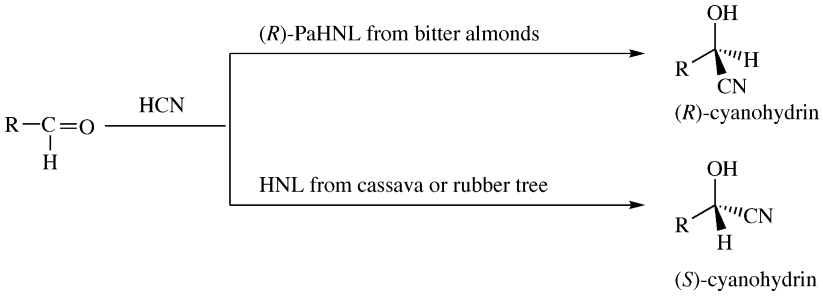

Fig. 4. Synthesis of optically active cyanohydrins using biocatalysts.

Oral Presentations

Microbial deracemization of α-substituted carboxylic acids

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The deracemization techniques to prepare the optically active α-methylcarboxylic acids and α-amino acids were realized by different types of chiral inversion processes, i.e., “enantioselective racemization” and “enantioselective inversion of configuration” (Fig. 5).

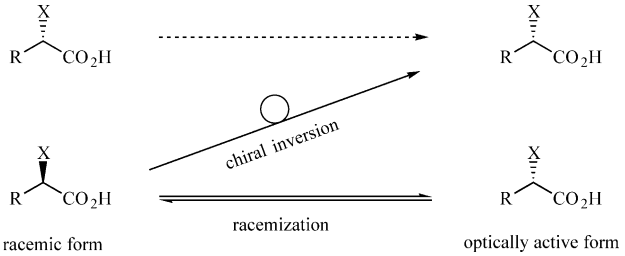

Fig. 5. Two types of approaches to realize the deracemization reaction.

Production of picolinic acid derivatives using biphenyl degradating enzymes

Kazutoshi Shindo[a,*], Ayako Osawa[a], Ryoko Nakamura[a], Kensuke Furukawa[b], Norihiko Misawa[c]
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[b]Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Fukuoka 812-8581, Japan
[c]Marine Biotechnology Institute, 3-75-1, Heita, Kamaishi-shi 026-0001, Japan. E-mail: kshindo@fc.jwu.ac.jp

Picolinic acid derivatives were prepared from phenyl-substituted aromas using *Escherichia coli* cells expressing modified BphA, BphB, and BphC (Fig. 6).

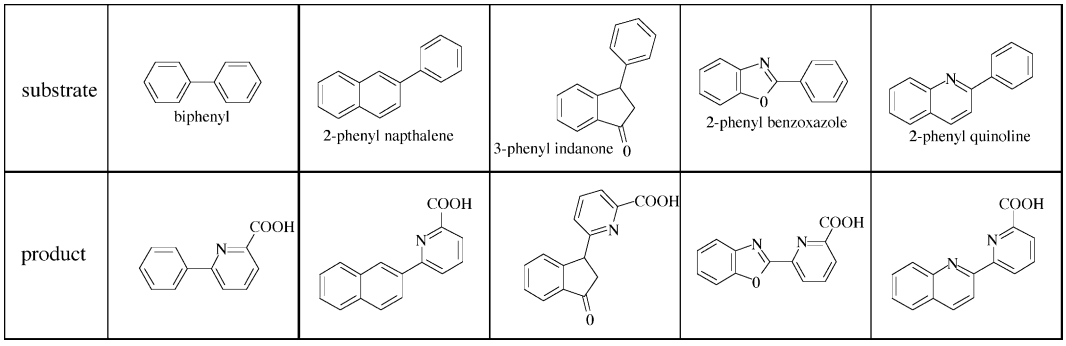

Fig. 6. Results of the bioconversion on various aromatics.

Improvement of cell-free expression system and its application to molecular evolution

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Toyota Central R&D Labs., Inc., 41-1 Yokomichi, Nagakute, Aichi 480-1192, Japan. E-mail: chiem@mosk.tytlabs.co.jp

Cell-free expression system was improved in order to fold unfolded proteins using chaperone-overexpressed *Escherichia coli* S30 extract, thus enabling molecular evolution method called single-molecule-PCR-linked in vitro expression (SIMPLEX) (Fig. 7).

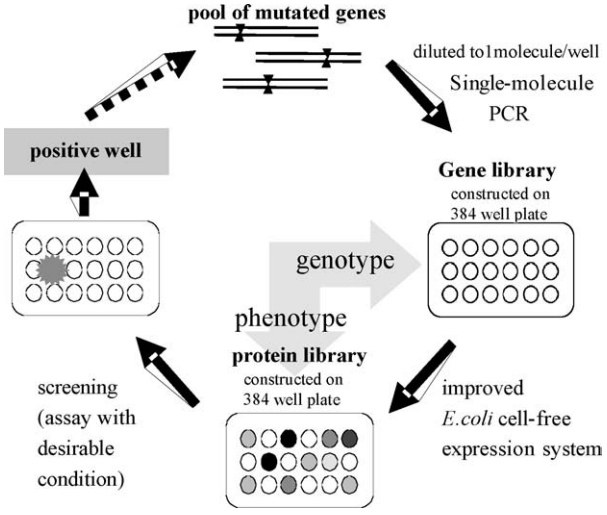

Fig. 7. The strategy of SIMPLEX using improved cell-free system.

Poster Presentations

Stability of lipase immobilized on surface-functionalized mesoporous silicates

Yuki Morioka[a], Katsuya Kato[a,*], Takao Saito[a], Sindhu Seelan[a], Yoshiyuki Yokogawa[a], Haruo Takahashi[b], Hideo Nakano[c], Tuneo Yamane[c]

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[b]Toyota Central R&D Laboratory, Nagakute, Aichi 480-1192, Japan

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Lipase from *Burkholderia cepacia* was immobilized on phenyl-substituted mesoporous silicates and the stability of immobilized lipase was evaluated in various conditions (Fig. 8).

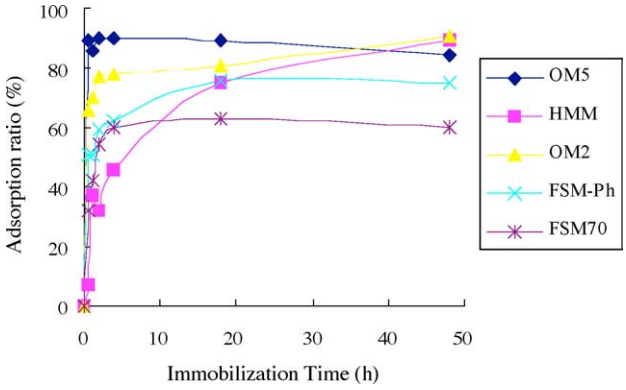

Fig. 8. Adsorption time of lipase on different mesoporous silicates.

Rapid purification of aldoxime dehydratase from *Bacillus* sp. OxB-1 and spectroscopic analysis of the enzyme

Yasuo Kato [a,*], Katsuaki Kobayashi[b], Shigetoshi Aono[b], Yasuhisa Asano[a]

[a]Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 939-0398, Japan

[b]Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, 5-1 Higashiyama, Myodaiji, Okazaki 444-8787, Japan. E-mail: yasu@pu-toyama.ac.jp

A novel heme-containing lyase, phenylacetaldoxime dehydratase, from *Bacillus* sp. OxB-1 was overexpressed in *Escherichia coli*, efficiently purified by TALON column with its His_6-tagged form, and its heme-environment was studied by analyzing electroabsorption, EPR, and resonance Raman spectra (Fig. 9).

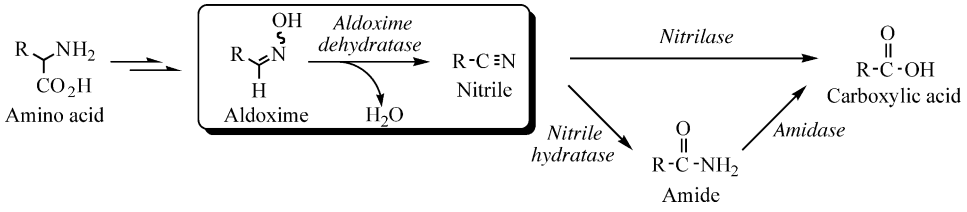

Fig. 9. Aldoxime-nitrile pathway in microorganisms.

Immobilization of catalytic antibody on large-pore mesoporous silicas

Katsuya Kato[a,*], Tadashi Hasegawa[b], Goro Yasuda[b], Takao Saito[a], Yoshiyuki Yokogawa[a]

[a]National Institute of Advanced Industrial Science and Technology (AIST), 2266-98 Anagahora, Shimoshidami, Moriyama-ku, Nagoya 463-8560, Japan

[b]Department of Applied Chemistry, Aichi Institute of Technolgy, Yachigusa, Yagusa-cho, Toyota 470-0392, Japan. E-mail: katsuya-kato@aist.go.jp

Catalytic antibody 84G3 was immobilized on the mesoporous silicates with the pore size from 3 to 40 nm (Fig. 10).

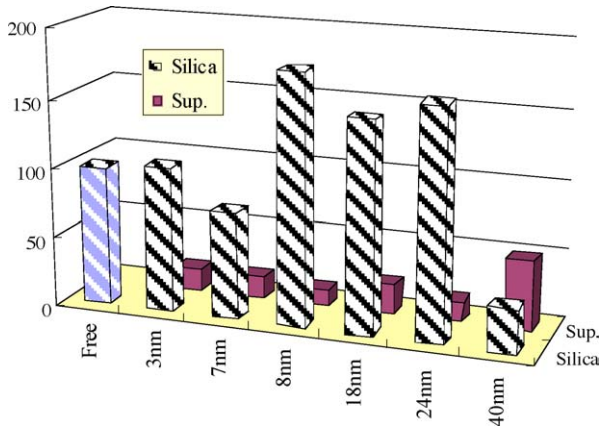

Fig. 10. Immobilization ratios of 84G3 on mesoporous silicates.

Screening for novel plant hydroxynitrile lyase (HNL)

Samik Nanda, Ai Kayo, Kenichiro Tamura, Yasuhisa Asano*
Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 939-0398, Japan. E-mail: asano@pu-toyama.ac.jp

Several plant hydroxynitrile lyases (HNL) having both (*R*)- and (*S*)-selectivity were isolated, purified and screened based on HPLC based enantioselective assay technique. Applications of these HNLs for the asymmetric synthesis of structurally different cyanohydrins were accomplished (Fig. 11).

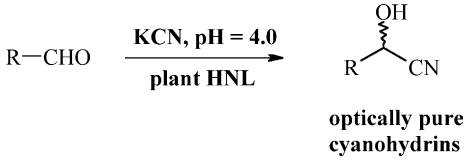

Fig. 11. Asymmetric synthesis of cyanohydrins with plant HNLs.

Lipase-catalyzed enantioselective acylation of amines in ionic liquids

Katsuya Kato*, Roxana Irimescu, Takao Saito, Yoshiyuki Yokogawa
National Institute of Advanced Industrial Science and Technology (AIST), 2266-98 Anagahora, Shimoshidami, Moriyama-ku, Nagoya 463-8560, Japan. E-mail: katsuya-kato@aist.go.jp

Lipase-catalyzed enantioselective acylation of 1-phenylethylamine and 2-phenyl-1-propylamine was performed by reacting the amines with carboxylic acids in a non-solvent system or in ionic liquids as reaction media (Fig. 12).

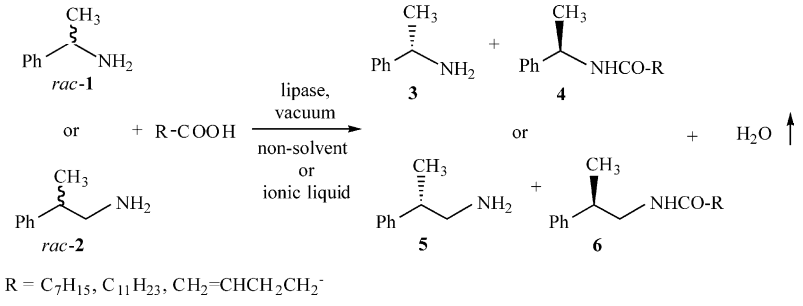

Fig. 12. Enantioselective acylation of amines under reduced pressure.

The useful glycosylation by plant cultured cells

Daisuke Kabusaki[a], Hiroki Hamada[b,*]
[a]Department of Applied Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan
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The biotransformation of bioactive compounds, such as (+)-catechin, β-thujaplicin (hinokitiol), Vitamin E derivative, raspberry ketone, and vanillin, were carried out for functionalization of these compounds, using the plant cultured cells of *Eucalyptus perriniana*. As the result, it was found that the cultured cells of *E. perriniana* predominantly glycosylated these compounds (Fig. 13).

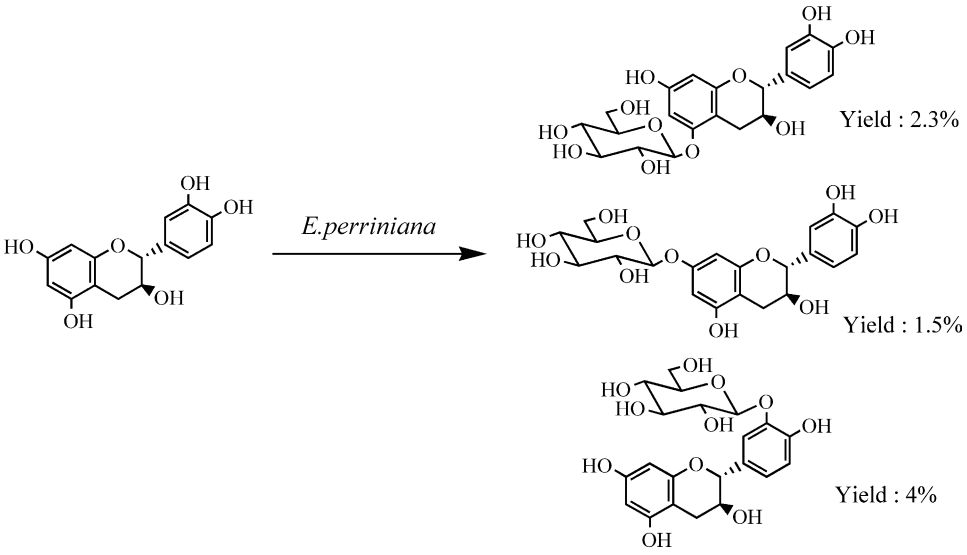

Fig. 13. Regioselective glycosylation of (+)-catechin by plant cultured cells.

Production of N^{α}-Z-DL-aminoadipic acid (N^{α}-Z-DL-AAA) from N^{α}-Z-DL-lysine with *Rhodococcus* sp. AIU Z-35-1

Kimiyasu Isobe[a,*], Keigo Tokuta[a], Yuuki Narita[a], Akira Matsuura[b], Takehiko Sakaguchi[b], Norio Wakao[a]
[a]Department of Agro-bioscience, Iwate University, 3-18-8 Ueda, Morioka 020-8550, Japan
[b]Sanyo Fine Co. Ltd., 1 Hirano-machi, Chuo-ku, Osaka 541-0046, Japan. E-mail: kiso@iwate-u.ac.jp

Approximately 40 mM of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA were produced from 50 mM N^{α}-Z-L-lysine and N^{α}-Z-D-lysine, respectively, by a reaction with new isolated strain, *Rhodococcus* sp. AIU Z-35-1, at 30 °C for 4 days (Fig. 14).

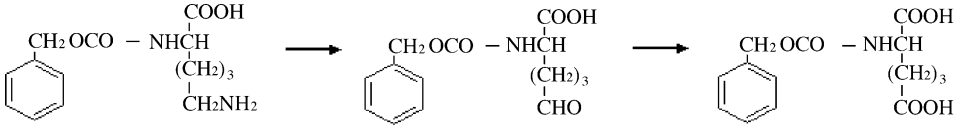

Fig. 14. Production of N^{α}-Z-L-AAA from N^{α}-Z-L-lysine by *Rhodococcus* sp. AIU Z-35-1.

Changing the function of arylmalonate decarboxylase (AMDase) by point mutation

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Department of Biosciences and Bioinformatics, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan.
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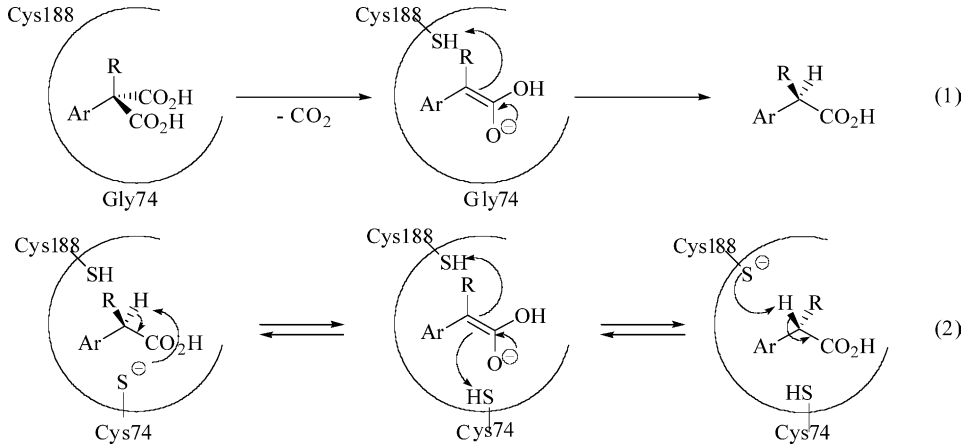

Fig. 15. The reaction mechanism of (1) decarboxylation of wild type AMDase and (2) racemization of G74C single mutant AMDase.

By the introduction of only single mutation based on the comparison with the reaction mechanism of glutamate racemase, arylmalonate decarboxylase has been endowed with racemase activity in addition to its original decarboxylase activity (Fig. 15).

Expression of nitrile hydratase from *Rhodococcus rhodochrous* NBRC15564 in *E. coli*

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Department of Biosciences and Informatics, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan.
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We have succeeded the expression of nitrile hydratase from *Rhodococcus rhodochrous* NBRC15564 in *Escherichia coli* by constructing a plasmid containing the genes of α- and β-subunits as well as the activator of the enzyme, and confirmed the activity via hydrolysis of benzonitrile (Fig. 16).

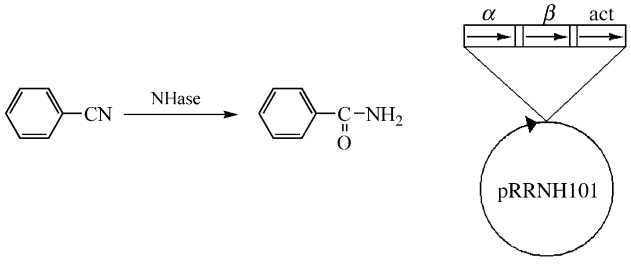

Fig. 16. Constructed vector for NHase.

Purification and characterization of a novel esterase from the thermoacidophilic archaeon *Sulfolobus tokodaii* strain 7

Yoichi Suzuki, Kenji Miyamoto, Hiromichi Ohta*
Department of Biosciences and Informatics, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan.
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We have isolated and characterized a protein expressed from the putative esterase gene (ST0071), which selected from the total genome analysis of the thermoacidophilic archaeon *Sulfolobus tokodaii* strain 7 (Fig. 17).

Enzyme-mediated synthesis of optically active β-amino acid derivatives

Tadayoshi Konegawa, Yasuhito Yamamoto, Kazuma Sakata, Hiroyuki Miyata*
Ube Laboratory, Ube Industries, Ltd., 1978-5, Kogushi, Ube, Yamaguchi 755-8633, Japan. E-mail: 27869u@ube-ind.co.jp

The racemic methyl 3-benzylamino-4-methylpentanoate (**1**) was efficiently resolved by hydrolysis with *Candida antarctica* lipase B to give the corresponding β-amino acid (*S*)-**2** in enantiomerically pure form (>99% e.e.) (Fig. 18).

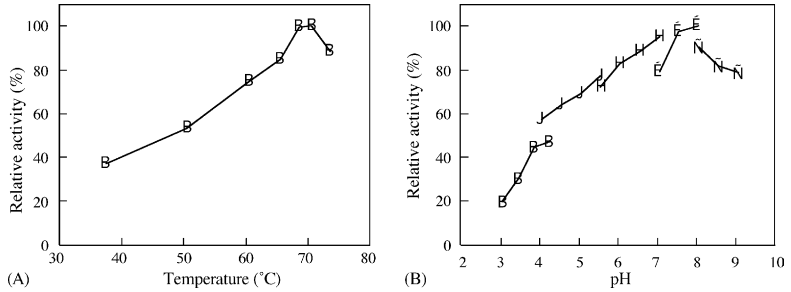

Fig. 17. The effect of temperature (A) and pH (B) on the esterase activity of *S. tokodaii* esterase (substrate: *p*-nitrophenyl butanoate).

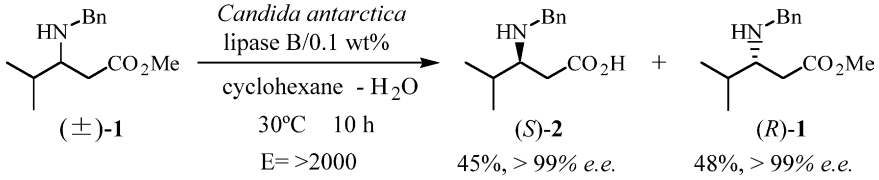

Fig. 18. Enzyme-mediated enantioselective hydrolysis of racemic β-amino ester.

Papain-catalyzed peptide synthesis through segment condensation using the carbamoylmethyl ester

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Several segment condensations have been achieved generally in high yields and without racemization through the kinetically controlled approach mediated by papain employing the carbamoylmethyl ester as an acyl donor (Fig. 19).

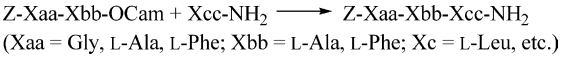

Fig. 19. Papain-catalyzed segment condensations.

Synthesis of optical active (+)- and (−)-bicyclofarnesol based on enzymatic function and its application to the synthesis of natural products

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School of Pharmaceutical Scieneces, Toho University, 2-2-1 Miyama, Funabashi, Chiba 275-8510, Japan.
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Lipase catalysed enantioselective acetylation of ketal-alcohol (±)-**1** gave (8a*R*)-**1** and its acetate (8a*S*)-**2**, which were converted to (−)-subersic acid (**4**) and (+)-norseterterpendiene ester (**3**), respectively (Fig. 20).

Substrate specificities of the HexPP synthase of *Bacillus subtilis* and HepPP synthase of *Micrococcus luteus* B-P26

Masanori Takekawa[a], Miho Kusakari[a], Ryo Ohba[a], Eri Shimizu[a], Mikiya Sato[a], Norimasa Ohya[a], Masahiko Nagaki[b], Tanetoshi Koyama[c], Yuji Maki[a,*]
[a]IMRAM, Tohoku University, Sendai 9980-8577, Japan
[b]Department of Material and Biological Chemistry, Yamagata University, Yamagata 990-8560, Japan
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The substrate specificities of the HexPS of the *Micrococcus luteus* B-P26 and the HepPS of the *Bacillus subtilis* were studied by using DMAPP analogs, GPP analogs, and FPP analogs having the chain with a various length and oxygen atom in their prenyl chain (Fig. 21).

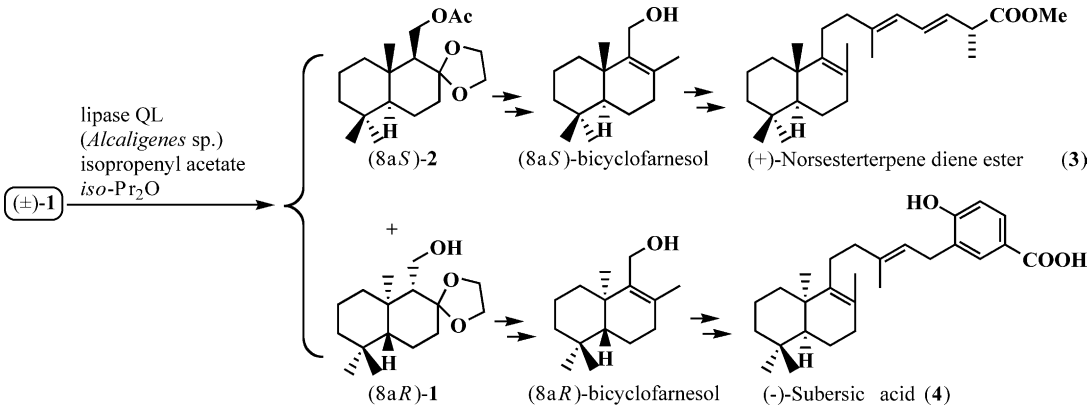

Fig. 20. Enantioselective acetylation of (±)-**1** by lipase and its application to the synthesis of (+)-**3** and (−)-**4**.

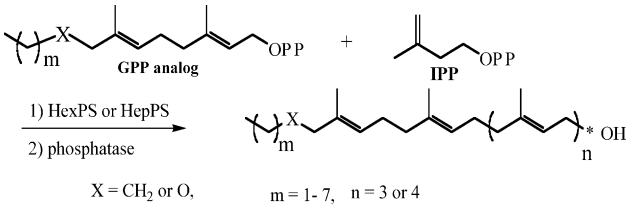

Fig. 21. Reaction of substrate analogue with HexPS or HepPS.

Treatment of germinated wheat to increase levels of GABA and IP_6 catalyzed by endogenous enzymes

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The IP_6 (or GABA) from wheat germinated in the presence of high (or low), controlled levels of dissolved oxygen was almost 3 times (or 18 times) greater than that from wheat germinated with no supplemental oxygen (or that from non-supplemented wheat) (Fig. 22).

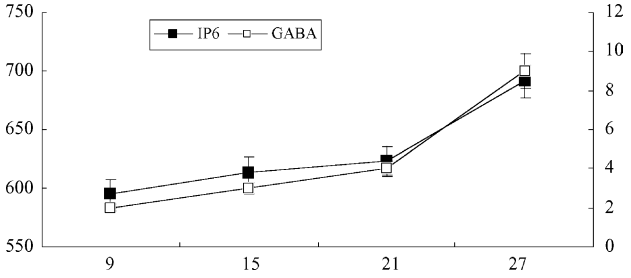

Fig. 22. GABA and IP_6 content during the germination of dark northern spring wheat with supplemental oxygen and water.

Direct construction of randomly mutated plasmid library by error-prone rolling circle amplification

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E-mail: rfujii@nfri.affrc.go.jp

We invented a simple and easy random mutagenesis technique named error-prone rolling circle amplification, which consists of only two steps to yield plasmid library with 3–4 kbp^{-1} random mutations (Fig. 23).

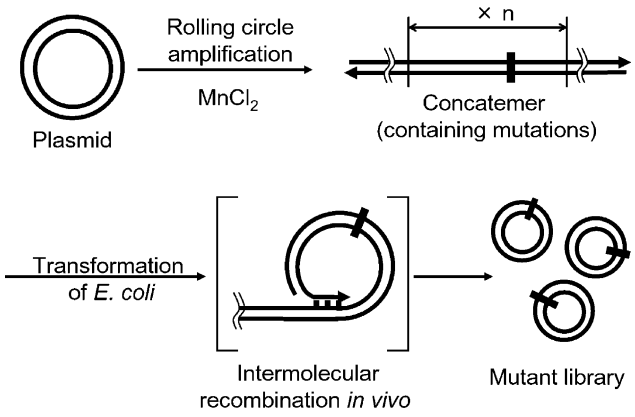

Fig. 23. Process of error-prone rolling circle amplification.

Enhancement of the efficiency in lipase-catalyzed resolution of primary alcohols and consideration of the enantioselectivity

Takashi Sakai*, Liu Yu, Toshinobu Korenaga, Tadashi Ema

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Lipase-catalyzed resolution of (2*R**,3*S**)-3-methyl-3-phenyl-2-aziridinemethanol (±)-**1** with lipase PS-C II at low temperatures gave (2*R*,3*S*)-**1** and its acetate (2*S*,3*R*)-**1a** with (2*S*)-selectivity, while a similar reaction of (2*R**,3*R**)-isomer (±)-**2** gave (2*R*)-selectivity (Fig. 24).

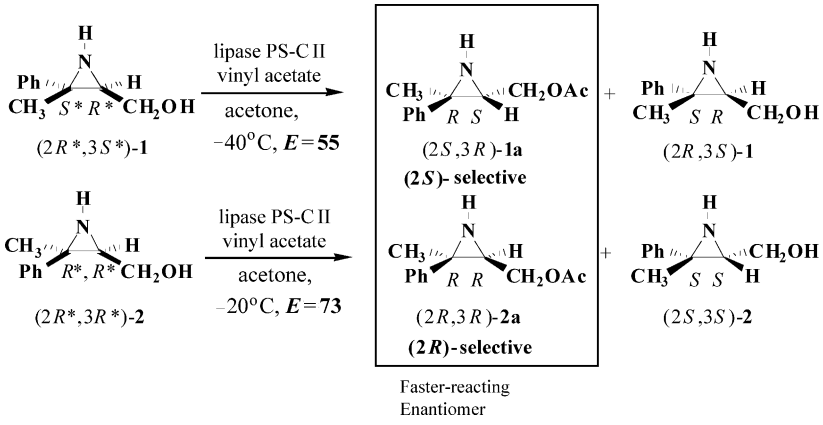

Fig. 24. Lipase-catalyzed kinetic resolution of 2-aziridinemethanol derivatives.

In silico screening for identification of peptide substrates of papain

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Docking simulation between papain and the peptide substrates, which were selected from a random library by using a phage-display technology, resulted in the finding of sensitive peptide sequences (Fig. 25).

Construction of a recombinant *E. coli* as a versatile chiral reduction biocatalyst

Tadashi Ema*, Hideo Yagasaki, Nobuyasu Okita, Toshinobu Korenaga, Takashi Sakai

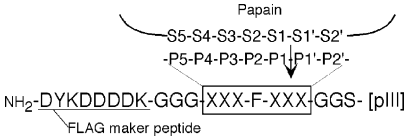

Fig. 25. Phenylalanine-containing peptide library displayed on pIII protein of phage.

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A recombinant *Escherichia coli* that overproduces a *Sacharomyces cerevisiae* carbonyl reductase (SCR) and a *Bacilus megaterium* glucose dehydrogenase was created and used for highly enantioselective whole-cell reduction of variety of ketones (Fig. 26).

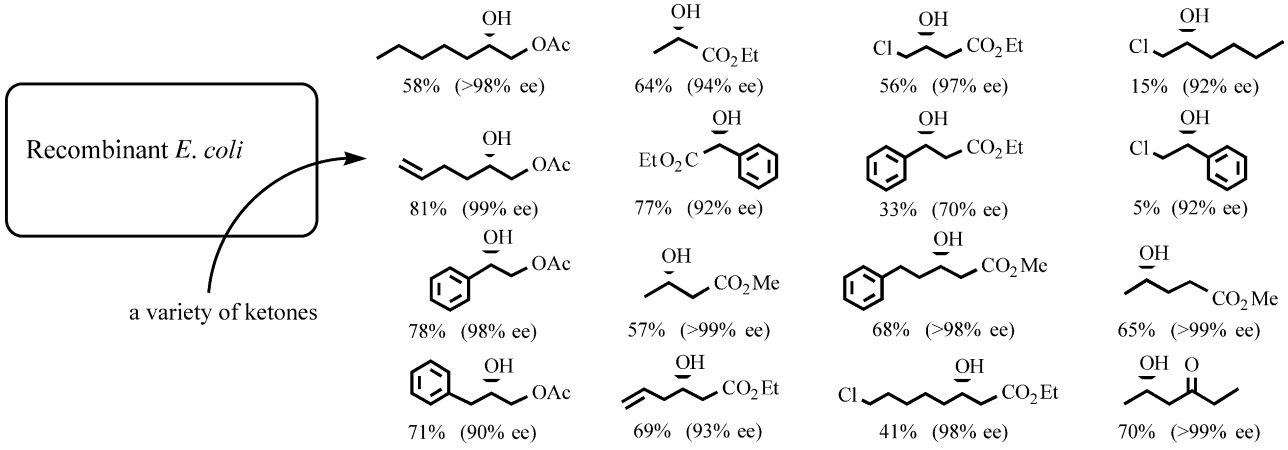

Fig. 26. Highly enantioselective reduction of ketones with a recombinant *E. coli*.

The study on the development of new agricultural chemicals by use of prenyltransferase

Masahiko Nagaki[a,*], Emiko Kakizaki[a], Yukio Harada[b], Tomokazu Handa[b], Yuji Maki[c], Tanetoshi Koyama[d]

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[b]Department of Bioproduction, Faculty of Agricultural and Life Science, Hirosaki University, 1 Bunkyo-cho, Hirosaki, Aomori 036-8560, Japan

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As we have been working on the enzymatic syntheses of novel isoprenoids homologs, which might show some antimicrobial activity against some plant pathogens such as *Colletotrichum acutatum*, *Fusarium* sp. and *Cochriobolus miyabeanus*, we want to develop some novel type antibacterial chemicals by using our libraries of new isoprenoid homologs accumulated to date in our laboratory.

Reduction of acetophenone derivatives by *Cyanidioschyzon*

Takamitsu Utsukihara[a], Wen Chai[a], Nakahide Kato[a], Osami Misumi[b], Tsuneyoshi Kuroiwa[b], C. Akira Horiuchi[a,*]

[a]Department of Chemistry, Rikkyo (St. Paul's) University, Nishi-Ikebukuro, Toshima-Ku, Tokyo 171-8501, Japan

[b]Department of Life science, Rikkyo (St. Paul's) University, Nishi-Ikebukuro, Toshima-Ku, Tokyo 171-8501, Japan.
E-mail: horiuchi@rikkyo.ac.jp

Biotransformation of acetophenone derivatives using *Cyanidioschyzon merolae* 10D gave the corresponding (*S*)-alcohols with excellent enantioselectivity in good yield (Fig. 27).

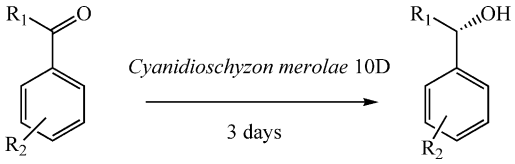

Fig. 27. Reduction of acetophenone derivatives by *C. merolae* 10D.

Enantio-selective reduction of ketones by rat liver 3α-HSD

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The enantio-selective reduction of various ketones to sec-alcohols was investigated kinetically with 3α-hydroxysteroid dehydrogenase (3α-HSD) from rat liver (Table 1).

Table 1
Kinetic parameters in the reduction of arylketones by rat liver 3a-HSD

Substrate	K_m[a]	V_{max}[b]	V_{max}/K_m[c]	Ratio	e.e. (%) by liver S-9
2-Acetylpyridine					
R	18.53	0.005	0.27	62.41	93
S	37.74	0.636	16.85		
3-Acetylpyridine					
R	402.00	0.730	1.82	4.31	84
S	75.52	0.592	7.84		
4-Acetylpyridine					
R	12.98	0.018	1.39	111.52	94
S	22.65	3.511	155.01		
Acetophenone					
R	N.D.[d]	N.D.[d]	N.D.[d]	N.D.[d]	96
S	4.55	0.040	8.79		

[a] mM.
[b] μmol/(mg protein min).
[c] μL/(mg protein min).
[d] N.D.: not determined.

Repetitive production of oligosaccharides from nonstarchy polysaccharides using the immobilized cells of a human intestinal *Clostridium*

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An anaerobic human intestinal bacterium, *Clostridium butyricum-beijerinckii* (isolated from human feces) secretes the non-starchy polysaccharide-degrading enzymes (extracellular and induced enzymes). We have developed cell immobilization techniques of the bacterium on cellulose-foam carriers that are effective for continuous production of the oligosaccharides from the dietary fibers in a fed-batch reactor system (Fig. 28).

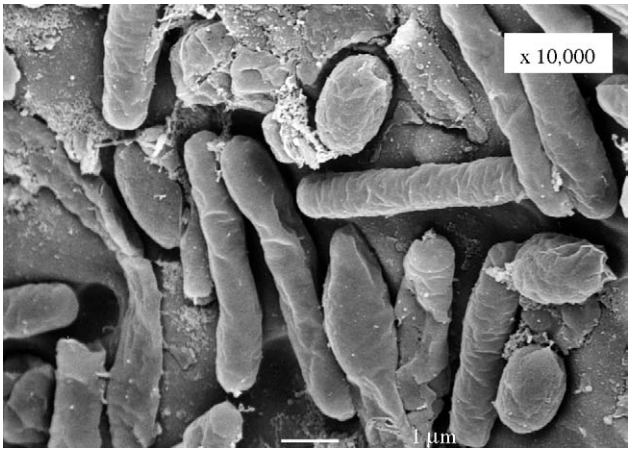

Fig. 28. Scanning electron micrograph of immobilized *Clostridium* cells on cellulose-form carriers.

Mechanism of *Candida antarctica* lipase B (CALB)-catalyzed hydrolysis of acetates of secondary alcohols: change of rate-determining step in acylation and proton inventory study

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Department of Materials Science, The University of Shiga Prefecture, 2500 Hassaka, Hikone 522-8533, Japan.
E-mail: hirohara@mat.usp.ac.jp

We have examined kinetics and thermodynamics of the acylation of *Candida antarctica* lipase B (CALB) by the acetates of single enantiomer chiral secondary alcohols and have also performed the proton inventory study to elucidate the mechanism of action of the enzyme (Table 2).

Table 2
Summary of thermodynamics and proton inventory study

Rate enhanced by	Rate-determining step	Shape of proton inventory
$\Delta S^{\ddagger}\uparrow\uparrow$	Formation of ET	Bowl shaped, downward bulging
$\Delta H^{\ddagger}\downarrow\downarrow$	Breakdown of ET	Dome shaped, upward bulging

Mechanism of *Candida antarctica* lipase B (CALB)-catalyzed hydrolysis of monohloroacetates of primary alcohols: Kinetic and thermodynamic study

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E-mail: hirohara@mat.usp.ac.jp

We have studied kinetics and thermodynamics of *Candida antarctica* lipase B (CALB)-catalyzed hydrolysis of the monochloroacetates of achiral and single enantiomers chiral primary alcohols to elucidate mechanism of the acylation under the consideration of the electrostatic and hydrophobic effects in the ground and the transition states (Fig. 29).

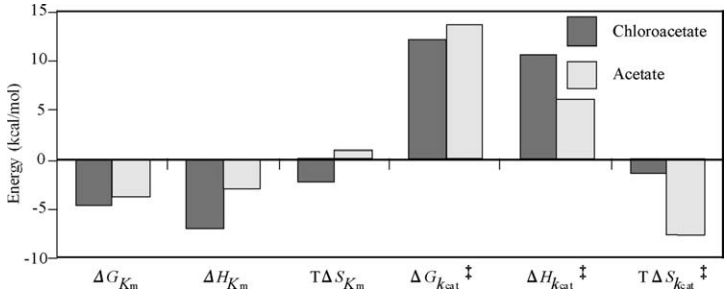

Fig. 29. Thermodynamic parameters of CALB-catalyzed hydrolysis.

Biotransformation of terpene and cycloalkanone by fungi

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[b]National Food Research Institute, 2-1-2 Kannondai, Tukuba, Ibaraki 305-8642, Japan. E-mail: horiuchi@rikkyo.ac.jp

Biotransformation of cycloalkanone using *Fusarium sporotrichioides* NFRI-1012 gave the corresponding allyl alcohol by the cleavage of carbon–carbon bond and in the case of β-ionone, 5,6-epoxy-β-ionone was obtained as major product (Fig. 30).

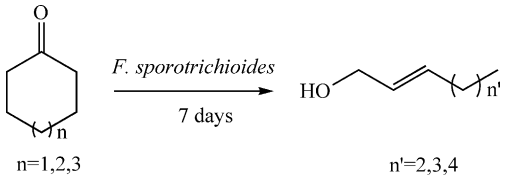

Fig. 30. Biotransformation of cycloalkanone by *F. sporotrichioides* NFRI-1012.

The development of highly active phenylacetaldehyde reductase (PAR) in concentrated 2-propanol

Yoshihide Makino*, Masatoshi Nakamura, Tohru Dairi, Nobuya Itoh

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The mutant of phenylacetaldehyde reductase (PAR) that can convert various ketones into corresponding chiral alcohols efficiently in concentrated 2-propanol, utilized for coenzyme regeneration, was successfully developed by combining advantageous amino acid substitutions (Fig. 31).

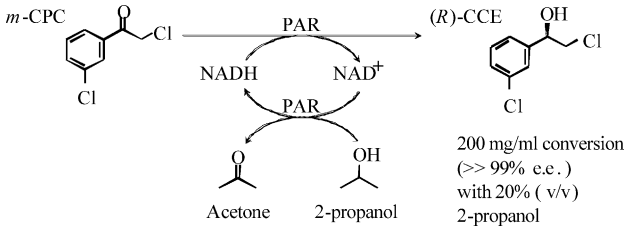

Fig. 31. Conversion of *m*-chlorophenacyl chloride (*m*-CPC) with engineered PAR.

Functional analysis of cytochrome P450 CYP153 family genes isolated from environmental samples

Mitsutoshi Kubota, Taku Uchiyama, Takahiro Maruyama, Miho Nodate, Mina Yasumoto-Hirose, Osamu Kagami, Norihiko Misawa*

Marine Biotechnology Institute Co., Ltd., 3-75-1 Heita, Kamaishi-shi 026-0001, Japan.
E-mail: norihiko.misawa@mbio.jp

Cytochrome P450 CYP153 family genes were isolated from environmental samples by the cassette PCR method, and then functionally analyzed in *Escherichia coli* (Fig. 32).

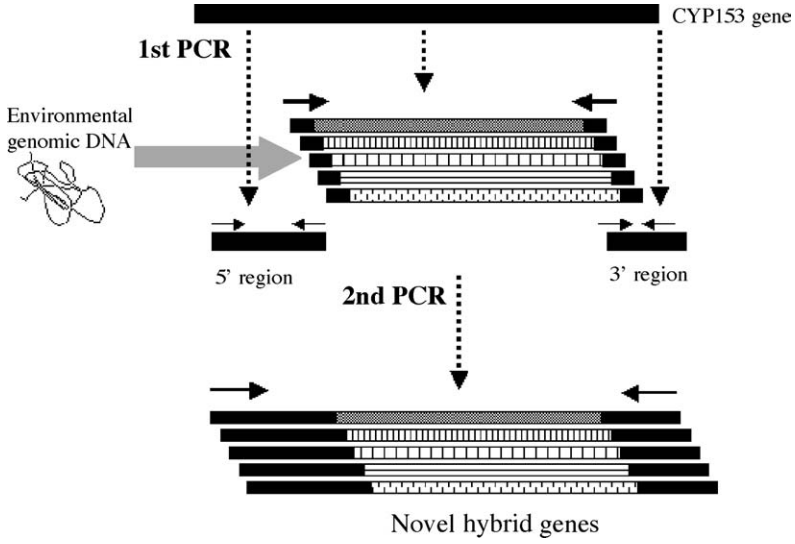

Fig. 32. Outline of the cassette PCR method.

The kinetic studies of chondroitin synthase on a 27 MHz quartz crystal microbalance

Ayaka Fujishima[a,*], Takanori Nihira[a], Nobuo Sugiura[b,c], Koji Kimata[b], Toshiaki Mori[a], Yoshio Okahata[a]

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[b]Institute of Molecular Science of Medicine, Aichi Medical University, Yazako, Nagakute, Aichi 480-1195, Japan

[c]Central Research Laboratories, Seikagaku Corporation, Tateno, Higashiyamato-shi, Tokyo 207-0021, Japan.

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We have kinetically analyzed the binding of chondroitin sulfate as acceptor substrate and the following chondroitin polymerization reaction by using the chondroitin synthase-immobilized 27 MHz quartz-crystal microbalance (Fig. 33).

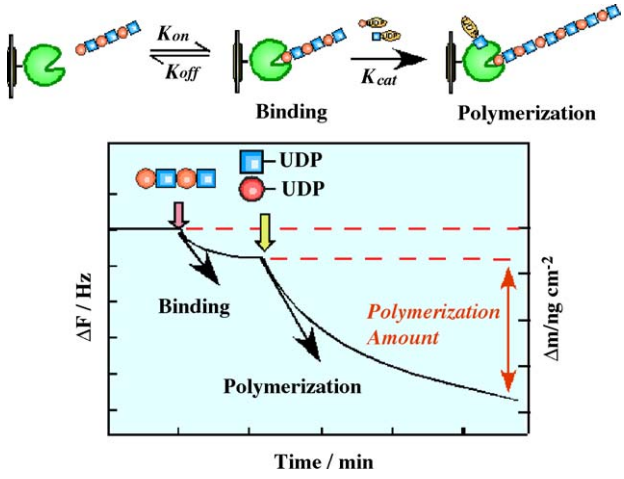

Fig. 33. Binding of acceptor and reaction process on chondroitin synthase-immobillized QCM.

Control of DNA polymerase activity by weak ultrasound irradiation

Yu Hoshino[*], Yu Mizushiro, Takayoshi Kawasaki, Yoshio Okahata

Department of Biomolecular Engineering, Tokyo Institute of Technology, Frontier, CREST, 4259 Nagatsuta-cyo, Midori-ku, Yokohama-shi, Kanagawa 226-8501, Japan. E-mail: yhoshino@bio.titech.ac.jp

We irradiated weak ultrasound to DNA polymerase reaction system and investigated the ultrasound effect on DNA polymerization activity and DNA polymerase binding kinetics to DNA by using a quarts crystal microbalance (27 MHz QCM) (Fig. 34).

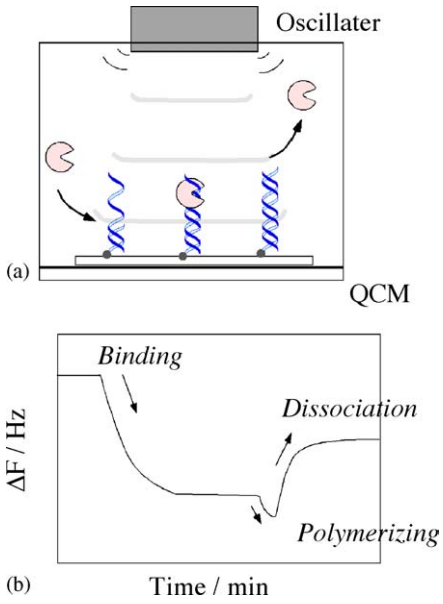

Fig. 34. (a) Schematic image of reaction system and (b) expected frequency change of a QCM during a reaction.

Direct monitoring of site-directed mutagenic glycosidase on a 27 MHz quartz-crystal microbalance

Toshiaki Mori*, Takanori Nihira, Yoshio Okahata

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To confirm that catalytic residues of isomaltodextranase, we constructed its mutants by the site-directed mutagenesis, and the kinetic parameters (k_{on}, k_{off}, K_d, and k_{cat}) could be determined using a dextran-immobilized 27 MHz quartz-crystal microbalance (QCM) (Fig. 35).

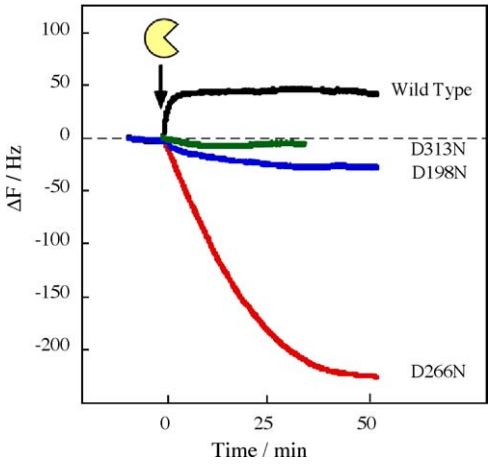

Fig. 35. Typical frequency changes (mass change) as a function of time of the dextran-immobilized QCM, responding to the addition of isomaltodextranase or its mutants in the aqueous solution.

Biotransformation of isoflavones by *Aspergillus niger*, as biocatalyst

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[b]Fuji Oil Ltd., Hannan R&D Center 1, Sumiyoshi-cho, Izumisano-shi, Osaka 598-8540, Japan.
E-mail: miyazawa@apch.kindai.ac.jp

Biotransformation of the isoflavones by *Aspergillus niger*, 6,7,4′-trimethoxyisoflavone (**1**) was transformed to 4′-hydroxy-6,7-dimethoxyisoflavone (**3**) and 5,7,4′-trimethoxyisoflavone (**2**) was transformed to 4′-hydroxy-5,7-dimethoxyisoflavone (**4**) (Fig. 36).

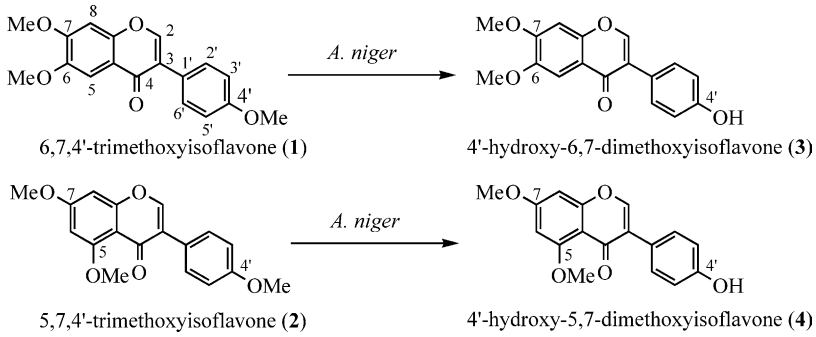

Fig. 36. Biotransformation of 6,7,4′-trimethoxyisoflavone (**1**) and 5,7,4′-trimethoxyisoflavone (**2**) by *A. niger*.

The baker's yeast reduction of β-keto esters in the presence of a thioglycoside

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Improved enantioselectivity was achieved in the free/immobilized baker's yeast reduction of β-keto esters in water using a thioglycoside as an additive (Table 3).

Table 3
The baker's yeast reduction of ethyl acetoacetate

Baker's yeast	Additive	Time (h)	*S*, e.e. (%)
Free	None	25	80
Free	Ethyl 1-thio-D-glucopyranoside	25	89
Immobilized	None	44	79
Immobilized	Ethyl 1-thio-D-ghicopyranoside	44	86

Activation of lipase PS by the ionic liquid coating

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A remarkable acceleration was recorded when *Pseudomonas cepacia* lipase was coasted by 1-butyl-1,2-dimethylimidazolium polyoxyethylene (10) cetyl sulfate (IL1) and used as catalyst for transesterification of 1-phenylethanol while maintaining excellent enantioselectivity (Fig. 37).

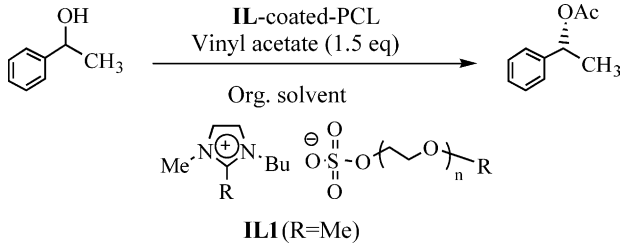

Fig. 37. Transesterification catalyzed by IL1-coated lipase PS.

The synthesis of medium cyclic keto-lactams with plant cell culture

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We have synthesized medium cyclic keto-lactams by the oxidative cleavages of substituted tetrahydrocarbazoles such as 6-methoxy-1,2,3,4-tetrahydrocarbazole (**1**) in the presence of H_2O_2 using plant cell cultures as a catalytic system (Fig. 38).

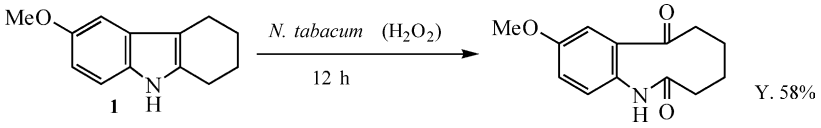

Fig. 38. The synthesis of medium cyclic keto-lactams using plant cell cultures.

Ionic liquid coated lipase PS-catalyzed enantioselective transesterfication

Yuichi Matsushita, Han Shi Hui, Shohei Wada, Toshiyuki Itoh*
Department of Materials Science, Faculty of Engineering, Tottori University, 4-101 Koyama Kinami, Tottori 680-8552, Japan. E-mail: titoh@chem.tottori-u.ac.jp

Transesterification of secondary alcohols was demonstrated using IL1-coated *Pseudomonas cepacia* lipase in the presence of vinyl acetate as acyl donor; the reaction was significantly dependent on the substrate and it reached a thousand times of acceleration of commercial lipase for several alcohols (Fig. 39).

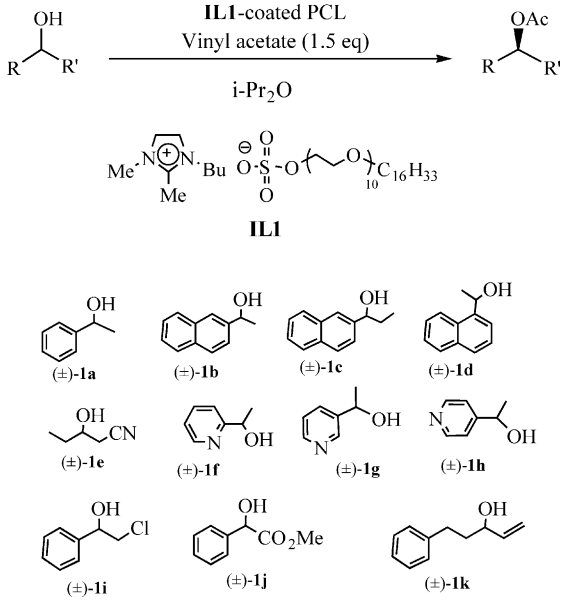

Fig. 39. Transesterification of various types of alcohols by IL1-coated lipase PS.

Transglycosylation reaction catalyzed by α-amylase using supramolecular substrate complex

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Ultrasonic irradiation onto starch–glycolipid mixture solution, which induced the proximity effect between glycosyl donor and acceptor, enhanced a transglycosylation reaction activity of α-amylase having low transglycosylation efficiency (Fig. 40).

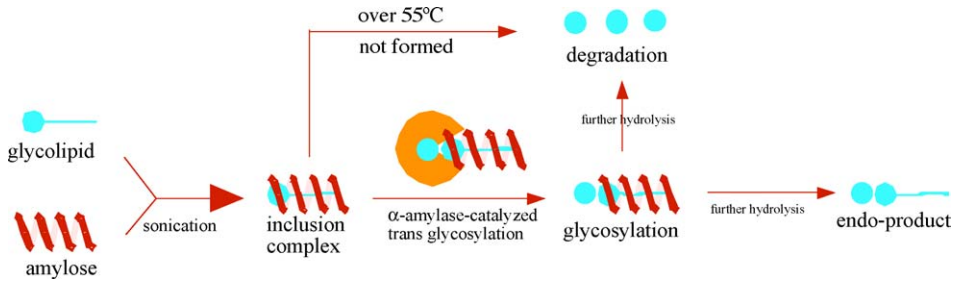

Fig. 40. Schematic depiction of α-amylase catalyzed transglycosylation with or without ultrasound-irradiated substrate mixture.

A novel strategy for the optical resolution of axially chiral binaphthol derivatives using lipase-catalyzed reaction

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Various types of binaphthol derivatives were subjected to lipase-catalyzed hydrolysis or transesterification; the results were significantly dependent on the alkyl chin length between the binaphtyl ring and terminal hydroxyl group (Fig. 41).

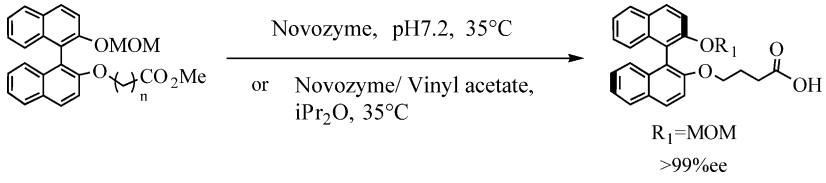

Fig. 41. Kinetic resolution of a binaphtyl compounds via lipase-catalyzed hydrolysis.

Asymmetric synthesis of 4-chromanones: Synthesis of chiral intermediate by lipase-catalyzed reaction

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4-Chromanones were synthesized in optically active form from chiral intermediates, which were obtained by lipase-catalyzed reactions (Fig. 42).

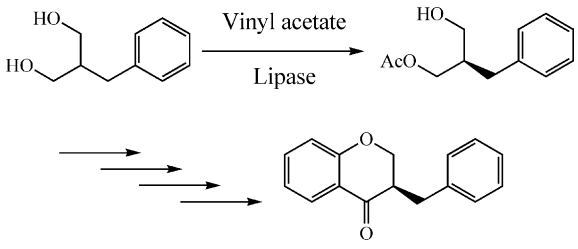

Fig. 42. Asymmetric synthesis of 3-benzyl-4-chromanone.

Regioselective hydroxylation of adamantane through microbial oxidation system

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Hydroxylation of adamantane by whole cells of *Streptomyces* strains was highly regioselective to give 1-adamantanol (Fig. 43).

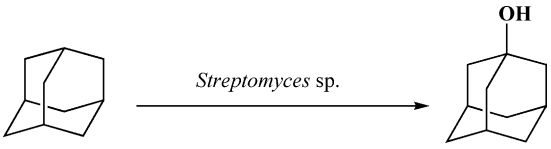

Fig. 43. Hydroxylation of adamantane by *Streptomyces* strains.

Lipase-catalyzed regioselective transesterification of D-allose

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The acylation of the rare sugar, D-allose (C-3 epimer of D-glucose), with fatty acid vinyl esters was successfully carried out using *Candida antarctica* lipase in acetonitrile to give D-allose 6-alkanoates with high regioselectivity in good yields (Fig. 44).

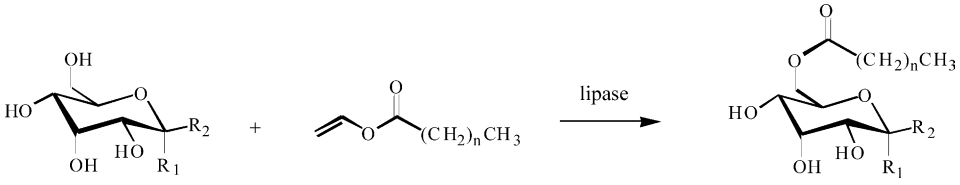

Fig. 44. Lipase-catalyzed acylation of D-allose with fatty acid vinyl esters.

Stereochemistry of decarboxylation of arylmalonic acid catalyzed by a mutant enzyme

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The enantiotopos differentiating selectivity of the mutant arylmalonate decarboxylase (S36N, G74C, and C188S) was revealed to be the same as that of the wild type enzyme, in spite of the fact that two enzymes gave the opposite enantiomer with each other as the products (Fig. 45).

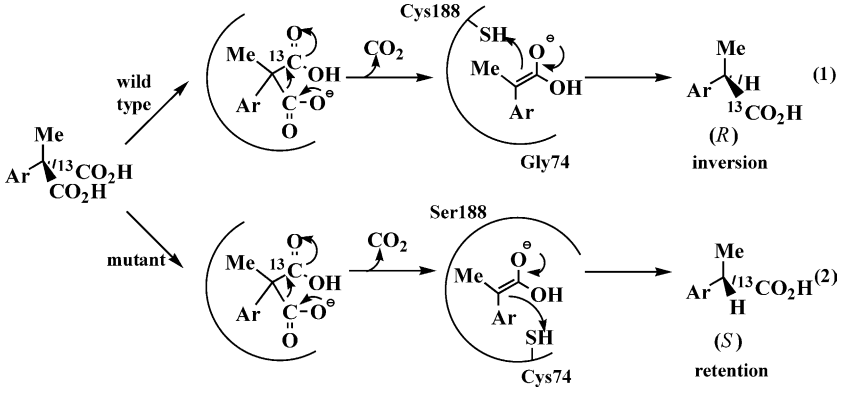

Fig. 45. The reaction mechanism of arylmalonate decarboxylase.