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Abstracts

Selected abstract from the 10th Japanese Symposium on the Chemistry of Biocatalysis

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Introduction

The 10th Symposium on Biocatalyst Chemistry Japan (BSJ) was held on 14 and 15, December 2006 at Kitakyushu International Conference Center, Kokura, Kitakyushu, Japan. The active attendees to the symposium were about 50 persons from academia, 20 from industries and 30 students, i.e., 100 persons in total. The symposium consisted of 18 contributed oral presentations, and 58 poster presentations. Each poster presenter introduced his or her research by 1-min oral presentation in English before the poster session. Three posters were selected for poster award. The panel discussion was specially programmed, the past and future in the field of biocatalyst chemistry research of Japan were discussed as the chairmen of Prof. Hiromichi Ohta of Keio University and Associate Prof. Kaoru Nakamura of Kyoto University. The next symposium will be organized by Prof. Toshiyuki Itoh of Tottori University and held on 25 and 26, January 2008 at Tottori, Japan.

Oral Presentations

Chemoenzymatic synthesis of naturally occurring β-glycosides by immobilized β-glucosidase from almond

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Naturally occurring β-glycosides such as benzyl glycosides, sacranosides and kenposide were synthesized by chemoenzymatic methods based on combination of enzymatic glucosidation using immobilizing β-glucosidase and chemical glycosidation (Fig. 1).

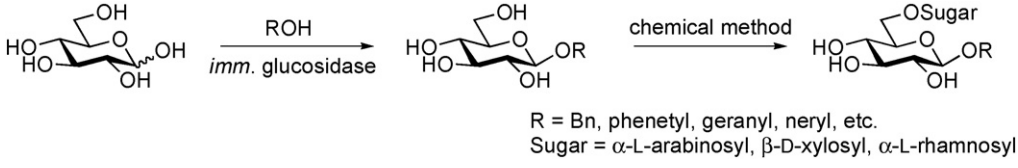

Fig. 1. Chemoenzymatic synthesis of natural occurring glycosides.

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E-mail address: asano@pu-toyama.ac.jp (Y. Asano).
[1] Symposium organizer.

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doi:10.1016/j.molcatb.2007.05.006

Chemoenzymatic synthesis of γ-alkyl-γ-butenolide

Mikio Fujii[a,b,*], Motonori Fukumura[a], Yumiko Hori[a], Yasuaki Hirai[a], Hiroyuki Akita[b], Kaoru Nakamura[c], Kazuo Toriizuka[a], Yoshiteru Ida[a,d]

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The *rac*-1-alkylallylalcohols were converted to highly optically active γ-alkyl-γ-butenolides by chemoenzymatic method based on the combination of lipase-catalyzed enantioselective transesterification and ring-closing metathesis (Fig. 2).

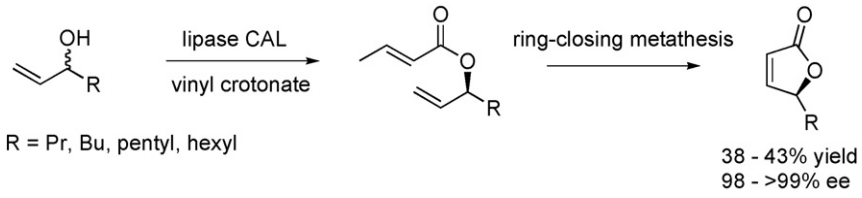

Fig. 2. Chemoenzymatic synthesis of chiral γ-alkylbutenolides.

Asymmetric synthesis of curcuphenol using lipase-catalyzed reaction

Masashi Kawasaki[a,*], Chieko Shimazaki[a], Yuko Hayashi[a], Hiroko Kakuda[b], Naoki Toyooka[b], Akira Tanaka[a], Hiroyuki Akita[c], Michimasa Goto[d], Tadashi Kometani[d]

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The highly enantioselective kinetic resolution of a racemic primary alcohol (±)-**4** by lipase-catalyzed transesterification with vinyl 3-phenylpropanoate afforded the optically pure primary alcohol (*R*)-**4** which can be intermediate of curcuphenol (Fig. 3).

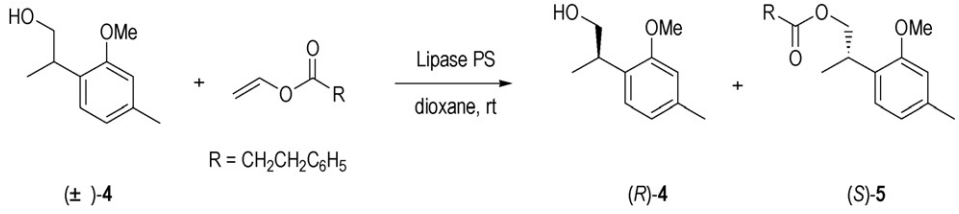

Fig. 3. Lipase-catalyzed transesterification of (±)-**4** with vinyl 3-phenylpropanoate.

Directed evolution for increasing amidase activities of lipase from *Pseudomonas aeruginosa*

Atsuko Hasegawa, Yuichi Nakagawa, Jun Hiratake[*], Kanzo Sakata

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Lipase from *Pseudomonas aeruginosa* TE3285 was subjected to directed evolution for improved amidase activity. The triple mutant Sat252 (F207S/A213D/L252F) exhibited 13.6-fold higher amidase activity toward amide **1** than that of wild-type lipase mainly by the increase in k_{cat} (Fig. 4).

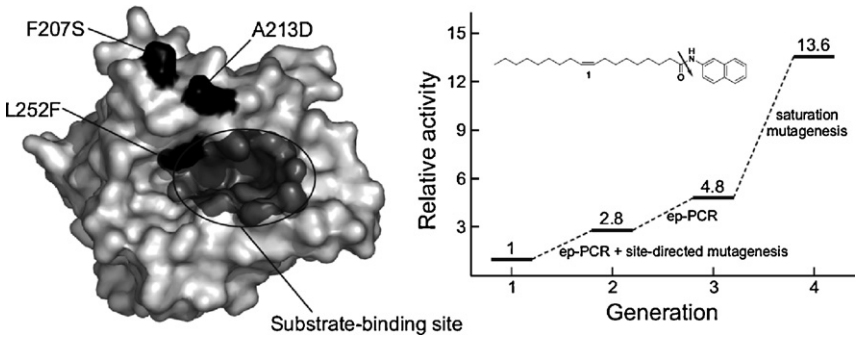

Fig. 4. Tertiary model of the mutant lipase Sat252 and progression of amidase activities.

Stopped-flow study of the conversion of α-hydroxyheme to verdoheme by heme oxygenase-1

Hiroshi Sakamoto[a,*], Kenichi Takahashi[b], Yuichiro Higashimoto[b], Saori Harada[b], Masato Noguchi[b]

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O_2-dependent reactions of the ferric and ferrous forms of α-hydroxyheme complexed with a water-soluble form of rat heme oxygenase-1 were examined by rapid-scan stopped-flow measurements under anaerobic conditions (Fig. 5).

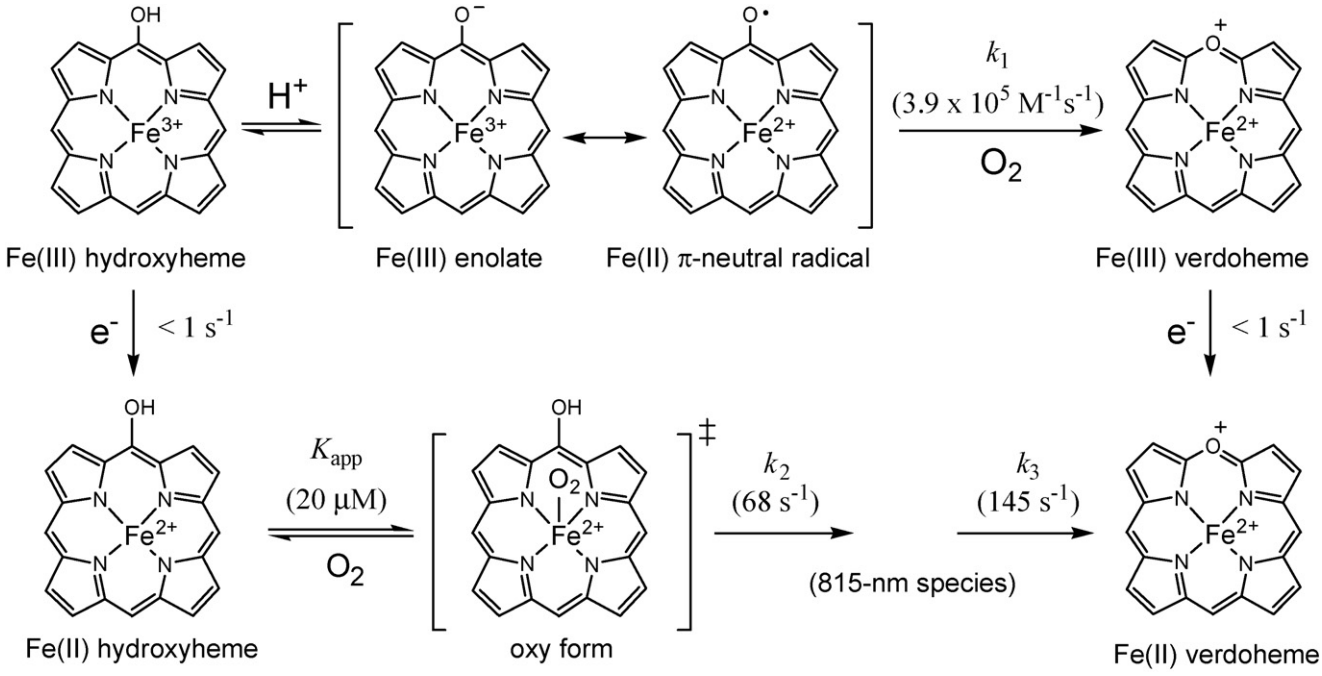

Fig. 5. Proposed reaction pathways of conversion of α-hydroxyheme to verdoheme.

Promiscuity of arylmalonate decarboxylase

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It has been revealed that arylmalonate decarboxylase (AMDase) exhibits catalytic promiscuity, i.e., racemase and aldolase-like activity in addition to its original decarboxylase activity (Fig. 6).

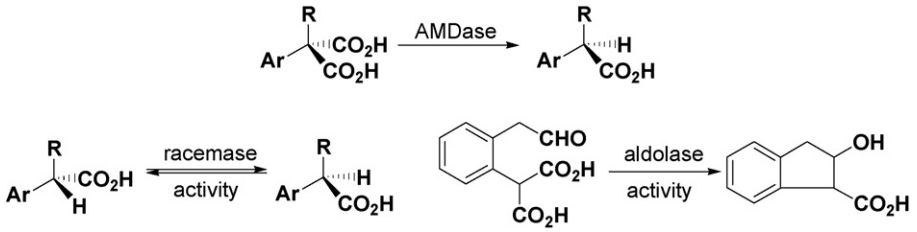

Fig. 6. The catalytic activities of AMDase.

Glucosylation of sucrose laurate with cyclomaltodextrin glucanotransferase

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The present study demonstrated that sucrose monolauroyl esters were found to serve as a substrate for cyclomaltodextrin glucanotransferase (CGTase)-catalyzed transglucosidation reactions affording new sucrose esters that have additional 1–3 glucose residues on the pyranose ring of the sucrose moiety in the ester (Fig. 7).

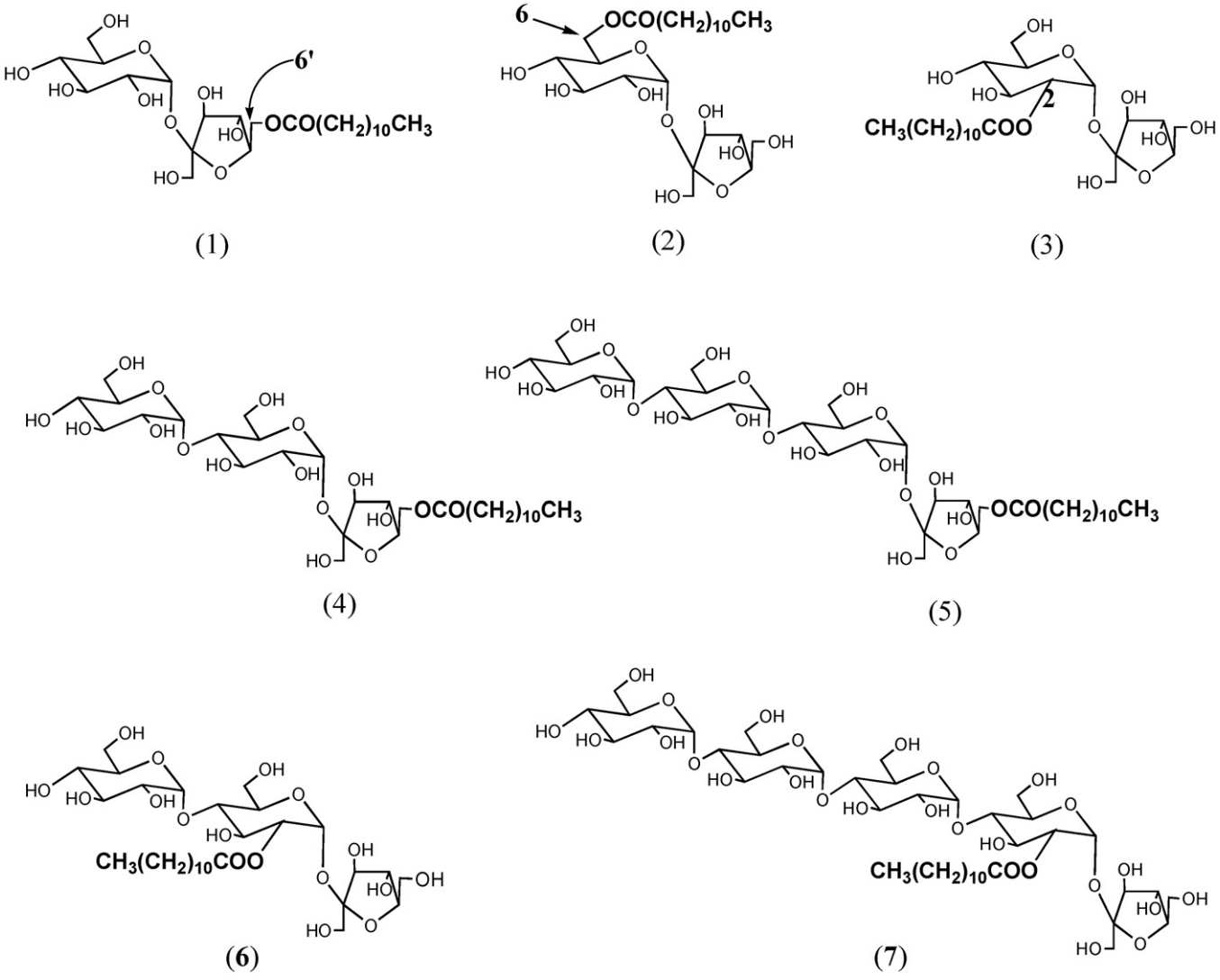

Fig. 7. Structure of sucrose monolaurates (**1**)–(**3**) and reaction products (**4**–**7**) by CGTase catalyzed glucanotransfer reactions.

Kinetic analyses of mutagenic hyperthermostable glycogen phosphorylase from *Aquifex aeolicus* on a 27 MHz quartz-crystal microbalance

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We found it was possible to detect directly and quantitatively each step of the amylose synthesis by thermophilic phosphorylase from *Aquifex aeolicus* in the aqueous solution by using the maltodextrin-immobilized 27 MHz quartz-crystal microbalance (QCM) (Fig. 8).

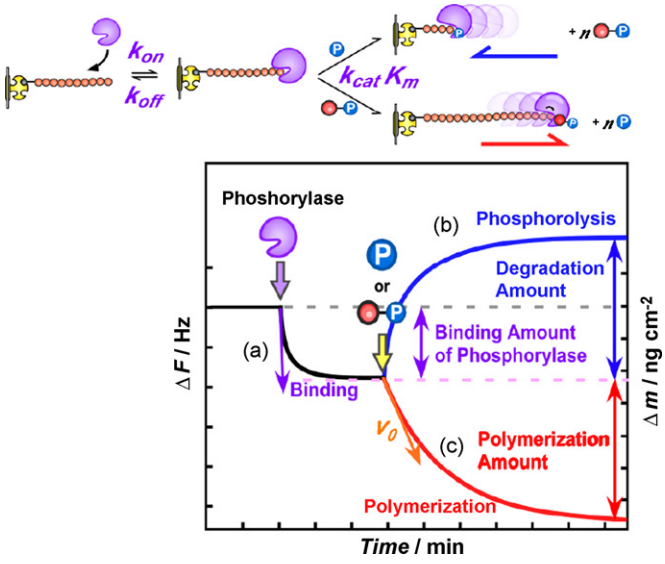

Fig. 8. Typical time courses of frequency changes of the amylopectin-immobilized QCM, responding to additions of (a) phosphorylase, (b) phosphoric acid, and (c) glucose 1-phosphate.

Microwave effect on the rolling circle amplification

Takeo Yoshimura, Kunitada Nishida, Keiichi Uchibayashi, Shokichi Ohuchi*

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The rolling circle amplification is an effective method of DNA amplification having tandem repeated sequences. In this study, the microwave was irradiated to RCA reaction on controlling the temperature (Fig. 9).

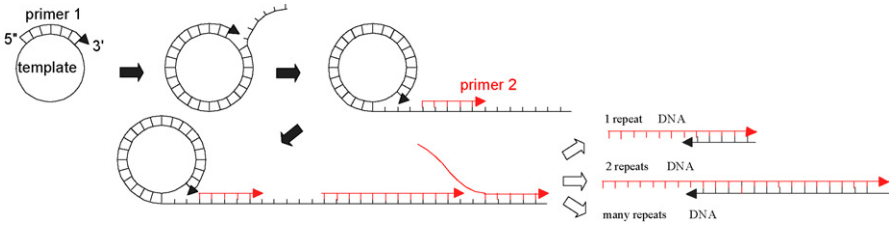

Fig. 9. Procedure of rolling circle amplification.

Microwave assisted enzymatic reaction with *Flavobacterium* biocatalyst

Satoko Matsuo, Kensuke Chikuda, Shokichi Ohuchi*

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We irradiated microwave for the transesterification reaction utilized *Flavobacterium* sp. with phosphotriesterase (Fig. 10).

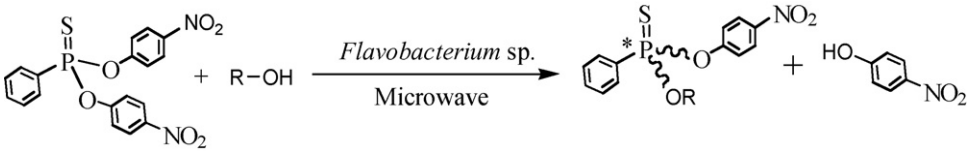

Fig. 10. Phosphotriesterase-catalyzed transesterification.

Asymmetric hydrogen-transfer bioreduction and evolutionary mechanism of alcohol dehydrogenase to increase the activity in polar organic solvent

Nobuya Itoh*, Tomomi Odan, Kousuke Inoue, Yoshihide Makino

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We have developed highly efficient bioreduction process which uses two NAD^+-dependent alcohol dehydrogenases (ADH): *Leifsonia* ADH (LSADH) and *Rhodococcus* phenylacetaldehyde reductase (PAR), which could efficiently reproduce NADH when concentrated 2-propanol (10 (w/v)% or more) was used as a hydrogen donor (Fig. 11).

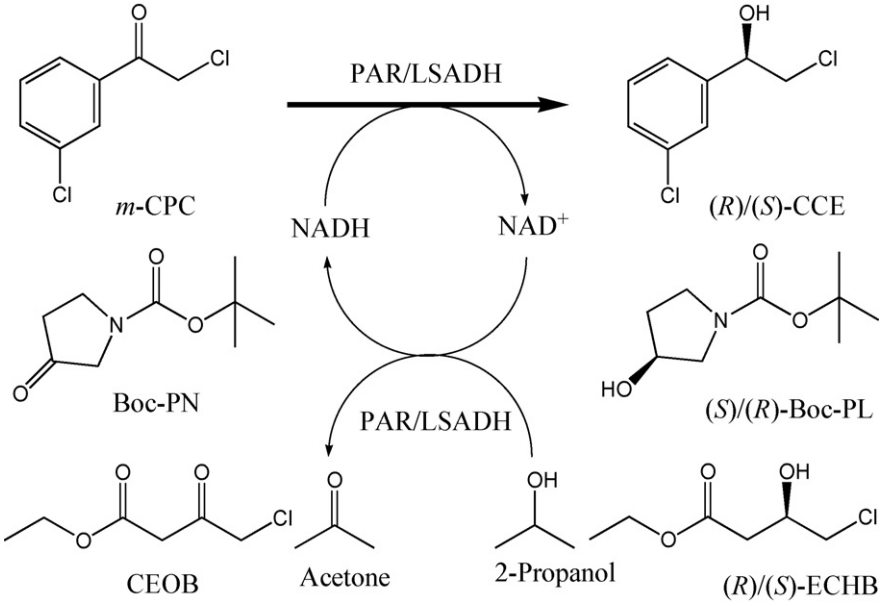

Fig. 11. Asymmetric hydrogen-transfer bioreduction.

Production of useful catechin derivatives by enzymatic treatment of green tea extract

Yuji Katsube[a], Keiichi Yamamoto[a], Kenzaburo Yoshida[a], Noriyuki Nakajima[b], Nobuya Itoh[b,*]

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Two compounds produced were identified as epitheaflagallin (**1**) and epitheaflagallin 3-*O*-gallate (**2**), respectively, when we treated green tea extract in the presence of gallic acid with laccase (Amano Enzyme Inc.) (Fig. 12).

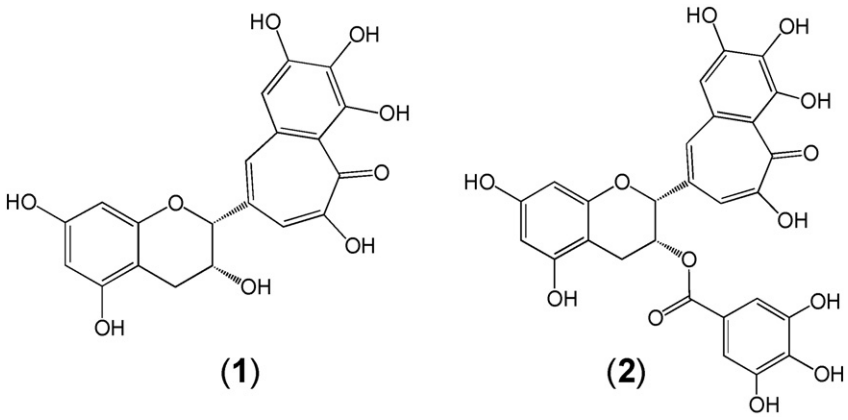

Fig. 12. Structure of epitheaflagallins.

Integration of organic synthesis and lipases—Total synthesis of symbioramide and (*S*)-azetidinecarboxylic acid as the examples

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Integrated use of organic synthesis and lipase-catalyzed reactions is emphasized, in the topics of total synthesis of symbioramide, a marine-origin ceramide (Tetrahedron Lett. 46 (2005) 3291), and the preparation of enantiomerically pure (*S*)-azetidinecarboxylic acid (Biosci. Biotechnol. Biochem. 69 (2005) 1892) (Fig. 13).

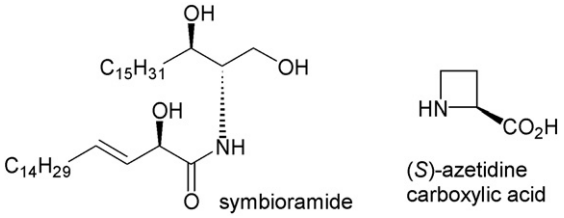

Fig. 13. Symbioramide and (*S*)-azetidinecarboxylic acid.

The synthesis of (*R*)-bicalutamide based on epoxide hydrolase-catalyzed kinetic resolution

Aya Fujino[a], Masayoshi Asano[a], Hitomi Yamaguchi[b], Masaya Ikunaka[b], Takeshi Sugai[a,*]

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A novel chemo-enzymatic synthesis of (*R*)-bicalutamide, a synthetic antiandrogen, was achieved by an engineered *Bacillus* epoxide hydrolase-catalyzed reaction as the key step (Tetrahedron Lett. 48 (2007) 979) (Fig. 14).

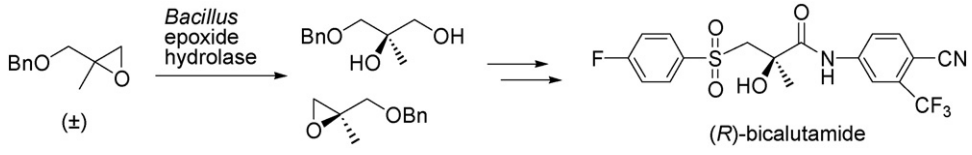

Fig. 14. Synthesis of (*R*)-bicalutamide.

Poster Presentations

Identification of enzyme catalyzing conversion of N^{α}-Z-L-lysine to N^{α}-Z-L-aminoadipic-δ-semialdehyde in *Rhodococcus* sp. AIU Z-35-1 and its application

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The enzyme catalyzing conversion of N^{α}-Z-L-lysine to N^{α}-Z-L-aminoadipic-δ-semialdehyde was identified as an L-amino acid oxidase with broad substrate specificity that oxidized N^{α}-acyl-L-lysine, N^{ε}-acyl-L-lysine, L-lysine and many other L-amino acids (Fig. 15).

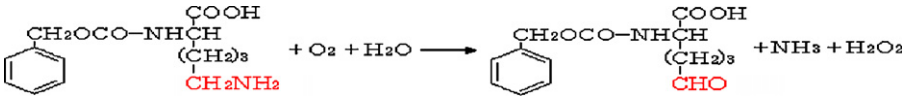

Fig. 15. Conversion of N^{α}-Z-L-lysine into N^{α}-Z-L-aminoadipic-δ-semialdehyde by *Rhodococcus* sp. AIU Z-35-1.

Regioselective hydroxylation of alkanes in a liquid–liquid interface bioreactor

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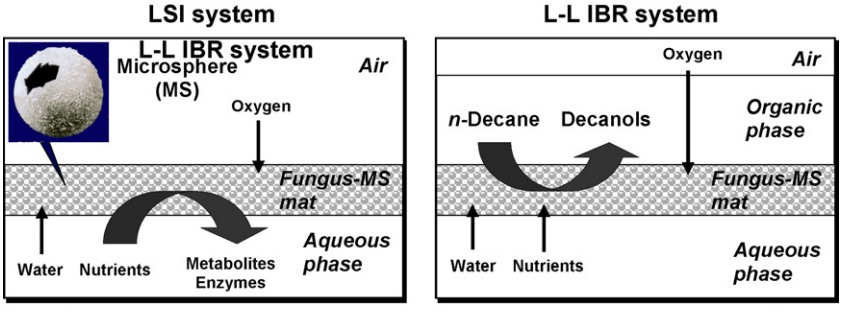
Novel fungal cultivation (liquid-surface immobilization, LSI) and bioconversion (liquid–liquid interface bioreactor, L-L IBR) systems were developed with a balooned microsphere (MS), and the L-L IBR was applied to the regioselective hydroxylation of *n*-alkanes such as *n*-decane with various fungi (Fig. 16).



Strain	Hydroxylated position	Regioselectivity (%)
Trichoderma sp.	C-1	100
Colletotrichum linicolum	C-2	66
Aspergillus tsunetana	C-3	66
Moniliella sp.	C-4	99
Rhizopus oryzae	C-5	90

Fig. 16. Regioselective hydroxylation of *n*-decane in liquid–liquid interface bioreactor.

Purification and characterization of carbonyl reductase from actinomycete

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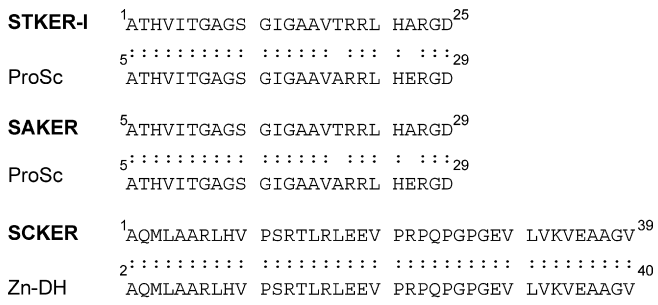
We have purified and characterized of an α-keto ester reductase (SAKER) from *Streptomyces averimitilis* NBRC14893 whole cells. This enzyme was dependent on NADPH as a coenzyme and reduced ethyl pyruvate to the corresponding (*S*)-hydroxy ester with high e.e. (98%) (Fig. 17).



STKER-I: a keto ester reductase from *S. thermocyaneoviolaceus*.
SAKER: a keto ester reductase from *S. avermitilis*.
SCKER: a keto ester reductase from *S. coelicolor* A3(2).
ProSc: a probable oxidoreductase in *S. coelicolor* A3(2).
Zn-DH: a possible zinc-containing alcohol dehydrogenase in *S. coelicolor* A3(2).
The underlined sequences are putative coenzyme binding site.

Fig. 17. Comparison of N-terminal amino acid sequences.

Reduction of acetophenone derivatives by *Nostoc mintum*

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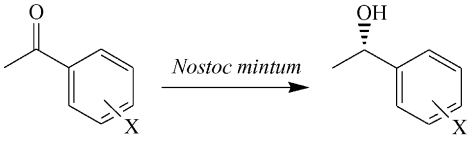
The reduction of acetophenone derivatives using photosynthetic microbes (*Nostoc mintum*) was investigated. It was found that reduction of acetophenone derivatives with *Nostoc mintum* gives the corresponding (*S*)-alcohols with excellent enantioselectivity (Table 1).

Table 1
Biotransformation of acetophenone derivatives using *N. Mintum*



X	7 days			9 days		
	Yield (%)	e.e. (%)	Configuration	Yield (%)	e.e. (%)	Configuration
H	4.1	90	*S*	4.5	88	*S*
o-Cl	38.0	95	*S*	35.7	96	*S*
m-Cl	13.7	87	*S*	22.2	94	*S*
p-Cl	29.5	95	*S*	19.1	91	*S*
o-Br	12.7	100	*S*	25.1	100	*S*
m-Br	13.7	87	*S*	13.7	80	*S*
p-Br	40.5	95	*S*	36.1	94	*S*

Oxidation of hydroxy derivatives with *Spirulina subsalsa*

Shinnosuke Okada, Asuka Kiyama, Takamitsu Utsukihara, Nakahide Kato, C. Akira Horiuchi*

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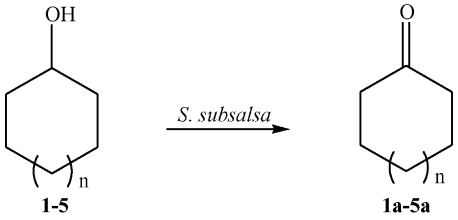
Biotransformation of some alcohols using *Spirulina subsalsa* gave the corresponding ketones and *S. subsalsa* showed high oxidation activity for cyclopentanol and cycloheptanol. It was found that this new synthetic method is convenient and eco-friendly synthetic method (Table 2).

Table 2
Biotransformation of cycloalkanols (**1**–**5**) with *S. subsalsa*



1-5 **1a-5a**

1, **1a** : n=0 **2**, **2a** : n=1
3, **3a** : n=2 **4**, **4a** : n=3 **5**, **5a** : n=7

Entry	Substrate	Days	Products (%)
1	**1**	5	**1a** (100)
2	**2**	7	**2a** (81)
3	**3**	4	**3a** (100)
4	**4**	7	**4a** (100)
5	**5**	7	**5a** (12)

Purification and characterization of cyclo (Leu–Phe) oxidase of *Streptomyces albulus* expressed in *Escherichia coli*

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Recombinant cyclo (Leu–Phe) oxidase catalyzing the conversion of cyclo (Leu–Phe) to albonoursin was purified and characterized, and its character was compared with that of the native CFL oxidase (Fig. 18).

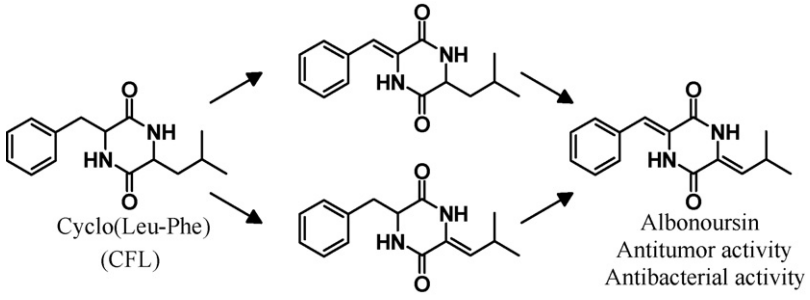

Fig. 18. Albonoursin biosynthetic pathway by CFL oxidase.

Purification and characterization of NADH oxidase from 2-phenylethanol-assimilating *Brevibacterium* sp. KU1309

Jun-Ichiro Hirano, Kenji Miyamoto, Hiromichi Ohta*

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NADH oxidase was purified from 2-phenylethanol-assimilating *Brevibacterium* sp. KU1309 and characterized. This enzyme had high thermal stability and is active in broad range of pH. Accordingly, it is useful for cofactor regeneration (Fig. 19).

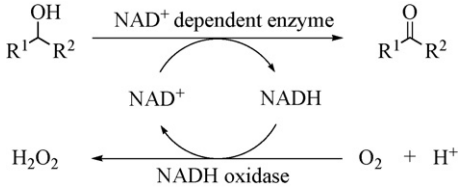

Fig. 19. NAD^+ regenerating system by NADH oxidase.

Kinetic resolution of 2-substituted propanol by 2-phenylethanol-assimilating bacteria

Kumiko Fujimori, Jun-Ichiro Hirano, Kenji Miyamoto, Hiromichi Ohta*

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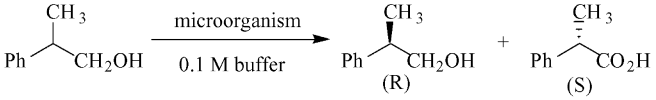
Microorganisms that degrade 2-phenylethanol were screened and those which enantioselectively oxidized 2-phenylpropanol to 2-phenylpropanoic acid were selected. By optimizing the cultural conditions and reaction conditions, various optically active α-substituted carboxylic acids were obtained (Table 3).

Table 3
Enantioselective oxidation of 2-substituted propanol by microorganisms



Strain	Alcohol		Carboxylic acid		*E*-value
	Recovery (%)[a]	e.e. (%)[b]	Yield (%)[a]	e.e. (%)[b]	
Brevibacterium sp. KU1320	48	>99	46	91	50
Arthrobacter sp. KU1321	48	87	38	93	49

[a] Determined by GC.
[b] Determined by HPLC.

Purification and characterization of new arylmalonate decarboxylases

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Arylmalonate decarboxylase (AMDase) producers were screened and isolated from soil samples. Purification and reactivity of the enzymes were compared with that of the original AMDase (Fig. 20).

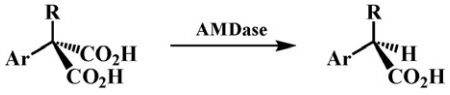

Fig. 20. Asymmetric decarboxylation of α-arylmalonate derivative.

Mechanism of action of *Burkholderia cepacia* lipase: Origin of rate enhancement and enantioselectivity

Yasuhiro Tsukamoto, Katsuhiko Tsuchida, Yūki Yoshimura, Tomoaki Yokota, Yoshinori Inoue, Hirohara Hideo*

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We have examined kinetics, thermodynamics and solvent isotope effects for *Burkholderia cepacia* lipase-catalyzed hydrolysis reactions of acetic acid esters of achiral or single enantiomer chiral alcohols to elucidate mechanism of action of the enzyme (Fig. 21).

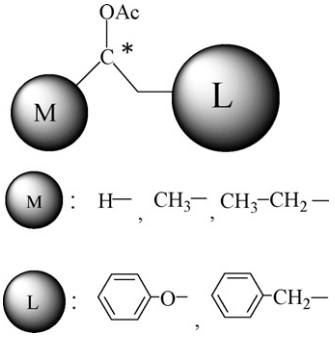

Fig. 21. Achiral or chiral acetic acid esters.

Mechanism of action of *Candida antarctica* lipase B: Specific rate Enhancement and enantioselectivity in hydrolysis of monochloroacetate

Keisuke Harada, Nozomi Ito, Yasuyuki Shimomachi, Atsushi Tanikawa, Yoshinori Inoue, Hideo Hirohara*

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We have examined kinetics, thermodynamics and solvent isotope effects of *Candida antarctica* lipase B (CALB)-catalyzed hydrolysis of monocholoroacetic acid esters of achiral and single enantiomer chiral secondary alcohols to elucidate mechanism of action of the enzyme (Fig. 22).

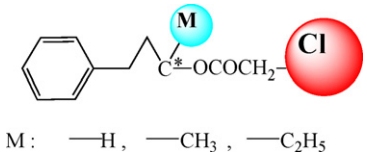

Fig. 22. Substrates.

On the β-replacement reactions catalyzed by vitamin B6 dependent enzymes

Tsuyoshi Yukumura, Atsushi Inada, Masafumi Doi, Kazuya Sada, Shin-Ichi Ozaki*

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We have studied two B6-dependent enzymes, human cystathionine β-synthase (CBS) and *O*-acetylserine sulfhydrylase (OASS), to consider the reason for CBS to have heme as an additional cofactor which is not retained in OASS (Fig. 23).

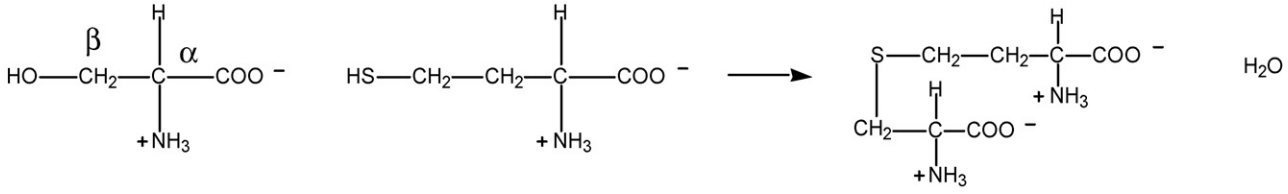

Fig. 23. The reaction catalyzed by CBS.

Correlation of lyophilized enzyme and bacterium on phosphotriesterase catalytic optical resolution

Kensuke Chikuda, Satoko Matsuo, Shokichi Ohuchi*

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We researched to correlation of lyophilized enzyme and bacterium on phosphotriesterase catalytic optical resolution (Fig. 24).

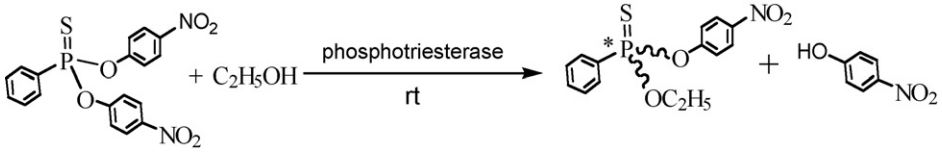

Fig. 24. Phosphotriesterase catalyzed optical resolution.

Enantioselectivity of lipase-catalyzed ring-opening polymerization of substituted lactones

Rieko Tani[a], Makoto Kasai[a], Minako Fujioka[b], Shin-Ichi Ueji[a,b,*]

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We studied the reactivity of lipase-catalyzed ring-opening polymerization of substituted lactones and it was found that high reaction temperature decreases the enantioselectivity of the reaction and the addition of alcohol resulted in a slight improvement of enantioselectivity (Fig. 25).

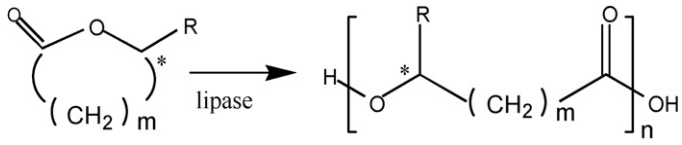

Fig. 25. Lipase catalyzed enantio-selective ring-opening polymerizaton of substituted lactones.

Enhanced enantioselectivity in the esterification catalyzed by lipase lyophilized with various ionic compounds

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Lipase lyophilized with various ionic compounds (ex. SDS, previously used as additives) dramatically enhanced the enantioselectivity in the esterificaion, and the enantioselectivity was found to be controlled by the characteristics of ionic compounds and the reaction temperature (Fig. 26).

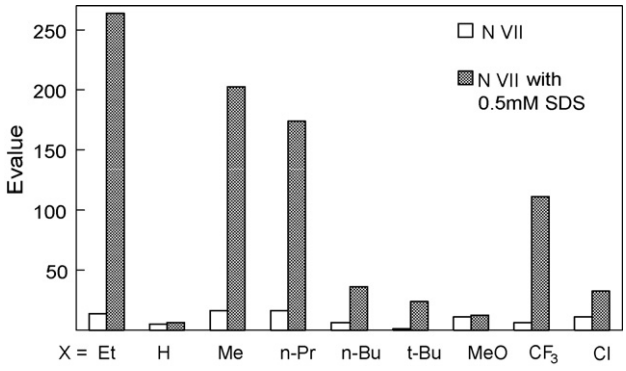

Fig. 26. Enhancement of the enantioselectivity in the esterification of 2-(4-substrated phenoxy) propanoic acid catalyzed by Lipase VII lyophilized with SDS.

Gene cloning and functional analysis of novel short-chain *cis*-prenyltransferase

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cis-Prenyltransferase (*cis*-PT) catalyze *cis*-condensation of isopentenyl diphosphate (IPP) to allylic diphosphate. We cloned novel short-chain *cis*-PT gene from three kinds of organisms and characterized enzyme activity (Fig. 27).

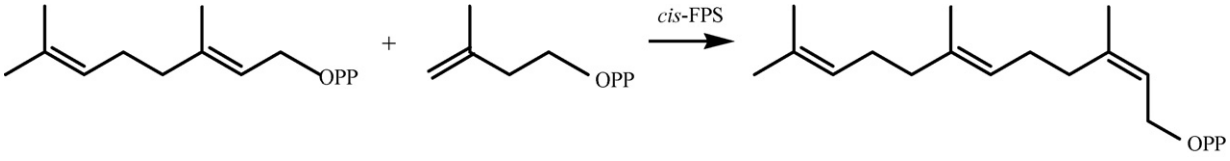

Fig. 27. Reaction of short-chain *cis*-PT (*cis*-farnesyl diphosphate synthase).

Product chain-length determination mechanism of short-chain *cis*-prenyltransferase from *Mycobacterium tuberculosis*

Motoyoshi Noike*, Takanori Ambo, Sayaka Kikuchi, Satoshi Yamashita, Seiji Takahashi, Hirofumi Kurokawa, Tanetoshi Koyama

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cis-Prenyltransferase catalyze the consecutive condensation of isopentenyl diphosphates with allylic prenyl diphosphates. In this study, we tried to elucidate the product chain-length determination mechanism of *cis*-farnesyl diphosphate synthase from *Mycobacterium tuberculosis* (Fig. 28).

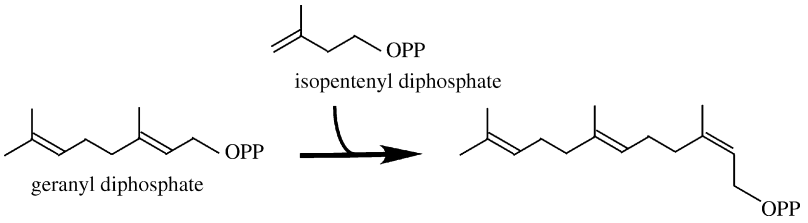

Fig. 28. Reaction of *cis*-farnesyl diphosphate synthase.

Biotransformation (−)-fraxinellone to (−)-fraxinellonone by *Aspergillus niger* and biological activity of the metabolites

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(−)-Fraxinellone was converted to the two products, (−)-(6*S*)-6-hydroxyfraxinellone and (−)-fraxinellonone (Fig. 29).

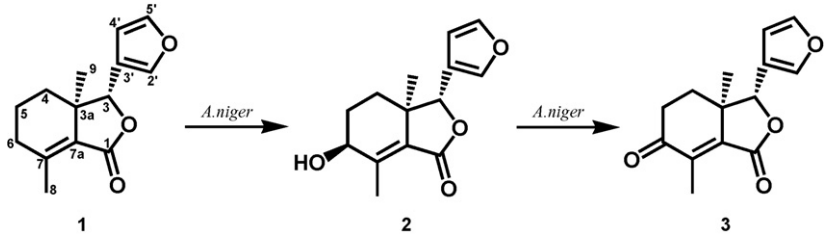

Fig. 29. Possible metabolic pathway of (−)-fraxinellone by *A. niger*.

Highly enantioselective reduction of ketones with recombinant *E. coli* cells

Tadashi Ema*, Nobuyasu Okita, Masahiro Takeda, Toshinobu Korenaga, Takashi Sakai

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The conditions for the asymmetric reduction of 2,4-octanedione with recombinant *E. coli* over producing a carbonyl reductase (SCR) and a glucose dehydrogenase (GDH) were optimized, and productivity reached 41 g/L (Fig. 30).

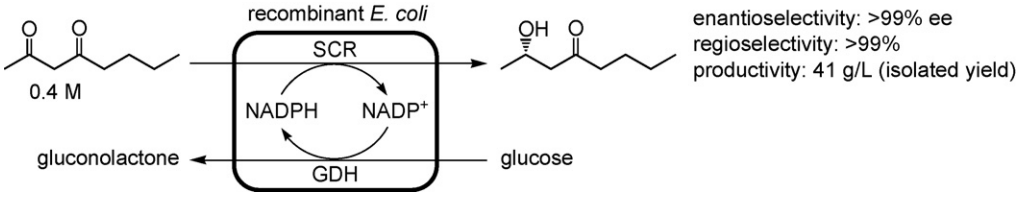

Fig. 30. Enantioselective and regioselective reduction of 2,4-octandione with a recombinant *E. coli*.

A new synthetic method for chiral 1,2-diamines and its application to the synthesis of bioactive piperidine derivatives

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A practical access to chiral 1,2-diamines and its application to the synthesis of NK-1 antagonists CP-99,994 and L-733,060 have been accomplished starting from enantiomerically pure 4-(*t*-butylcarbamoyl)-1-alken-3-ols which can be obtained through the lipase-catalyzed kinetic resolution (Fig. 31).

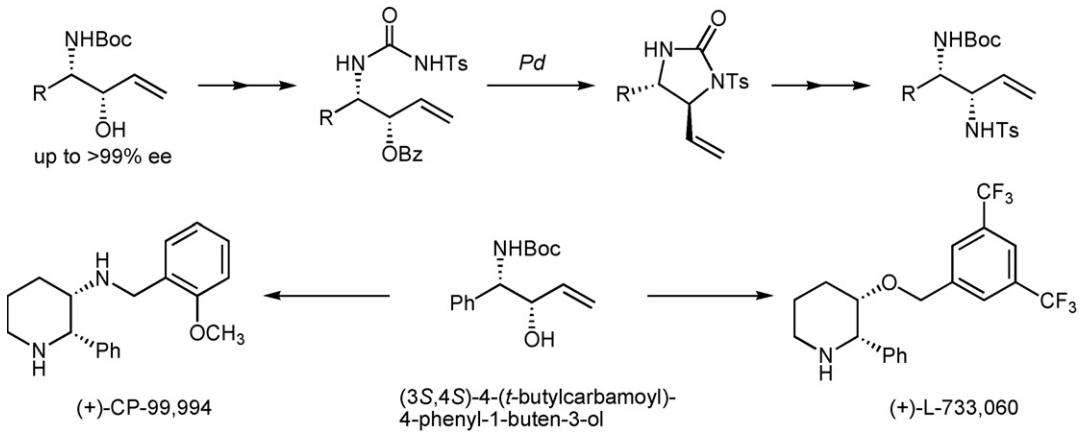

Fig. 31. A new synthetic method for chiral 1,2-diamines and its application to the synthesis of bioactive piperidine derivatives.

Production of malic acid by baker's yeast: Fixation of carbon dioxide

Yuki Kubota[a], Tomoko Matsuda[b], Tadao Harada[a,*], Kaoru Nakamura[c]

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[b]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan

[c]Tokyo Tech, School and Graduate School of Bioscience and Biotechnology, Yokohama-shi, Kanagawa 226-8501, Japan. E-mail: harada@rins.ryukoku.ac.jp

Fixation of carbon dioxide is one of the most important target in research of chemical and biological fields. Here, we would like to report that baker's yeast can immobilize carbon dioxide and produce malic acid. The reaction proceeded under carbon dioxide atmosphere than under air atmosphere. The product, malic acid, was detected by capillary zone electrophoresis and also by the enzymatic method.

Biotransformation of trifluoroacetophenone by lichen mycobionts

Rie Tanaka[a], Miki End[a], Kojiro Hara[a], Masashi Komine[a], Yoshikazu Yamamoto[a,*], Kaoru Nakamra[b]

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By screening of cultured lichen mycobionts, trifluoroacetophenone was converted to the corresponding (*S*)- and (*R*)-alcohol with highest enantioselectivity in excellent yields by BAM/AB-1 isolated from *Dibaesis absoluta* and PPY/ZA-1 isolated from *Pseudopyrenula* sp., respectively (Table 4).**Regioselectivity in the lipase-catalyzed reactions of dihydric phenols**

Table 4
Asymmetric reduction by lichen mycobionts

Strain	%Yield (%e.e.)	*R*/*S*
BAM/AB-1	97.3 (96.3)	*S*
PPY/ZA-1	97.1 (90.5)	*R*

Toshifumi Miyazawa*, Manabu Hamada, Ryohei Morimoto, Takashi Murashima, Takashi Yamada

Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan. E-mail: miyazawa@base2.ipc.konan-u.ac.jp

A systematic investigation has been carried out on the regioselectivity during the *Candida antarctica* lipase B-catalyzed acylation or deacylation (Scheme 1) toward the hydroxyls of substituted dihydric phenols, i.e., hydroquinones and resorcinols (Fig. 32).

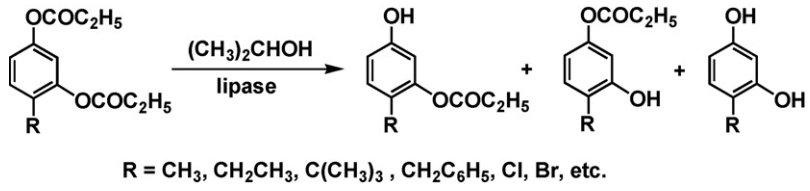

Fig. 32. Lipase-catalyzed regioselective deacylation of resorcinol derivatives.

Biotransformation of flavonoids

Yoshiharu Okuno[a,*], Hirotoshi Utsunomiya[b], Mitsuo Miyazawa[c]

[a]Second Department of Pathology, Wakayama Medical University, Kimiidera, Wakayama, Wakayama 641-0012, Japan

[b]Central Research Center, Wakayama Medical University, Kimiidera, Wakayama, Wakayama 641-0012, Japan

[c]Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University Kowakae, Higashiosaka-shi, Osaka 577-8502, Japan. E-mail: dueap620@sakai.zaq.ne.jp

The biotransformation of polymethoxyflavonoids by microorganism, *Aspergillus niger*, and insect, larvae of *Spodoptera litura*, were investigated and the main reaction was demethylation in *A. niger*, and was glucosylation in larvae of *S. litura* (Fig. 33).

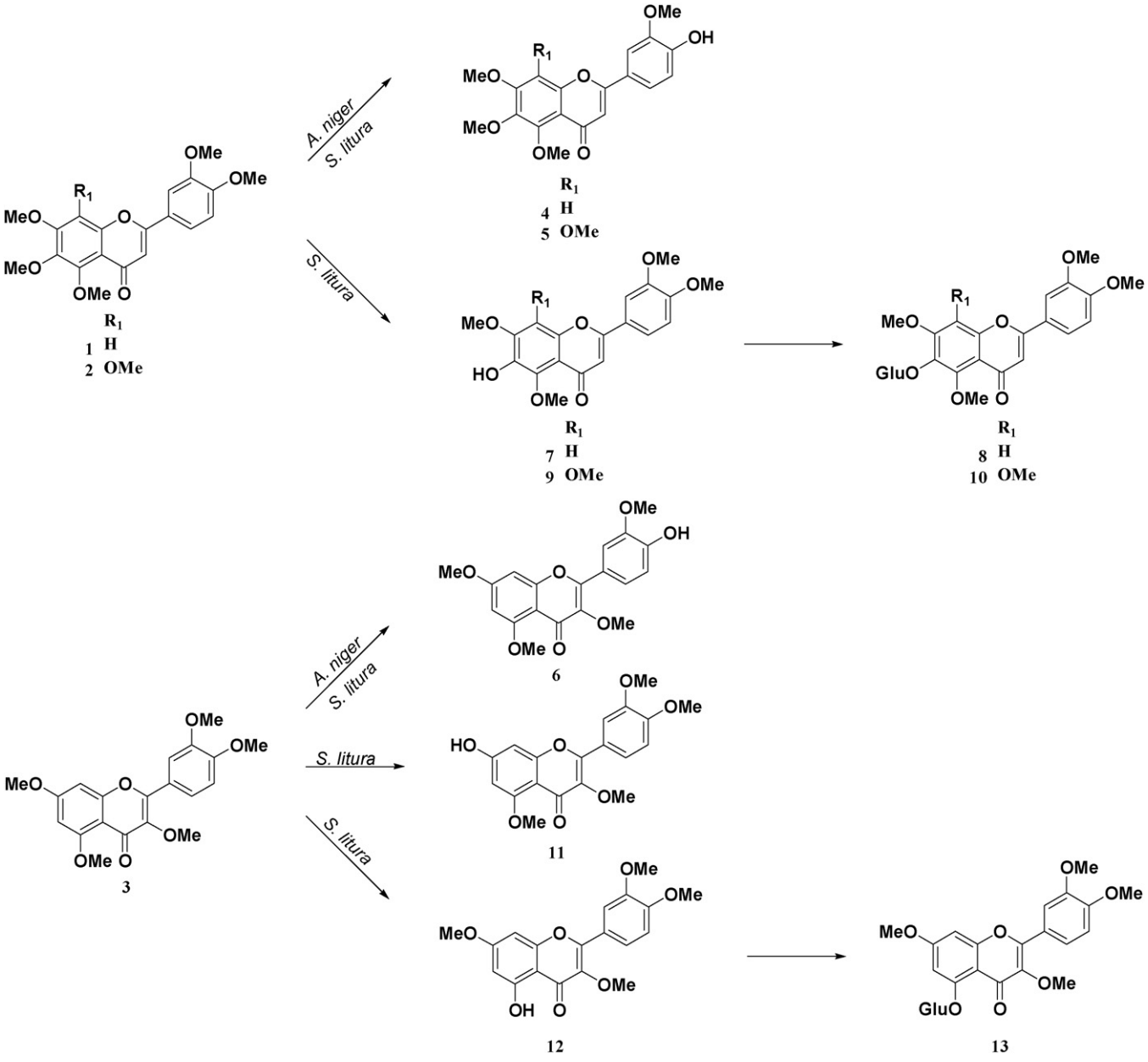

Fig. 33. Biotransformation of polymethoxyflavonoids by *A. niger* and *S. litura*.

Gene design for expression of β-structural phosphotriesterase

Seiji Kurisu, Satoko Matsuo, Hiroya Osoegawa, Shokichi Ohuchi*

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Diisopropylfluorophosphatase from *Loligo vulgaris* was designed on the basis of codon optimized gene strategy (Fig. 34).

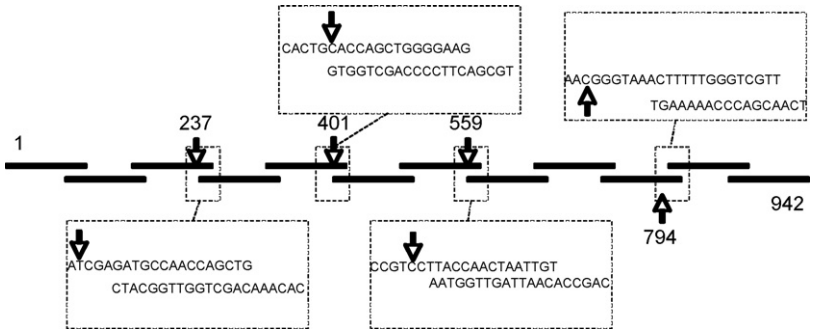

Fig. 34. Gene design of diisopropylfluorophosphatase.

Synthesis of optically active phosphorus compounds using lipase-catalyzed optical resolution

Kosei Shioji*, Nobuaki Ueda, Kentaro Okuma

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The enantioselectivity in the optical resolution of 1-hydroxymethylalkylphenylphosphine oxide (**1**) and phosphine borane (**3**) was improved by using co-lyophilized lipases with modified β-cyclodextrins (Fig. 35).

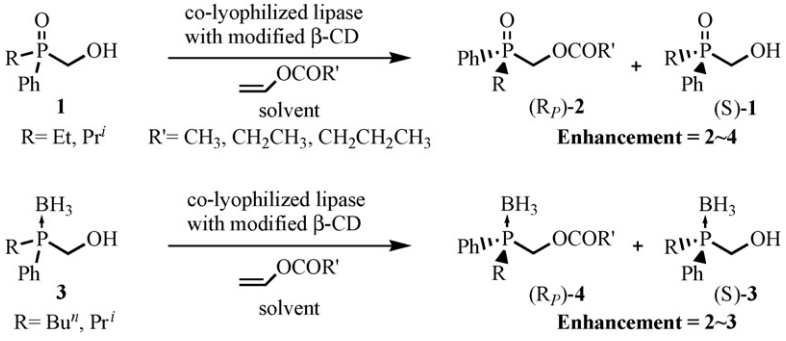

Fig. 35. Co-lyophilized lipase-catalyzed optical resolution of 1-hydroxymethylalkyl-phenylphoshpine oxide and phosphine borane.

Biotransformation of monoterpene alcohols by plant cultured cells

Sou Sakamoto[a], Kei Shimoda[b], Kohji Ishihara[c], Hiroki Hamada[a,*]

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[b]Department of Pharmacology and Therapeutics Chemistry, Faculty of Medicine, Oita University, Hasama-machi, Oita 879-5593, Japan

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We investigated the biotransformation of (−)-perillyl alcohol using plant cultured cells.It was found that (−)-perillyl alcohol was converted to 1-perillyl-β-D-glucopyranoside by plant cells of *Eucalyptus perriniana*. Furthermore, 1-perillyl-β-D-glucopyranoside was glycosylated to the corresponding oligosaccharides by a cyclodextringlcanotransferase (Fig. 36).

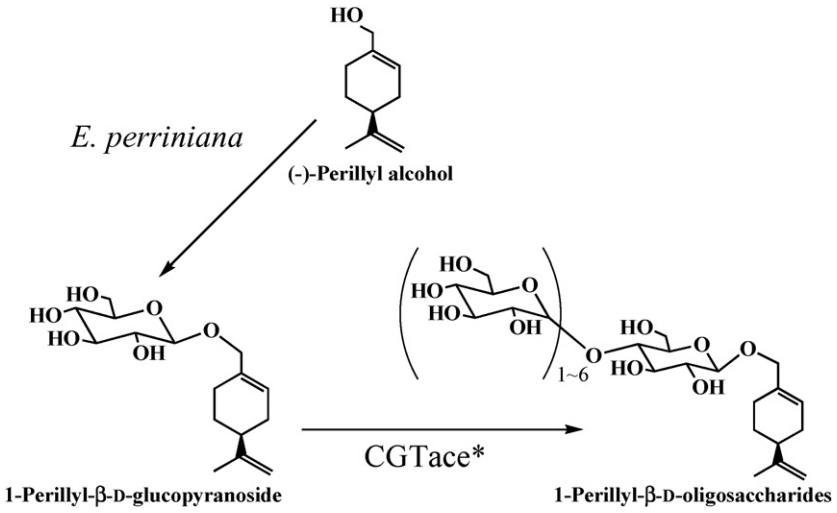

Fig. 36. Biotransformation of perillyl alcohol.

Biotransformation of taxifolin and quercetin by plant cultured cells of *Eucalyptus perriniana*

Fumiko Kasai[a], Kohji Ishihara[b], Kei Shimoda[c], Nobuyoshi Nakajima[d], Hiroki Hamada[a,*]

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[b]Department of Life Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan

[c]Department of Pharmacology and Therapeutics Chemistry, Faculty of Medicine, Oita University, Hasama-machi, Oita 879-5593, Japan

[d]Industry, Government, and Academic Promotional Center, Regional Cooperative Research Organization, Okayama Prefectural University, 111 Kuboki, Soja, Okayama 719-1197, Japan. E-mail: hamada@das.ous.ac.jp

Plant cultured cells have the ability to glycosylate the hydroxyl group of polyphenols. To analyze the regioselectivity of the glycosylation, we investigate the biotransformation of two flavonoids by plant-cultured cells of *Eucalyptus perriniana* (Fig. 37).

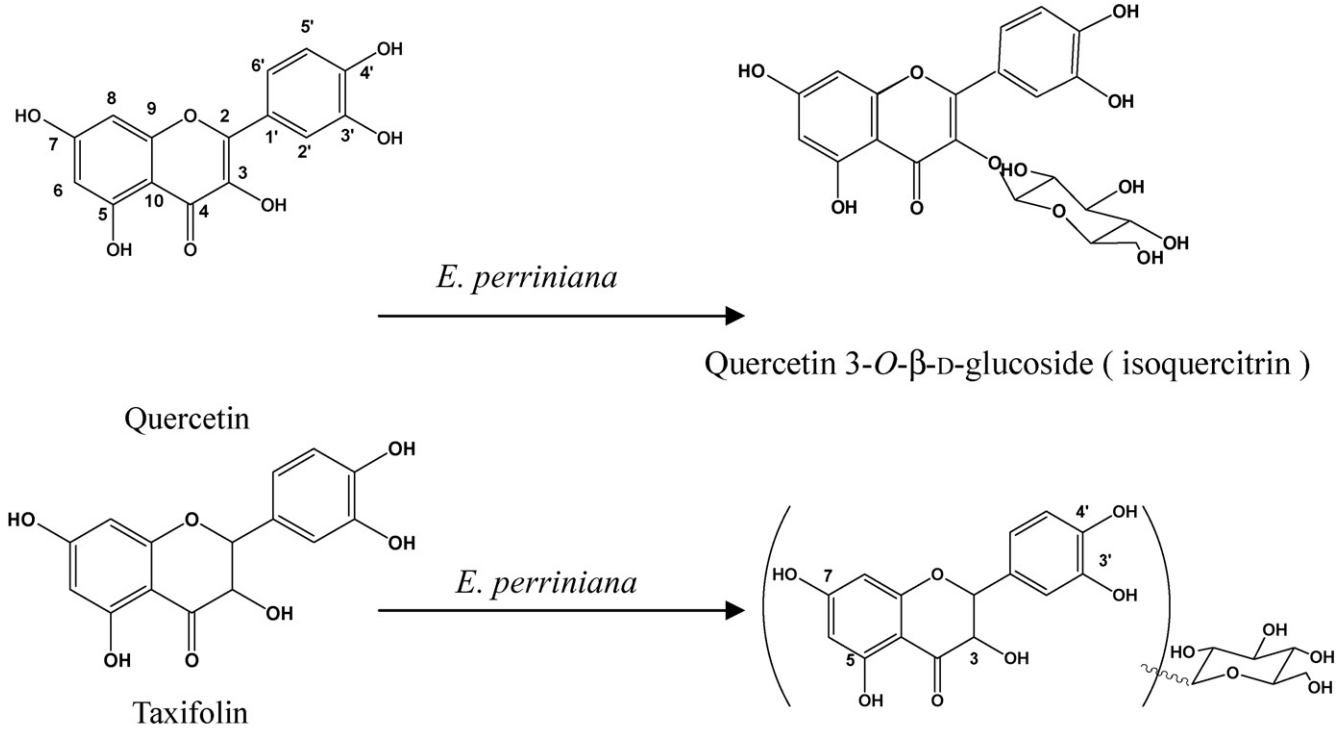

Fig. 37. Biotransformation of taxifolin and quercetin by *E. perriniana*.

Biotransformation of daidzein by plant cultured cells

Noriaki Sato[a], Kohji Ishihara[b], Nobuyoshi Nakajima[c], Hiroki Hamada[a,*]

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[b]Depertment of Life Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan

[c]Industry, Government, and Academic Promotional Center, Regional Cooperative Research Organization, Okayama Prefectural University, 111 Kuboki, Soja, Okayama 719-1197, Japan. E-mail: hamada@das.ous.ac.jp

Because daidzein, which is abundant in soybeans, exhibits estrogen-like function, it is expected to alleviate symptoms of osteoporosis occurring after menopause. To enhance the water-solubility of daidzein, we investigated the glycosylation of daidzein using various kinds of plant-cultured cells (Fig. 38).

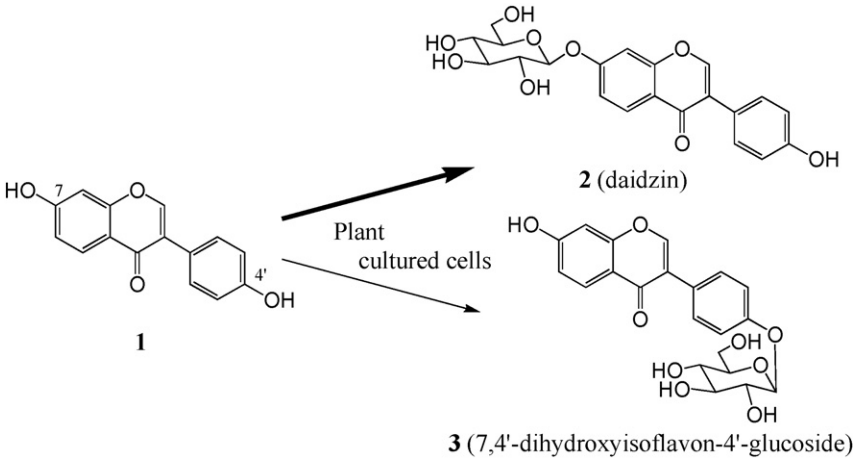

Fig. 38. Biotransformation of daidzein by plant cultured cells.