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Abstracts

Selected abstracts from the 12th Japanese Symposium on the Chemistry of Biocatalysis

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Introduction

'The 12th Japanese Symposium on the Chemistry of Biocatalyst' was successfully held in Toho University, Chiba, Japan, on December 4–5, 2008, organized by Professor Hiroyuki Akita of Toho University. The main subject was "material conversion by biocatalyst". There were eight invited lectures, eleven oral presentations, and 47 posters. About 160 participants from Universities, government research institutes, and companies, extensively discussed the bioconversion based on the gene technology, novel biocatalysts, mechanisms of their reactions and their application in organic synthesis, etc. Shown below are the selected short abstracts of the presentation.

Invited Lectures

Synthesis and *in vivo* evaluation of amyloid-β aggregation inhibitor

Hiroaki Okuno

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Developed were new Aβ aggregation disruptors composed of a stilbene derivative and rather hydrophilic peptide, and the evaluation *in vivo* with Tg mouse applying an Alzet administration method showed markedly disappeared senile plaques for the first time in the brain of the model mouse treated with DSB(EEX)3 compound (Fig. 1).

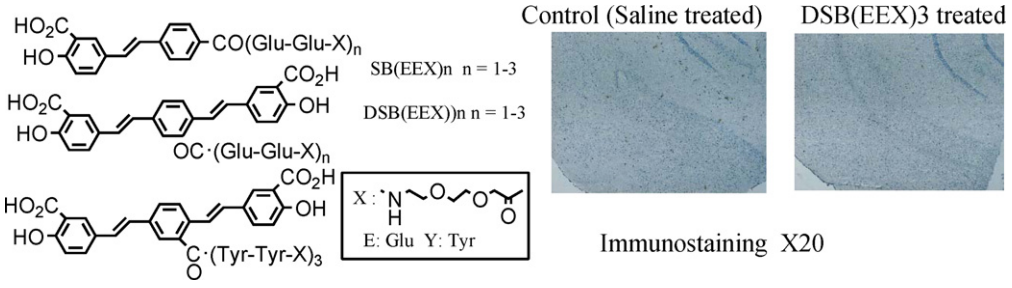

Fig. 1. Synthesis and *in vivo* evaluation of amyloid-β aggregation inhibitor.

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doi:10.1016/j.molcatb.2009.08.014

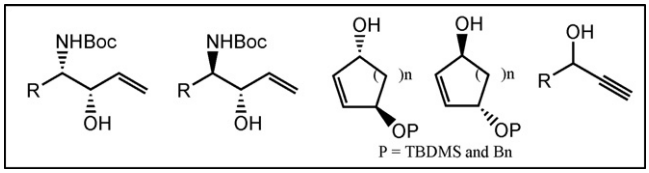

Fig. 2. Chirons synthesized by lipase-catalyzed reactions.

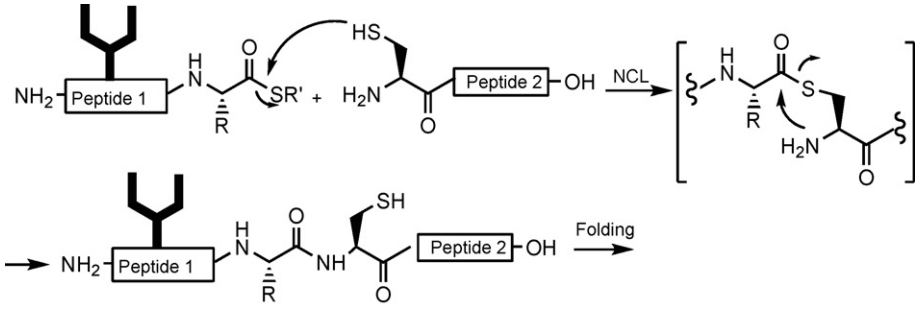

Fig. 3. Synthesis of glycoproteins.

Present and future's dream for the application of bioconversion processes on chemical industry

Junzo Hasegawa
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Now-a-days, almost chemical products are derived from oil. Exhaustion of oil in near future and increase of atmospheric carbon dioxide are very serious problems for human being. Bridge over these definite difficulties, development of distinguished technical innovations to use of renewable resources is essential. Biotechnology is also an important technology to solve these predicaments. Especially, biotechnology might serve in the fields of production of bio-energy, basic chemicals and bio-materials, and for energy saving measure and process innovation.

I would like to introduce our approach to the problems, a bio-polymer production and process innovation on APIs syntheses.

Future directions in biocatalysis

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Future directions in biocatalytic research are discussed. The situation using biocatalysts can be predicted to change as follows:

(1) Substrates for biocatalysis will be changed from petroleum products to biomass.
(2) Non-conventional reaction media such as ionic liquids, supercritical fluids, and dense gas will be frequently used.
(3) Engineered microbes will be widely used.
(4) In chemical industries, biocatalysts will be used in place of chemical catalysts.

The potential of biocatalysts in organic synthesis

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The power of lipase in kinetic resolution of alcohols has been demonstrated in the preparation of a variety of man-made chirons such as 1,2-aminoalcohols, 4-alkoxycycloalk-2-en-1-ols, and propargylic alcohols, which would provide easy access to several biologically active compounds of high synthetic value (Fig. 2).

Chemoenzymatic synthesis of diverse oligosaccharides and chemical synthesis of glycoprotein's

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Chemical synthesis of glycoprotein having a human complex type oligosaccharide such as chemokine (MCP-3) and several small glycoproteins were examined by use of native chemical ligation (Fig. 3).

Cloning of the novel gene for the vitamin D3 hydroxylase and its application to the biocatalytic process to produce active vitamin D3

Yoshikazu Fujii[a,c,*], Hiroki Kabumoto[a], Kenji Nishimura[a], Yoshiaki Yasutake[b], Koji Takeda[a], Tomohiro Tamura[b,c] Akira Arisawa[a]
[a]Mercian corporation, 1808 Nakaizumi, Iwata, Shizuoka, 438-0078, Japan

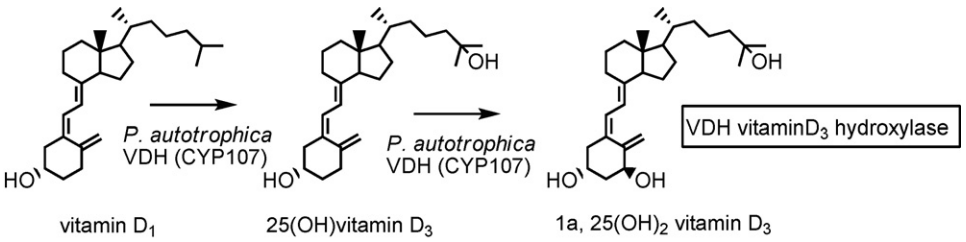

Fig. 4. Active vitamin D_3.

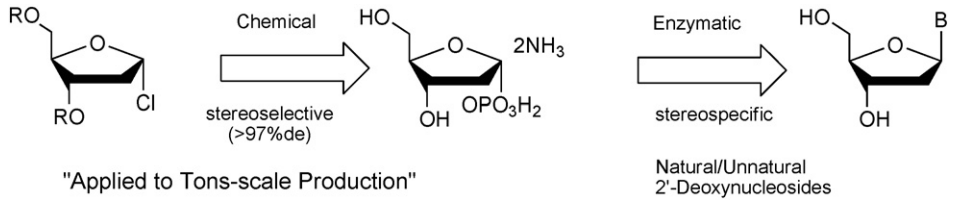

Fig. 5. Synthesis of deoxynucleosides.

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The enzyme responsible for 1α,25$(OH)_2$ vitamin D_3 production from vitamin D_3 was purified from *Pseudonocardia autotrophica* by multi-steps column chromatography and the gene for the enzyme was applied for improving vitamin D_3 biotransformation with recombinant system (Fig. 4).

Development of a simple process for deoxynucleosides by using an enzymatic reaction

Hironori Komatsu
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The method consists of three distinctive technologies: (i) stereoselective synthesis of 2-deoxyribose 1-α-phosphate (dRP) by crystallization-induced asymmetric transformation; (ii) an efficient method to expedite an enzymatic conversion by adding $Mg(OH)_2$; (iii) development of a new enzyme for the enzymatic synthesis of 2′-deoxycytidine (Fig. 5).

Bioprocesses in non-aqueous media

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We investigated *Kocuria rhizophila* DC2201,whose cell was tolerant to over 25 different types of organic solvent including alcohols, hydrocarbons, acetic acid esters, and ketones, and expected DC2201 could be a good host cell of industrial bioconversion to produce chemicals in non-aqueous media.

Oral Presentation

Structure-based engineering of plant polyketide synthases

Kiyofumi Wanibuchi[a], Hiroyuki Morita[a], Hiroshi Noguchi[a], Ikuro Abe[a,b,*]
[a]School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
[b]PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan. E-mail: abei@u-shizuoka-ken.ac.jp

On the basis of the crystal structures of wild-type and M207G mutant pentaketide chromone synthase (PCS) from *Aloe arborescens*, F80A/Y82A/M207G triple mutant was constructed and shown to produce an unnatural novel nonaketide naphthopyrone by sequential condensations of nine molecules of malonyl-CoA (Fig. 6).

Enzymatic synthesis of unnatural novel compounds by plant polyketide synthases

Hiroyuki Morita[a], Shepo Shi[a], Hiroshi Noguchi[a], Ikuro Abe[a,b,*]
[a]School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
[b]PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan. E-mail: abei@u-shizuoka-ken.ac.jp

A C_{19} hexaketide stilbene and a C_{21} heptaketide chalcone were synthesized by a plant type III polyketide synthase; octaketide synthase (OKS) from *Aloe arborescens* (Fig. 7).

Asymmetric synthesis of chiral compounds using a carbonyl reductase

Tadashi Ema*, Sayaka Ide, Taro Kadoya, Kumiko Akihara, Toshinobu Korenaga, Takashi Sakai*
Graduate School of Natural Science and Technology, Okayama University, Tsushima, Okayama 700-8530, Japan. E-mail: ema@cc.okayama-u.ac.jp

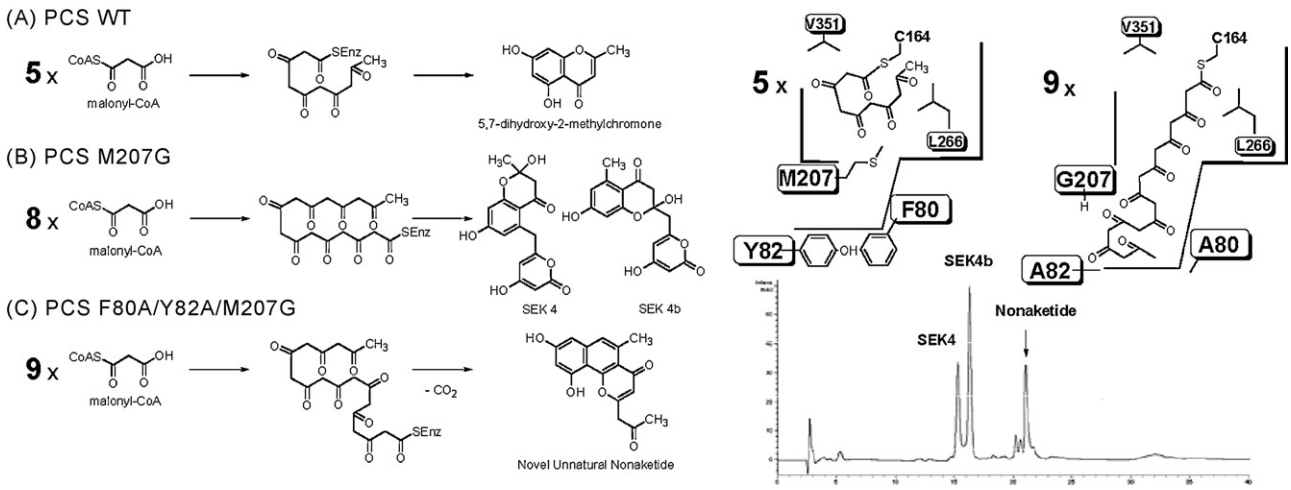

Fig. 6. Structure-based engineering of plant polyketide synthases.

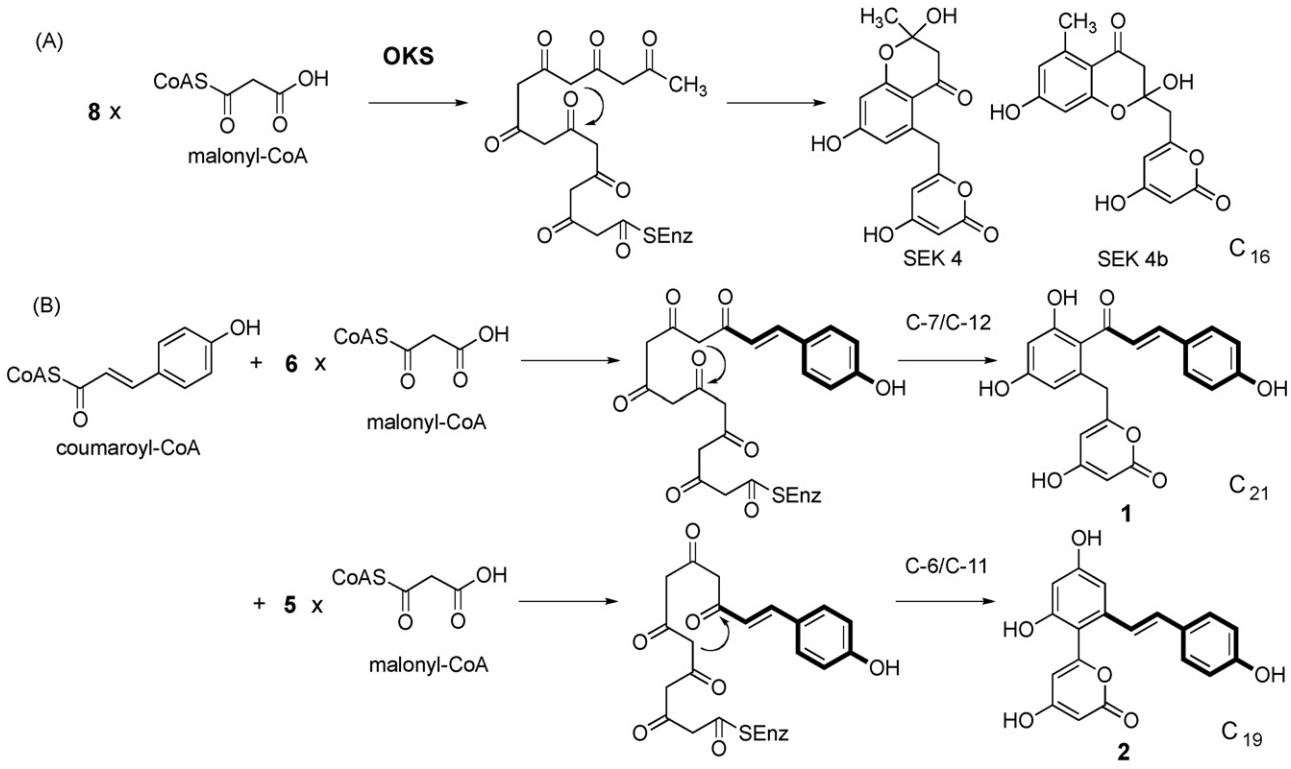

Fig. 7. Enzymatic synthesis by plant polyketide synthases.

A recombinant *E. coli* overproducing a versatile carbonyl reductase called SCR was used to prepare optically active alcohols containing the difluoromethylene group (Fig. 8).

Development of optically active tryptophan synthesis using acylase and 3-step total synthesis of clavicipitc acid

Yuusaku Yokoyama[a,*], Hidemasa Hikawa[a], Yasuoki Murakami[b], Hiroaki Okuno[a]

[a]Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba, 274-8510, Japan. E-mail: yokoyama@phar.toho-u.ac.jp

[b]Faculty of Pharmacy, Chiba Institute of Science

We have succeeded in a three-step synthesis of optically active clavicipitic acid (**5**) from 4-bromoindole (**1**) and DL-serine (**2**) using enzymatic kinetic resolution with acylase followed by Pd-catalyzed Heck reaction of the resulted optically active 4-bromotryptoptohan (**4**) (Fig. 9).

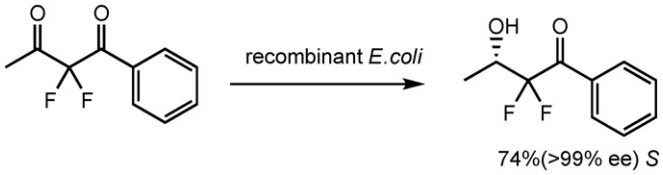

Fig. 8. Asymmetric synthesis of chiral compounds using a carbonyl reductase.

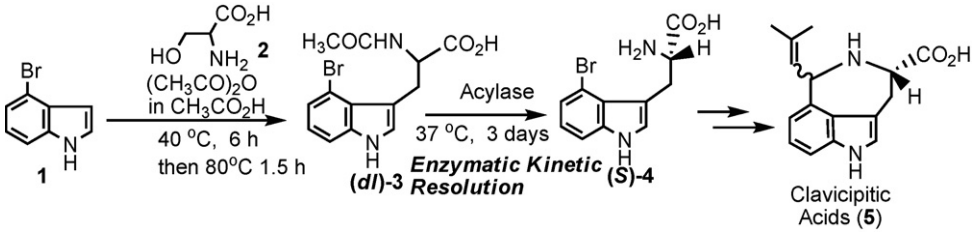

Fig. 9. Acylase catalyzed 3-step total synthesis of clavicipitc acid.

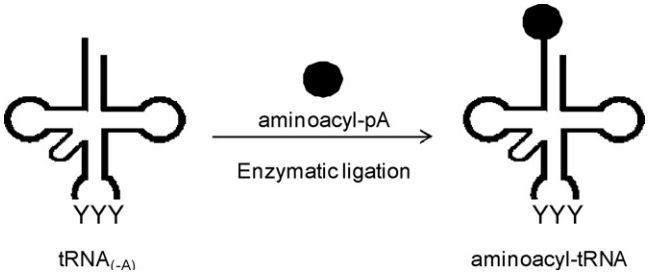

Fig. 10. Preparation of aminoacyl-tRNAs.

Very simple method for the preparation of aminoacyl-tRNAs

Dai-ichiro Kato*, Junichi Ohsako, Takeshi Matsumoto, Masahiro Takeo, Seiji Negoro

Department of Materials Science and Chemistry, Graduate School of Engineering, University of Hyogo, 2167 Shosha, Himeji, Hyogo 671-2201, Japan. E-mail: kato@eng.u-hyogo.ac.jp

We have established a new simpler method for the preparation of aminoacyl-tRNA by the enzymatic ligation of aminoacyl-pA and $tRNA_{(-A)}$, which will make a strong site-specific amino acid mutagenesis tool for a wide range of researchers in the field of pharmaceutical and bioorganic chemistry (Fig. 10).

Production of novel macrolide antibiotics by mycinose biosynthesis gene of *Micromonospora griseorubida* producing mycinamicin

Yojiro Anzai[a,*], Yohei Iizaka[a], Wei Li[a], Naoki Idemoto[a], Kazuo Koike[a], Kenji Kinoshita[b], Fumio Kato[a]

[a]Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan. E-mail: yanzai@phar.toho-u.ac.jp

[b]School of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Kyuban-cho, Koshien, Nishinomiya, Hyogo 663-8179, Japan

19-Deformyl-5-*O*-mycaminosyltylonolide (**1**) was converted to 19-deformyldesmycosin (**2**) and the novel compound 19-deformyl-12,13-epoxydesmycosin (**3**) by hybrid biosynthesis with the polyketide synthase inactivated strain *M. griseorubida* M7A21 which parent strain *M. griseorubida* A11725 produces 16-memberd macrolide antibiotics mycinamicin. Mycinose biosynthesis gene (*mycCI*, *mycCII*, *mycD*, *mycE*, *mycF*, *mydH*, and *mydI*) in *M. griseorubida* A11725 was introduced into *Micromonospora rosaria* IFO13697 producing rosamicin (**4**), and the resulting strain *M. rosaria* TPMA0001 produced "unnatural" natural mycinosyl compounds IZI, 23-*O*-mycinosyl-20-deoxo-20-dihydro-12,13-deepoxyrosamicin, (**5**) and IZII, 23-*O*-mycinosyl-20-deoxo-20-hydroxy-12,13-deepoxyrosamicin, (**6**) (Fig. 11).

Activation of enzymatic reactions by chiral imidazolium alkyl PEG sulfate

Yoshikazu Abe, Takuya Hirakawa, Shuichi Hayase, Motoi Kawatsura, Toshiyuki Itoh*

Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, 4-101Koyama Minami, Tottori 680-8552, Japan. E-mail: titoh@chem.tottori-u.ac.jp

Chiral pyrrolidine-substituted imidazolium cetyl-PEG-sulfate (D-ProMe) derived from D-proline worked as an excellent activating agent of *Burkholderia cepacia* lipase; it is particularly interesting that the D-isomer of the imidazolium salt worked better than the L-isomer (Fig. 12).

The synthesis of bioactive compounds using plant cultured cells -glycosylation-

Hiroki Hamada*, Azusa Ohiro, Mai Kondo, Daisuke Sato, Nobuyuki Nakajima, Kei Shimoda

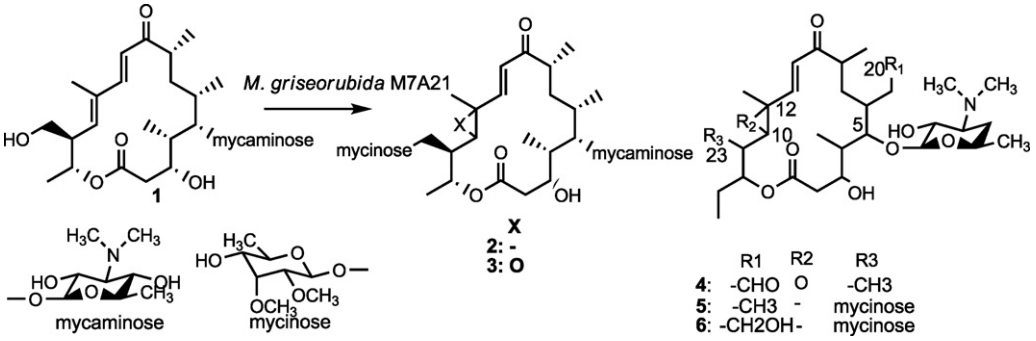

Fig. 11. Production of novel macrolide antibiotics by *Micromonospora griseorubida*.

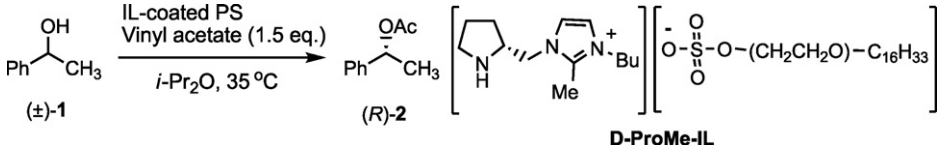

Fig. 12. Activation of enzymatic reactions by chiral imidazolium alkyl PEG sulfate.

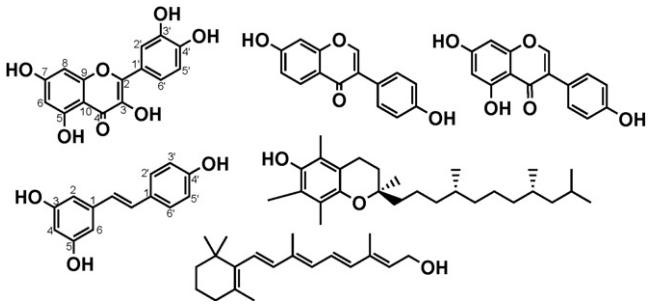

Fig. 13. Bioactive compounds for glycosylation by plant cultured cells.

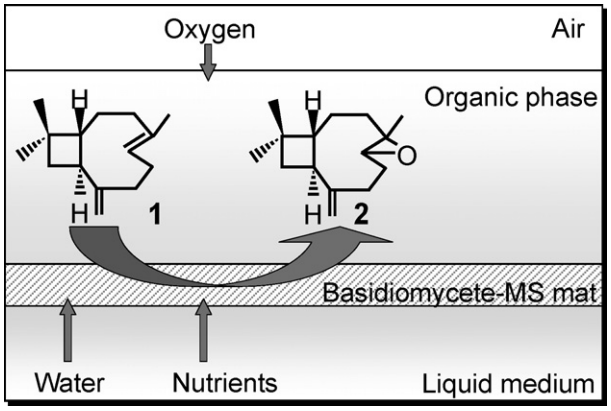

Fig. 14. Regioselective epoxidation in a liquid–liquid interface bioreactor (L–L IBR).

Department of Life Science, Okayama University of Science, Ridai-cho Okayama 700-0005, Japan. E-mail: hamada@dls.ous.ac.jp

Glycosylation of various phenols such as quercetin, daidzein, resveratrol and vitamins A and E using plant cultured cells have been carried out to give the corresponding glycosylated products regioselectively (Fig. 13).

Regioselective epoxidation of β-caryophyllene with *Nemania aenea* SF 10099-1 in a liquid–liquid interface bioreactor (L–L IBR)

Shinobu Oda*, Shinichi Ohashi

Genome Biotechnology Laboratory, Kanazawa Institute of Technology, 3-1 Yatsukaho, Hakusan, Ishikawa 924-0838, Japan. E-mail: odas@neptune.kanazawa-it.ac.jp

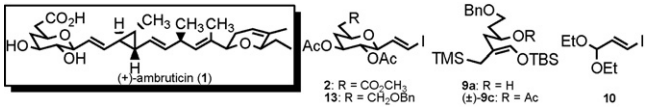

Fig. 15. Synthesis of optically active left fragment of ambruticin.

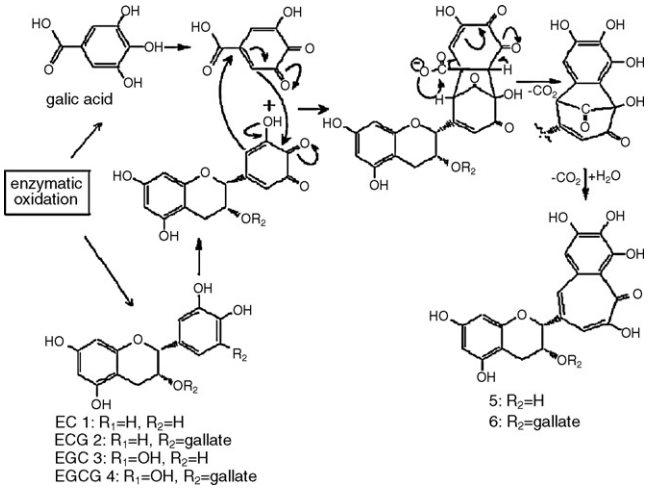

Fig. 16. Biocatalytic conversion of green tea catechins.

β-Caryophyllene (**1**) was regioselectively epoxidized to β-caryophyllene oxide (**2**, accumulation, >35 g/L-organic phase) with a basidiomycete, *Nemania aenea* SF 10099-1, in a liquid–liquid interface bioreactor (L–L IBR) (Fig. 14).

Synthesis of optically active left fragment of ambruticin via enzymatic resolution

Laurent Turet[a], Takuya Kumamoto[a,b], István E. Markó[a,*]

[a]Unité de Chimie Organique and Médicinale, Université catholique de Louvain, Place Louis Pasteuer 1, B-1348, Louvain-la-Neuve, Belgium

[b]Graduate School of Pharmaceutical Sciences, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba, Japan. E-mail: istvan.marko@uclouvain.be

Toward synthesis of optically active left fragment **2** of (+)-ambuticin (**1**), enzymatic resolution of acetate (±)-**9c** with Amano PS was successful to give the corresponding optically active alcohol **9a** in 22% and 84% ee, which was converted to (2*S*,3*S*,4*S*,6*R*)-**13** with desired stereochemistry via silyl-modified Sakurai cyclization with acetal **10** (Fig. 15).

Biocatalytic conversion of green tea catechins to epitheaflagallin and epitheaflagallin 3-O-gallate: Production of promising functional foods

Junji Kurokawa[a], Yuji Katsube[b], Keiichi Yamamoto[b], Takayuki Matsunaga[c], Masaru Ogasawara[c], Nobuya Itoh[a,*]

[a]Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, Imizu, Toyama 939-0398, Japan. E-mail: nbito@pu-toyama.ac.jp

[b]Kracie Pharma Ltd.

[c]Toyama Prefectural Institute for Pharmaceutical Research

Green tea which contain catechin derivatives such as (−)-epicatechin (EC) (**1**), (−)-epicatechin gallate (ECg) (**2**), (−)-epigallocatechin (EGC) (**3**), and (−)-epigallocatechin gallate (EGCg) (**4**) has been recognized as a useful functional food. Epitheaflagallin and epitheaflagallin 3-*O*-gallate, which are minor components of black tea, are preferentially synthesized from EGC and EGCg in green tea extracts in the presence of laccase and gallic acid and, thus, to improve the composition of the catechins present in green tea (Fig. 16).

Poster Presentations

Biotransformation of flavonoids by plant cultured cells

Eriko Kimura[*], Tatsunari Kobayashi, Nobuyoshi Nakajima, Kei Shimoda, Naoji Kubota, Hiroki Hamada

Department of Life Science, Okayama University of Science, Ridai-cho Okayama 700-0005, Japan. E-mail: hamada@dls.ous.ac.jp

One of plant cultured cells, *E. perriniana*, has been found to glycosylate regioselectively the hydroxyl group of polyphenols (flavonoids) such as fisetin and morin (Fig. 17).

Synthesis of indoleamine 2,3-dioxygenase (IDO) inhibitor

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[a]Department of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba, 274-8510, Japan.
E-mail: yokoyama@phar.toho-u.ac.jp

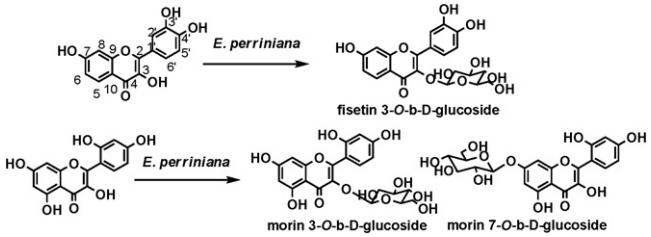

Fig. 17. Biotransformation of fisetin and morin by plant cultured cells.

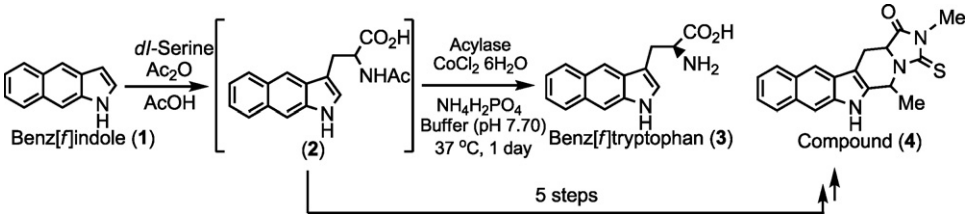

Fig. 18. Synthesis of Indoleamine 2,3-dioxygenase (IDO) inhibitor.

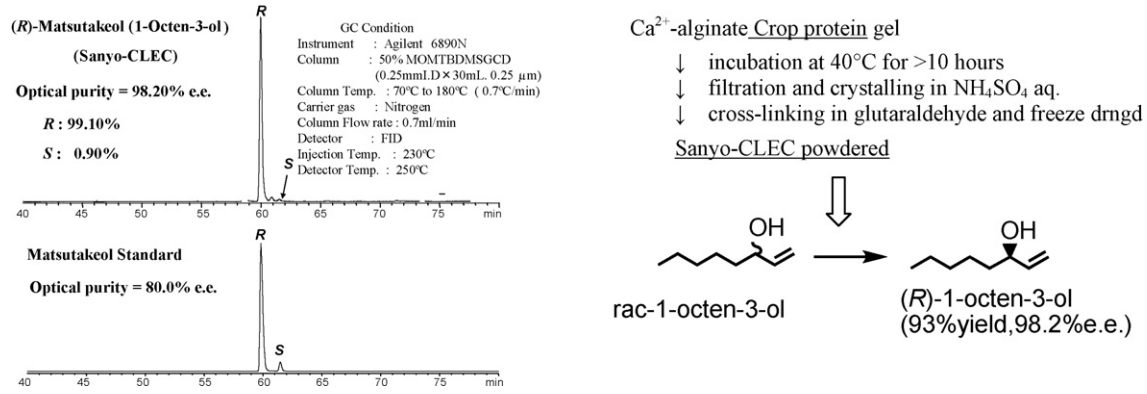

Fig. 19. Chromatography of (*R*)-1-octen-3-ol obtained with Sanyo cross-linked enzyme crystal.

[b]National Institute for Longevity Sciences

We synthesized optically pure benz[*f*]tryptophan (**3**) and its analogue (**4**) from benz[*f*]indole (**1**) and DL-serine through N-acetylbenz[*f*]tryptophan (**2**), and the compound (**4**) showed the potent IDO inhibitory activity (Fig. 18).

Deracemization to (*R*)-1-octen-3-ol with Sanyo-cross-linked enzyme crystals

Hiroyuki Nagaoka*

Sanyo Shokuhin Co., Ltd. R&D, 555-4 Asakura, Maebashi, Gunma 371-0811, Japan. E-mail: hnagaoka@sanyofoods.co.jp

The NAD(P)-alcohol dehydrogenase easily obtained from Ca^{2+}-alginate crop protein gel was crystallized, cross-linked, and freeze-dried, the Sanyo-CLEC catalyzed deracemization; that is, *S*-isomer in *rac*-1-octen-3-ol (Matsutakeol) was enantioselectively catalyzed oxidation to 1-octen-3-one and then catalyzed reduction to (*R*)-1-octen-3-ol with 93% yield, 98.2% e.e (Fig. 19).

Asymmetric synthesis of versatile monoepoxyzerumbone and monoepoxyzerumbol

Masataka Awata, Tsuji Azusa, Yasuhiko Yoshida, Yasushi Kawai, Takashi Kitayama*

Department of Advanced Bioscience, Graduate School of Agriculture, Kinki University, 3327-204, Naka-machi, Nara, 631-8505, Japan. E-mail: kitayama@nara.kindai.ac.jp

Versatile two kinds of optically active 6,7-monoepoxyzerumbone were synthesized by oxidation of corresponding optically active monoepoxyzerumbols prepared by lipase-catalyzed enantioselective transesterification of racemic one (Fig. 20).

Peptide deformylase- and methionine aminopepitdase-catalyzed formyl-methionine-removal toward ribosome-catalyzed non-natural peptide synthesis

Takashi Kawakami[a], Atsushi Ohta[a], Hiroshi Ashigai[a], Hiroshi Murakami[b], Hiroaki Suga[a,b,*]

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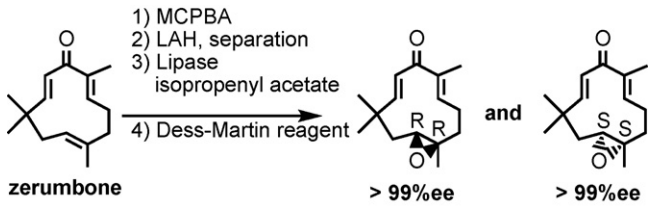

Fig. 20. Asymmetric synthesis of versatile monoepoxyzerumbone and monoepoxyzerumbol.

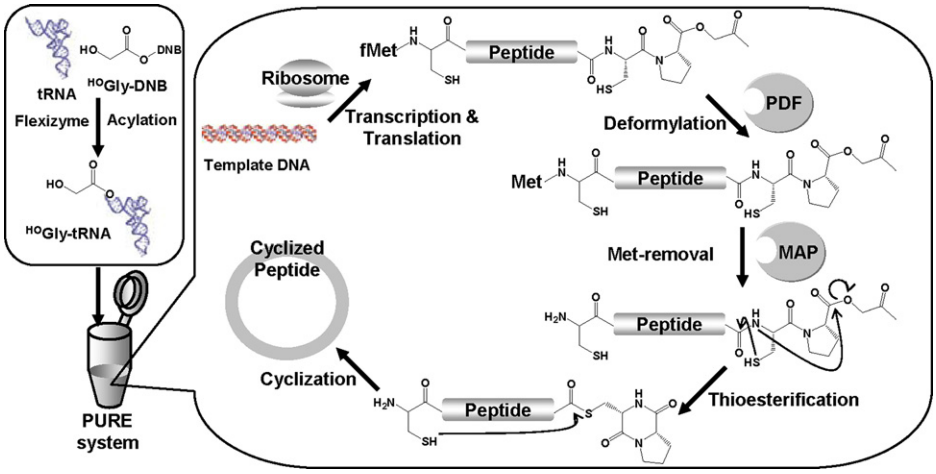

Fig. 21. Ribosomal synthesis of backbone-cyclized peptides via PDF- and MAP-catalyzed fMet-removal.

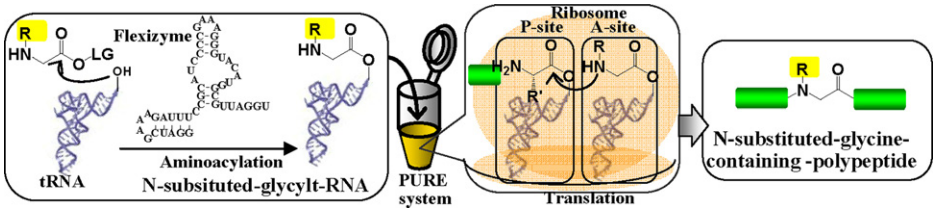

Fig. 22. Ribosomal incorporation of N-substituted-glycines into polypeptides.

We demonstrated ribosomal synthesis of backbone-cyclized non-natural peptides in an engineered *in vitro* translation system (PURE system) by means of intra-molecular native chemical ligation between an N-terminal cystein enzymatically constructed using peptide deformylase (PDF)- and methionine aminopeptidase (MAP)-catalyzed formyl-methionine (fMet)-removal and a C-terminal thioester chemically constructed using a spontaneous thioesterification of Cys-Pro-^{HO}Gly (^{HO}Gly; glycolic acid) introduced by the ribosomal incorporation of ^{HO}Gly charged onto tRNA by flexizyme (Fig. 21).

Ribosome-catalyzed incorporation of various N-substituted glycines into a nascent polypeptide backbone

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We demonstrated comprehensive screening of ribosomal incorporation of a variety of N-substituted glycines where N-substituents are alkyl, aryl, hydroxyl, amine, carboxyl, amide, cyano, ketone, ester, alkene, alkyne, and azide into a polypeptide backbone using the integration of tRNA-aminoacylating ribozyme (flexizyme) and reconstituted cell-free translation system (PURE system) (Fig. 22).

Asymmetric reduction of ketones by cultured cells of *Nicotiana tabacum*

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Chemical yields and enantioselectivities in asymmetric reduction of ketones by photoautotrophic cultured cells of *Nicotiana tabacum* under illumination of fluorescent lamp were largely influenced by concentrations of atmospheric carbon dioxide (Fig. 23).

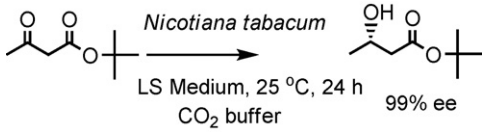

Fig. 23. Reduction of *t*-butyl acetoacetate by *Nicotiana tabacum* cultured cells.

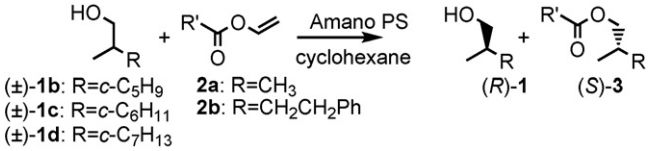

Fig. 24. Lipase-catalyzed transesterification of primary alcohols.

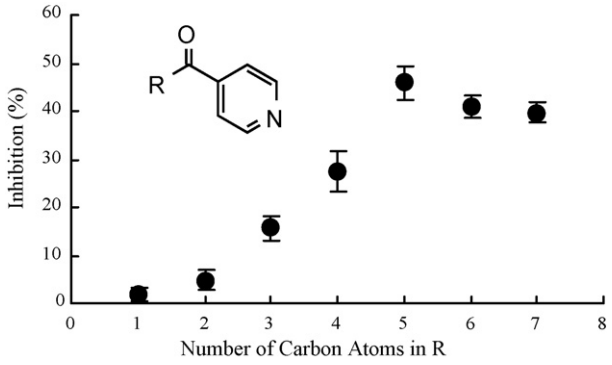

Fig. 25. Inhibitory effects of aryl alkyl ketones on carbonyl reductase (DHRS4) activity.

Lipase-catalyzed transesterification of primary alcohols

Masashi Kawasaki[a,*], Naoki Toyooka[b], Michimasa Goto[c], Tadashi Kometani[c]
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[b]Graduate School of Medicinal and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[c]Department of Chemical and Biochemical Engineering, Toyama National College of Technology, 13 Hongo, Toyama 930-8630, Japan
Lipase-catalyzed enantioselective transesterification of a variety of primary alcohols containing cycloalkane ring with vinyl esters was studied (Fig. 24).

Catalytic reaction of tetrameric carbonyl reductase (DHRS4) in pig heart: Structural characteristics of substrate-binding domain

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[b]Liberal Arts Education Center, Aso Campus, Tokai University, Kumamoto 869-1404, Japan
[c]Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan. E-mail: hshimada@gpo.kumamoto-u.ac.jp
The inhibitory effects of aryl alkyl ketones on tetrameric carbonyl reductase (DHRS4) activity were examined, using the cytosolic fraction of pig heart, and the result indicated that a hydro-phobic cavity corresponding to straight-chain alkyl group of five carbon atoms is located in its substrate-binding domain (Fig. 25).

Biotransformation of 2-haloacetophenone derivatives by *Nostoc minutum*

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[b]Hakodate Technical College, Tokura-cho, Hakodate 042-8501, Japan
[c]Department of Chemistry, Rikkyo (St. Paul's) University, Nishi-Ikebukuro, Toshimaku, Tokyo 171-8501, Japan
Transformation of 2-haloacetophenone derivatives (**1**–**6**) was investigated using *Nostoc minutum* and it was found that biotransformation of 2-haloacetophenone derivative gives the corresponding hydroxy compound (Fig. 26).

Resolution of 2-aryloxypropanoic acids using *Carica papaya* lipase as biocatalyst

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Department of Chemistry, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan. E-mail: miyazawa@konan-u.ac.jp

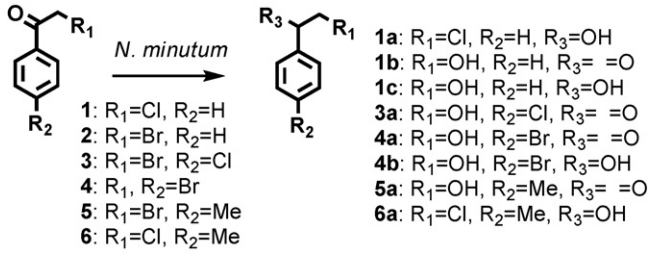

Fig. 26. Biotransformation of 2-haloacetophenone derivatives by *Nostoc minutum*.

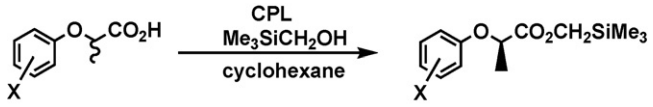

Fig. 27. CPL-catalyzed enantioselective esterification of 2-aryloxypropanoic acids.

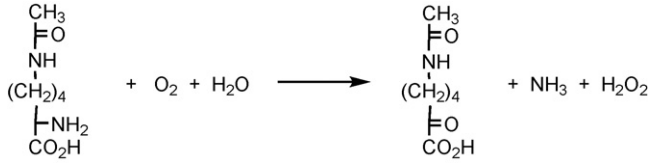

Fig. 28. Resolution of N^{ε}-acetyl-DL-lysine with *Rhodococcus* sp. AIU Z-35-1.

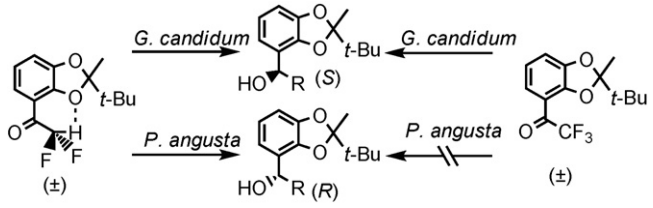

Fig. 29. Asymmetric reduction and kinetic resolution of fluorine-containing aromatic substrates.

The resolution of 2-aryloxypropanoic acids was investigated via transesterification and esterification mediated by *Carica papaya* lipase (CPL); the latter procedure depicted below generally afforded excellent enantioselectivities (Fig. 27).

Microbial resolution of N^{ε}-acetyl-DL-lysine with *Rhodococcus* sp. AIU Z-35-1

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Department of Biological Chemistry and Food Science, Iwate University, 3-18-8 Ueda, Morioka 020-8550, Japan. E-mail: kiso@iwate-u.ac.jp

Cell reaction with *Rhodococcus* sp. AIU Z-35-1 was useful for an enantioselective production of N^{ε}-acetyl-D-lysine and 6-acetylamino-2-oxohexanoic acid from N^{ε}-acetyl-DL-lysine (Fig. 28).

Study on asymmetric reduction and kinetic resolution of fluorine-containing aromatic substrates

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[b]Faculty of Pharmacy, Keio University, Kyoto University, 1-5-30 Shibakoen, Minato-ku, Tokyo, 105-8512, Japan

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Yeast-mediated asymmetric reduction of fluorine-containing aromatic ketones with remote chiral center was examined. Some contrasting changes such as reaction rate and enantiofacial selectivity were observed, between difluoro- and trifluoromethyl ketones (Fig. 29).

Study on hydrolase-catalyzed kinetic resolution of 2,2-disubstituted cyanoacetates

Yuki Tatsumi, Manabu Hamada, Toshinori Higashi, Takeshi Sugai*

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We have interested in the region- and enantioselectivity in hydrolase-catalyzed reactions on 2,2-disubstituted cyanoacetates with a quaternary chiral center. Contrasting selectivities between *Candida antarctica* lipase B and pig liver esterase were observed, as shown in Fig. 30.

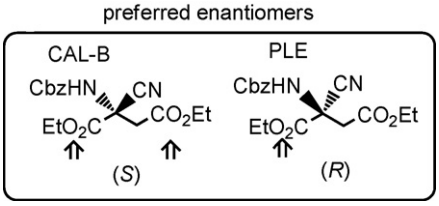

Fig. 30. Preferred enantiomers in hydrolase-catalyzed kinetic resolutin of 2,2-disubstituted cyanoacetates.

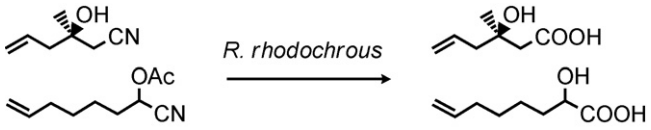

Fig. 31. Microbial hydrolysis of α- and β-hydroxy nitriles.

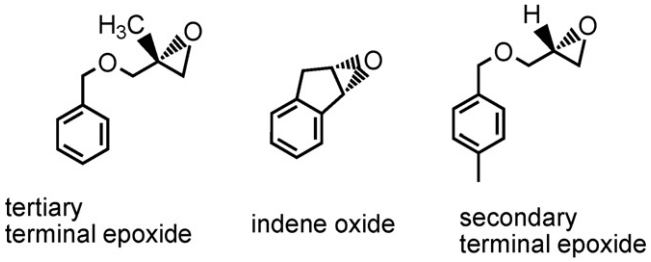

Fig. 32. Substrate specificity of epoxide hydrolase with different microbial origins.

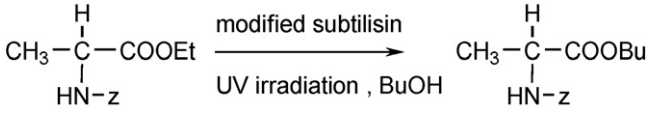

Fig. 33. Chemical modification of enzymes with azobenzene.

Preparation of α- and β-hydroxy acids based on microbial hydrolysis of nitriles

Atsuko Nakagomi[a], Aya Fujino[b], Toshinori Higashi[a,*], Takeshi Sugai[a]
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[b]Department of Chemistry, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo, 105-8512, Japan. E-mail: sugai-tk@pha.keio.ac.jp

Microbial enzymatic hydrolysis of nitriles to carboxylic acids works as mild functional group transformation. We applied cultured whole cells of *Rhodococcus rhodochrous* NBRC15564 on α- and β-hydroxy nitriles to obtain corresponding hydroxy acids (Fig. 31).

Study on substrate specificity of epoxide hydrolase with different microbial origins

Maki Sakamoto[a], Manabu Hamada[b], Aya Fujino[b], Toshinori Higashi[a,*], Takeshi Sugai[a]
[a]Faculty of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo, 105-8512, Japan
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The recent growing availability of epoxide hydrolase (EH) prompted us to examine the substrate specificity of EH with various origins, by applying tertiary and secondary terminal epoxides and indene oxide (Fig. 32).

Chemical modification of enzymes with azobenzene—Attempts to photomodulate the enzyme activity and enantioselectivity

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Graduate of Human Development and Environment, Kobe University, 3-11 Tsurukabuto, Nada, Kobe, 657-8501, Japan. E-mail: ueji@kobe-u.ac.jpThe methionine residue on the surface of subtilisin was chemically modified with azobenzene (AZB) and the influences of photochromism of AZB on the enzyme activity and enantioselectivity were investigated using transesterification of Z-L-Ala-OEt with BuOH under the UV irradiation (Fig. 33).

The use of bamboo powder on biocatalysis

Shin-ichi Nishiyma[a], Shin-ichi Ueji[a], Kaoru Nakamura[b,*]
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[b]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan. E-mail: nakamura@scl.kyoto-u.ac.jp

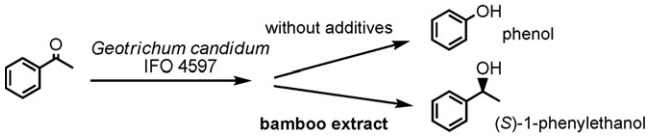

Fig. 34. Effect of bamboo on biotransformations.

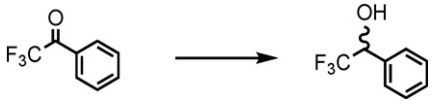

Fig. 35. Asymmetric reduction of α,α,α-trifluoroacetophenone by cyanobacteria.

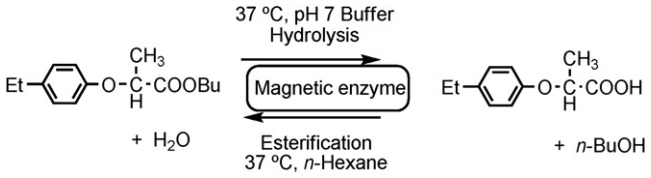

Fig. 36. Magnetic enzyme-catalyzed hydrolysis and esterification.

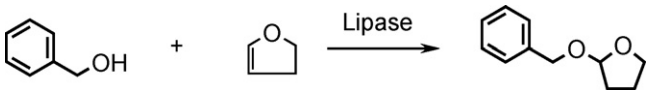

Fig. 37. Lipase-catalyzed addition reaction.

The use of bamboo powder accelerated reaction rates and improved enantioselectivities of biocatalyses such as asymmetric reduction and esterification (Fig. 34).

Asymmetric reduction of ketones by microalgae

Maoki Hama[a], Jyunya Horitsune[a], Shin-ichi Ueji[a], Kaoru Nakamura[b,*]
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[b]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan. E-mail: nakamura@scl.kyoto-u.ac.jp

Effect of pH on microalgae-mediated asymmetric reduction of α,α,α-trifluoroacetophenone has been investigated (Fig. 35).

Preparation of magnetic enzymes and its enzyme activity and enantioselectivity

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Graduate School of Human Development and Environment, Kobe University, 3-11Tsurukabuto, Nada, Kobe, 657-8501, Japan. E-mail: ueji@kobe-u.ac.jp

We prepared magnetic enzymes of *Candida rugosa* lipase and subutilisin by immobilization and coating with magnetic nanoparticles and its enzyme activity and enantioselectivity were investigated using the hydrolysis of butyl 2-(4-substituted phenoxy)propanoates and the esterification of the corresponding acid, the enantioselectivity of which was reversed in the magnetic subutilisin-catalyzed hydrolysis (Fig. 36).

Lipase-catalyzed addition reaction of benzyl alcohol to 2.3-dihydrofuran

Takeru Shigemura, Hiromi Yumoto, Shin-ichi Ueji*
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The native lipase was found to catalyze successfully the addition reaction of benzyl alcohol to 2.3-dihydrofuran, while the heat-denatured lipase resulted in a loss of its function to catalyze the addition reaction (Fig. 37).

Deodorization of bad-smelling compounds with biocatalysts

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[a]Department of Materials Chemistry, Faculty of Science and Technology, Ryukoku University, Otsu, Shiga 520-2194, Japan
[b]Tokyo Institute of Technology, Yokohama-shi, Kanagawa 226-8501, Japan
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Odor is often said as the third factor affecting the comfortable life. Then, deodorization of bad-smelling compounds is one of the target in environment improvement. We would like to report that bad-smelling compounds such as 2-octenal, body odor of old people or shield bug, could be deodorized by biocatalysts such as baker's yeast and *Geotrichum candidum*.

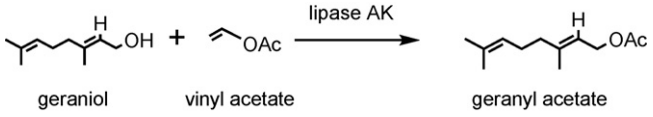

Fig. 38. Biotransformation of perfumes.

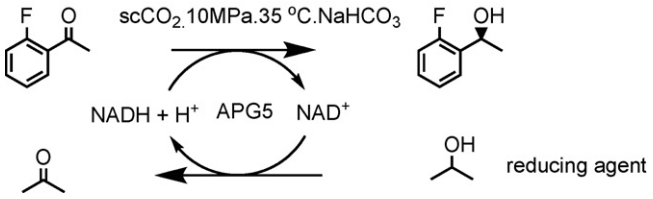

Fig. 39. Asymmetric reduction of ketone by alcohol dehydrogenase in scCO2/buffer (used reducing agent).

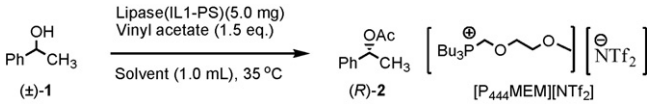

Fig. 40. Rapid transesterification of (±)-**1** by IL1-coated Lipase PS in [P_{444MEM}][NTf_2].

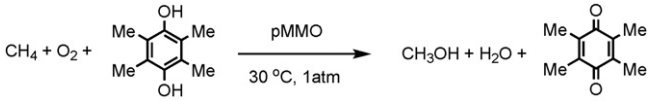

Fig. 41. Methane hydroxylation to methanol by pMMO *in vitro*.

Biotransformation of perfumes

Tomoya Nakamura[a], Yuki Kubota[a], Shuji Ohyama[a], Tadao Harada[a,*], Tomoko Matsuda[b], Kaoru Nakamura[c]
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[b]Tokyo Institute of Technology, Yokohama-shi, Kanagawa 226-8501, Japan
[c]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan. E-mail: harada@rins.ryukoku.ac.jp

Acetylation of (*rac*)-2-methyl-1-butanol, geraniol, and nerol was carried out using lipase AK immobilized on freeze-dried tofu, in the presence of vinyl acetate (Fig. 38).

Biocatalytic asymmetric reduction of ketones in supercritical carbon dioxide

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[a]Department of Materials Chemistry, Faculty of Science and Technology, Ryukoku University, Otsu, Shiga 520-2194, Japan
[b]Tokyo Institute of Technology, Yokohama-shi, Kanagawa 226-8501, Japan
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Although asymmetric reduction of ketones by a crude alcohol dehydrogenase from *Geotorichum candidum* could not proceed in supercritical carbon dioxide, the addition of sodium bicarbonate increased chemical yields of alcohols and turn over numbers of the coenzyme, and gave optically active alcohols in excellent ee (Fig. 39).

Design of phosphonium ionic liquids appropriate for lipase-catalyzed transesterification

Kazuhide Yoshiyama, Yoshikazu Abe, Keisuke Kude, Akihiko Ishioka, Shuichi Hayase, Motoi Kawatsura, Toshiyuki Itoh*
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Attempts have been made to evolve phosphonium ionic liquids appropriate for lipase-catalyzed reaction: very rapid lipase PS-catalyzed transesterification of secondary alcohols was accomplished when methoxyethoxymethyl(tri-n-butyl)phosphonium bis(trifluoromethanesulfonyl)amide ([P_{444MEM}][NTf_2]) was used as solvent (Fig. 40).

Inhibitory effect of hydrogen peroxide on particulate methane monooxygenase

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The generation of hydrogen peroxide (H_2O_2) by particulate methane monooxygenase (pMMO) and the inhibitory effect of H_2O_2 on pMMO activity were observed *in vitro*, suggesting that the inactivation of pMMO during its purification is caused by the H_2O_2 generated in the purification step (Fig. 41).

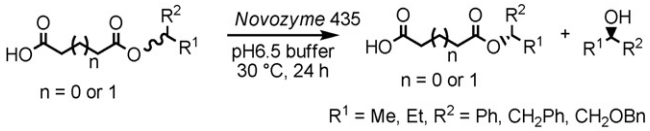

Fig. 42. Enantioselective hydrolysis of dicarboxylic acid mono esters.

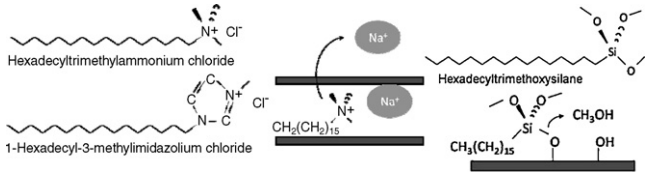

Fig. 43. Clays modified with hydrophobic chains.

Enzyme-mediated enantioselective hydrolysis of dicarboxylic acid mono esters

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The enzyme-mediated enantioselective hydrolysis of dicarboxylic acid mono esters proceeded with excellent enantioselectivity (*E* value >200) to afford the optically active alcohols (Fig. 42).

Structural and functional analysis of enone reductase p90 from cultured cells of *N. tabacum*

Hidetaka Nomura, Toshihiko Iwasaki, Akihito Matsushima, Takayoshi Fujii, Kazumasa Fujita, Naoaki Sakamoto, Takashi Yamamoto, Toshifumi Hirata, Shunsuke Izumi*
Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-hiroshima, 739-8526, Japan. E-mail: sizumi@sci.hiroshima-u.ac.jp

We have investigated the structure and function of the enone reductase that is involved in the reduction of the C–C double bond of verbenones in *N. tabacum* cells, by cDNA cloning, expression of recombinant reductase in *Escherichia coli*, and biotransformation of enones with the recombinant reductase.

Protein complex determines stereoselectivity of pulegone reductase

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Protein clusters rule the stereoselectivity of pulegone reductase; the pulegone reductase which bound with β-glucosidase and trypsin inhibitor catalyzed (1*R*,4*R*)-isomenthone preferentially rather than (1*R*,4*S*)-menthone from (*R*)-pulegone.

Immobilization of lipase on organic-modified clay compounds and their catalytic properties

Toshimitsu Kamiya[a], Daisuke Hirabayashi[a], Kenji Suzuki[a], Katsuya Kato[b,*], Keiichi Inukai[b]
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[b]National Institute of Advanced Industrial Science and Technology, 2266-98 Anagahora, Shimoshidami, Moriyama-ku, Nagoya 463-8560, Japan. E-mail: katsuya-kato@aist.go.jp

The lipase was immobilized on clays modified with hydrophobic chains, and the enzymatic activities were improved remarkably compared to non-modified clays (Fig. 43).

Recognition of nucleobase and ribosyl moiety with nucleoside-metabolism enzyme

Akihiko Hatano[a,b,*] Miki Yanagihara[b], Michiko Koike[b], Hideyuki Yamada[b], Yuichi Nakagomi[b]
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[b]Department of Materials and Life Science, Shizuoka Institute of Science and Technology, 2200-2 Toyosawa, Fukuroi, Shizuoka, Japan. E-mail: a-hatano@sic.shibaura-it.ac.jp

Thymidine phosphorylase (TP) is available to synthesize unnatural nucleosides, however, this enzyme recognized the structure of the substrate thymidine with high specificity. 4′-β-Thiothymidine was converted to 1-phosphate form in a 3.8% yield with TP, however hexopyranosyl analogue did not react (Fig. 44).

Synthesis of optically active alcohols containing the difluoromethylene group by using carbonyl reductases

Tadashi Ema*, Taro Kadoya, Kumiko Akihara, Toshinobu Korenaga, Takashi Sakai*
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Chiralscreen OH (Daicel Chemical Industries) was used to prepare both enantiomers of alcohols containing the difluoromethylene group by the asymmetric reduction of the corresponding ketones (Fig. 45).

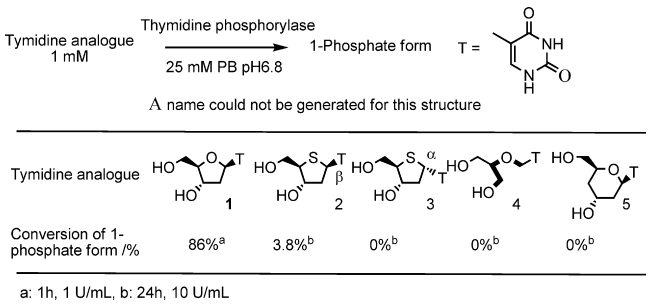

Fig. 44. Effect of ribosyl skeleton of the substrates with TP.

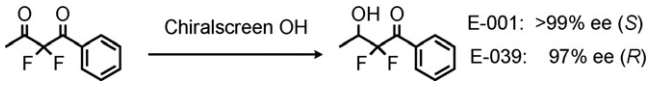

Fig. 45. Reduction of difluoroketones by carbonyl reductases.

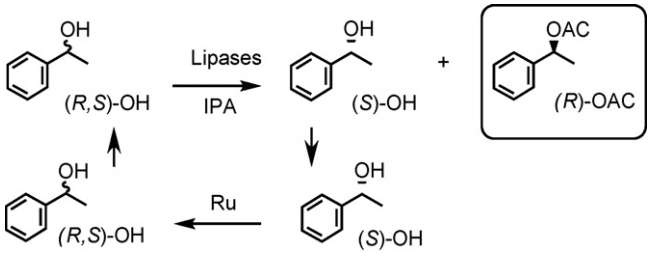

Fig. 46. Dynamic kinetic resolution of secondary alcohols by lipase and ruthenium catalyst in ILs.

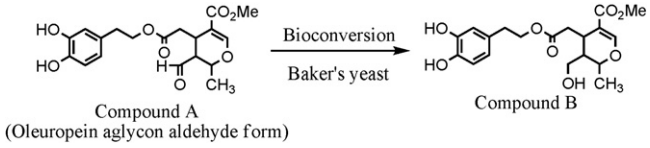

Fig. 47. Bioconversion of secondary metabolites from olive leaves by Baker's yeasts.

Dynamic kinetic resolution of secondary alcohols with immobilized lipases and ruthenium catalyst in ionic liquid

Shun Nakagaki*, Katsuya Kato, Kiyoshi Hirao

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Dynamic kinetic resolution of secondary alcohols with immobilized lipase and ruthenium catalyst was carried out in ionic liquids (ILs) under a nitrogen atmosphere to 80 °C (Fig. 46).

Conversion of secondary metabolites from olive leaves by Baker's yeasts and the antioxidative activity of the product

Erika Akihisa, Yoshihiro Harada, Kumi Kobayashi, Akiko Hirano, Teruhiko Nitoda, Hiroshi Kanzaki*

The Graduate School of Natural Science and Technology, Okayama University, 1-1-1 Tsushima-naka, Okayama, 700-8530, Japan. E-mail: hkanzaki@cc.okayama-u.ac.jp

We found that oleuropein aglycon aldehyde form (compound A) in extracts of olive leaves was converted by the Baker's yeasts catalyzed reduction to a novel alcohol compound B and that the product exhibited potent antioxidative activity in DPPH radical scavenging assay (Fig. 47).

Dynamic kinetic resolution of allyl alcohols by the combined use of lipases and $VO(OSiPh_3)_3$: Application to various substrates

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The title dynamic kinetic resolution was found to be applicable to various racemic allyl alcohols (**2** and **3**) to give the allyl acetates (*R*)-**4** in high chemical and optical yields (Fig. 48).

Purification and characterization of keto ester reductase from micro green algae

Rieko Iwai[a], Nobuyoshi Nakajima[b], Kohji Ishihara[a,*]

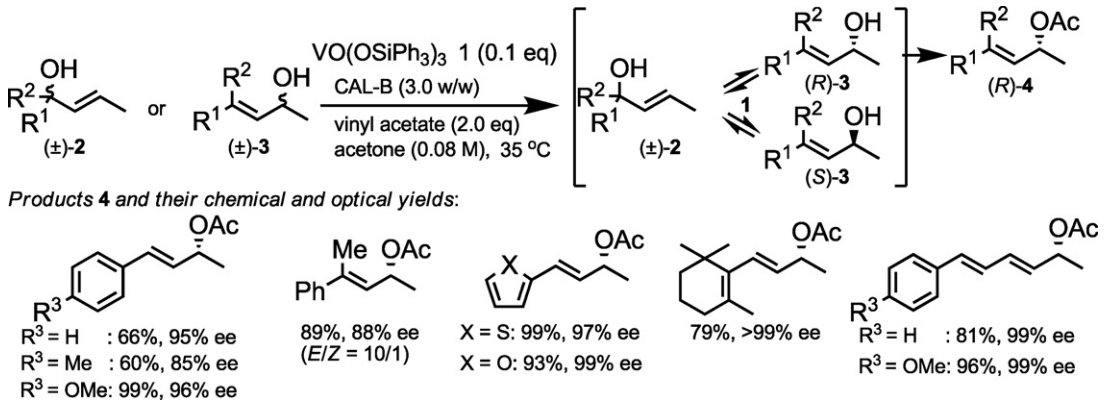

Fig. 48. Dynamic kinetic resolution of allyl alcohols.

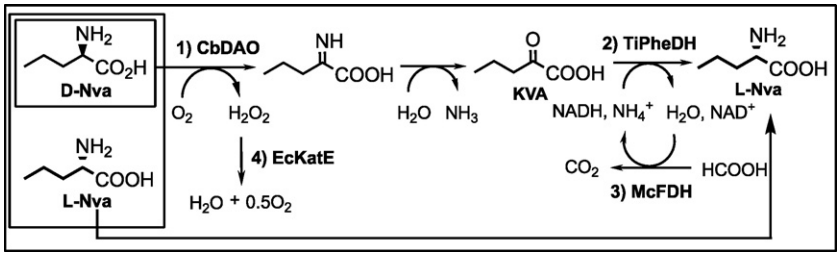

Fig. 49. One-pot synthesis of unnatural amino acid.

[a]Graduate School of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan
[b]Graduate School of Health and Welfare, Okayama Prefectural University, 111 Kuboki, Soja, Okayama 719-1197, Japan. E-mail: ishihara@dls.ous.ac.jp

We achieved the purification and characterization of a keto ester reductase from micro green algae, *C. sorokiniana* SAG 211-8k whole cells. The molecular mass of the native enzyme was estimated to be 30 kDa and 32 kDa by gel filtration chromatography and SDS-PAGE, respectively.

One-pot synthesis of unnatural amino acid with deracemization

Motoko Hayashi[a,*], Norihiro Kimoto[b], and Hiroaki Yamamoto[b]
[a]Life Science Development Center, CPI Company, Daicel Chemical Industries, Ltd., 1-1 Shinko-cho, Myoko, Niigata, 944-8550, Japan
[b]Corporate Research Center, R&D Management, Daicel Chemical Industries, Ltd., 1239 Shinzaike, Aboshi-ku, Himeji, Hyogo, 671-1283, Japan

One-pot deracemization of 80 g/l DL-Nva (DL-norvaline) with recombinant *E. coli* expressed four enzymes gave unnatural amino acid, L-Nva in 90% yield, >99%e.e. as a white crystal, and this multi-enzymatic one-pot deracemization can be applied to synthesis of other unnatural amino acids (Fig. 49).

Gene expression by *E. coli* of *Synechocystis* sp. PCC6803 for improvement of ketone reduction

Hidehiko Aida[a], Tetsuo Takemura[a,*], Tadahumi Horisaki[b], Atsushi Okada[b], Hideaki Nojiri[b], Kaoru Nakamura[c]
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[b]Biotechnology Research Center, University of Tokyo
[c]Institute for Chemical Research, Kyoto University. E-mail: ttakemur@rs.kagu.tus.ac.jp

Unknown genes expected to encode carbonyl reduction ability of *Synechocystis* sp. PCC6803 were overexpressed in *Escherichia coli* cells for the improvement of some ketone reductions.

Improvement of ketone reduction by deletion mutants of *Synechocystis* sp. PCC6803

Tetsuo Takemura[a,*], Nobuaki Umeno[a], Kaori Akiyama[a], Shinya Yamahira[a], Yukiko Tamai[a], Hisataka Ohta[a], Kaoru Nakamura[b]
[a]Faculty of Science, Tokyo University of Science, 1-3, Kagurazaka, Shinjuku-ku, Tokyo, 162-8601, Japan. E-mail: ttakemur@rs.kagu.tus.ac.jp
[b]Institute for Chemical Research, Kyoto University

Deletion mutants of *Synechocystis* sp. PCC6803 were prepared expecting the inactivation of enzyme genes corresponding to undesirable reactions in the microorganism to improve some ketone reductions (Fig. 50).

Cloning and expression of a novel NADH oxidase gene from *Brevibacterium* sp. KU1309

Kumiko Fujimori[*], Jun-ichiro Hirano, Hiromichi Ohta, Kenji Miyamoto, Daisuke Uemura

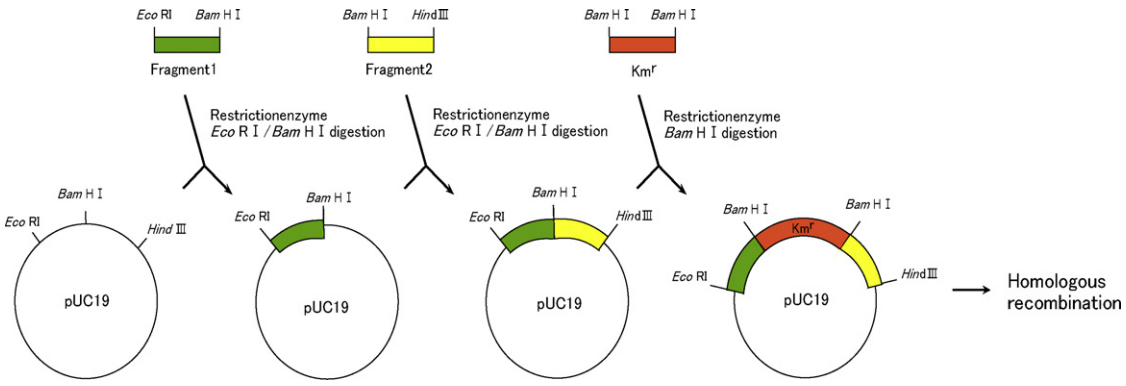

Fig. 50. Improvement of ketone reduction by deletion mutants of *Synechocystis*.

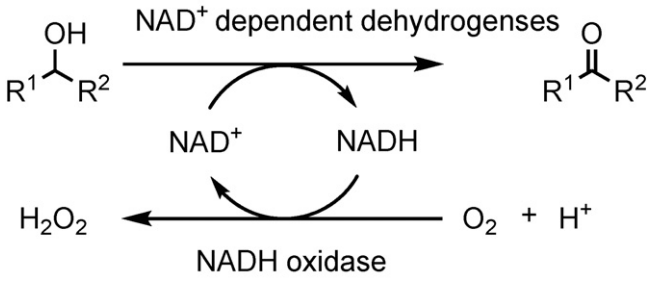

Fig. 51. Cloning and expression of a novel NADH oxidase gene.

Department of Biosciences and Informatics, Keio University, 3-14-1Hiyoshi Kohoku-ku, Yokohama 223-8522, Japan. E-mail: kmiyamoto@bio.keio.ac.jp

We have cloned and expressed a novel thermostable NADH oxidase gene from *Brevibacterium* sp. KU1309 and the recombinant enzyme showed high activity at wide range of pH (pH 8–11) (Fig. 51).

Chemo-enzymatic synthesis of efficient chiral building blocks using D-allose

Emi Uneyama, Rie Takahashi, Yumiko Takagi
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Enzyme-catalyzed reactions have been successfully utilized in selective deacylation or acylation of sugars and hence have provided an effective method of manipulating protecting-group strategies in carbohydrate synthesis. We described to novel synthesis of D-allose derivatives for efficient chiral building blocks using lipase technology.

Synthesis of optically active trifluoromethylalkanol using ionic liquids

Yumiko Takagi*, Hiroaki Ishihara, Tsutomu Ogawa, Fumi Yamana, Toshiyuki Itoh
Department of Chemistry, Faculty of Education, Kagawa University, 1-1 Saiwai-Cho, Takamatsu 760-8522, Japan. E-mail: ytakagi@ed.kagawa-u.ac.jp

Ionic liquids (ILs) were primarily explored for their applications in electrochemical technologies and as solvents in electronic. This characteristic is very interesting to perform enzymatic reactions with lipases which are well known to be active on interfaces. Investigated enantioselectivity in the enzymatic kinetic resolution of trifluoromethylalkanol employing immobilized lipase from *Candida antarctica* results from the use of the ionic liquids [bmim][PF_6] and [bmim][TFSI] as reaction medium. Hydrolysis reaction of 1,1,1-trifluoromethyl-2-acetate proceeded smoothly to provide corresponding to alcohol with efficient enantioselectivity. The influence of ionic liquid on the catalytic efficiency and selectivity has been studied.

Synthetic study of piericidin A_1 and B_1

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Piericidin A_1 and B_1, which are metabolites of *Streptomyces mobaraensis* and *S. pactam* and have been well known as inhibitors of the mitochondrial transport system, are synthesized from chiral synthon obtained based on lipase Amano A6 catalyzed enantioselective hydrolysis of acetate (Fig. 52).

Chemoenzymatic synthesis of tauranin

Sadayuki Ishii, Mikio Fujii, Hiroyuki Akita*
Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba, 274-8510, Japan. E-mail: akita@phar.toho-u.ac.jp

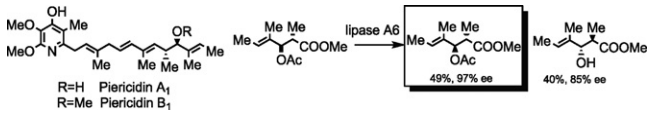

Fig. 52. Lipase-catalyzed synthesis of Piericidin A_1 and B_1.

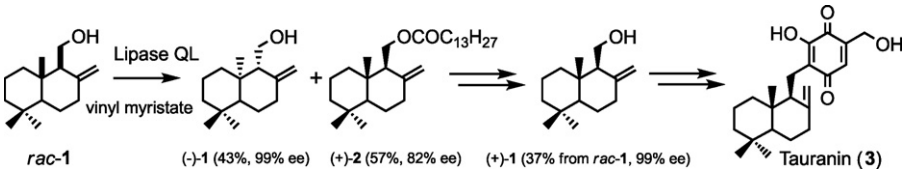

Fig. 53. Chemoenzymatic synthesis of tauranin.

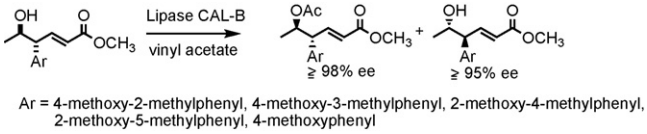

Fig. 54. Lipase-catalyzed synthesis of bisabolane sesquiterpenes.

Lipase-catalyzed transesterification of *rac*-albicanol (*rac*-**1**) by the use of vinyl mirystate afforded the both enantiomers of optical activity albicanol (**1**) and the synthesis of tauranin (*rac*-**3**) from *rac*-**1** was achieved (Fig. 53).

Synthetic study of bisabolane sesquiterpenes by using *Candida antarctica* lipase B

Mikio Fujii, Yuu Kitazawa, Hiroyuki Akita*

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Transesterification of (4*S**,5*S**)-methyl 4-aryl-5-hydroxyhex-2(*E*)-enoates was performed using *Candida antarctica* lipase-B (CAL-B) in the presence of vinyl acetate, and highly optically active (4*S*,5*S*)-methyl 4-aryl-5-hydroxyhex-2(*E*)-enoates and the corresponding (4*R*,5*R*)-acetates were obtained in excellent optical purities (Fig. 54).