Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 42 (2006) 114–135
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Abstracts

Selected abstracts from the 9th Japanese Symposium on the Chemistry of Biocatalysis

Yasuhisa Asano [a,*], Hideo Hirohara [b,1], Kohji Ishihara [c]

[a] *Biotechnology Research Center, Faculty of Engineering, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 930-0398, Japan*
[b] *Department of Material Science, The University of Shiga Prefecture, 2500 Hassaka-cho, Hikone 522-8533, Japan*
[c] *Department of Life Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan*

Available online 17 August 2006

Invited Lectures

The present situation of plant-based plastic for automobile and the future prospects

Haruo Takahashi
Toyota Central R&D Labs., Inc., 41-1 Aza Yokomichi, Oza Nagakute, Nagakute-cho, Aichi-gun, Aichi 480-1192, Japan.
E-mail: e1092@mosk.tytlabs.co.jp

Mass consumption has been a part of 20th century society. In order to create an environmental conscious system for the 21st century, we have developed the automotive plant-based plastic. Poly lactic acid is being developed as renewable alternative for conventional petroleum-based plastic. For mass production of optical pure lactic acid, we newly constructed a transgenic yeast strain that included multi-copies of bovine LDH gene on the genome.

Father of modern biotechnology, Jokichi Takamine, and his life

Yutaka Yamamoto
Shin Nihon Chemical Co., Ltd., 19-11 Showa-cho, Anzyo, Aichi 446-0063, Japan.
E-mail: yama@snc-enzymes.co.jp

Dr. Jokichi Takamine has been known as an inventor of "Taka-Diastase", the first commercial microbial enzyme in the world, and "Adrenalin", the first drug from animal organs in the world. Although he contributed to the world as a scientist, as an inventor, as an entrepreneur, and as a philanthropist, however, these contributions have not been known by the people. These contributions will be introduced.

Snake venom phospholipase A_2: Specificity, mechanism and evolution

Inn-Ho Tsai
Institute of Biological Chemistry, Academia Sinica, and College of Life Science, National Taiwan University, P.O. Box 23-106, Taipei, Taiwan.
E-mail: bc201@gate.sinica.edu.tw

Reaction mechanism of the 14 kDa secreted phospholipase A_2 (PLA) was discussed based on its 3D-structure. Various special natural mutations which affect the catalytic activities of snake venom PLAs were listed and discussed. Two examples were detailed

* Corresponding author. Tel.: +81 766 56 7500x530; fax: +81 766 56 2498.
E-mail address: asano@pu-toyama.ac.jp (Y. Asano).
[1] Symposium organizer.

1381-1177/$ – see front matter
doi:10.1016/j.molcatb.2006.06.025

to show the strategy of venom PLA-inactivation through evolution: (1) Pro31-substitution causing distorted oxyanion hole at the active site of five elapid venom PLAs; (2) the acidic subunit of a heterodimeic PLA having very low catalytic activity but causing vasodilation through endothelial cells.

Entropy an important component of enantioselectivity

Karl Hult
School of Biotechnology, Department of Biochemistry, Royal Institute of Technology (KTH), AlbaNova University Center, SE-106 91 Stockholm, Sweden.
E-mail: kale@biotech.kth.se

The stereospecificity pocket of lipase B from *Candida antarctica* (CALB) was redesigned (Trp104Ala) to be able to accommodate 1-phenylethanol. This mutation caused the strongly *R*-selective wild-type CALB into an *S*-selective mutant and the *S* selectivity increased with temperature, dominated by entropy.

Revolution in dipeptide manufacturing: Discovery and application of the synthesizing enzyme

Shin-ichi Hashimoto
Technical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1-1 Kyowa-cho, Hofu-shi, Yamaguchi 747-8522, Japan.
E-mail: shashimoto@kyowa.co.jp

Dipeptides are the compounds composed of two L-amino acids with α-peptide bonds. Despite their usefulness, commercial applications of dipeptides are limited due to the lack of their industrial manufacturing process. To breakthrough this situation, we screened a novel activity which can synthesize a dipeptide by forming α-peptide bond with two unmodified L-amino acids. A unique idea of screening resulted in the discovery of the gene coding for the novel enzyme. By using this enzyme, we constructed a revolutional process for dipeptide manufacturing, dipeptide fermentation. I will present the screening strategy of the enzyme, characteristics of the enzyme obtained, and the outline of our dipeptide manufacturing process.

Molecular enzymologic studies on the synthesis and processing of a multi-functional polyamino acid, poly-g-glutamate

Makoto Ashiuchi
Department of Bioresources Science, Faculty of Agriculture, Kochi University, 2-5-1 Akebono-cho, Kochi 780-8520, Japan.
E-mail: ashiuchi@cc.kochi-u.ac.jp

Some peptide biopolymers including poly-ε-L-lysine and cyanophycin (multi-L-arginyl-poly-β-L-aspartate) are synthesized non-ribosomally and often called "polyamino acids". Poly-g-glutamate (PGA), a main component of *natto* mucilage, likewise belongs to the family of polyamino acids; this multi-anionic polyisopeptide exhibits the multi-functionalities and meets the demands of the times with the respect to the new biomaterial industry that is friendly to life and the environment. The PGA synthetic mechanism in *Bacillus subtilis*, the characterization of the PGA synthetase, and the properties of the new useful biocatalyst, i.e. PGA-processing enzyme that produces the value-added biopolymers (high-molecular-mass L-PGA and low-molecular-mass D-PGA) from *B. subtilis* PGA, will be presented.

Using genomics to expand biocatalysis

Jon D. Stewart
Department of Chemistry, University of Florida, Gainesville, FL 32611, USA.
E-mail: jds2@chem.ufl.edu

We used genome mining to clone and express libraries of new biocatalysts for carbonyl and alkene reductions. The substrate- and stereoselectivities of these enzymes were defined, and examples of how they could be used in total synthesis projects were presented.

The protein scaffold of CALB as host for new enzyme reactions

Karl Hult
School of Biotechnology, Department of Biochemistry, Royal Institute of Technology (KTH), AlbaNova University Center, SE-106 91 Stockholm, Sweden.
E-mail: kale@biotech.kth.se

The lipase B from *Candida antarctica* (CALB) is a very versatile enzyme and its enantioselectivity has been altered by mutation. The enzyme was also changed to catalyze new reactions, such as aldol reaction, a Michael type addition reaction.

Poster Presentations

Hyper-accumulation of glutamine in tissue cultured watercress

Kayo Mitamura[b], Mayuko Yamamoto[b], Yasuo Kato[a,*], Shinjiro Ogita[a]
[a]Faculty of Engineering, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan
[b]College of Technology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan.
E-mail: yasu@pu-toyama.ac.jp

We succeeded in efficient proliferation of multiple shoots and adventitious roots of watercress (*Nasturtium officinale* R.Br.) by culturing them with cytokinine and auxin, respectively, and found that glutamine was hyper-accumulated up to 80% of the total soluble amino acids in the tissue culture cells (Fig. 1).

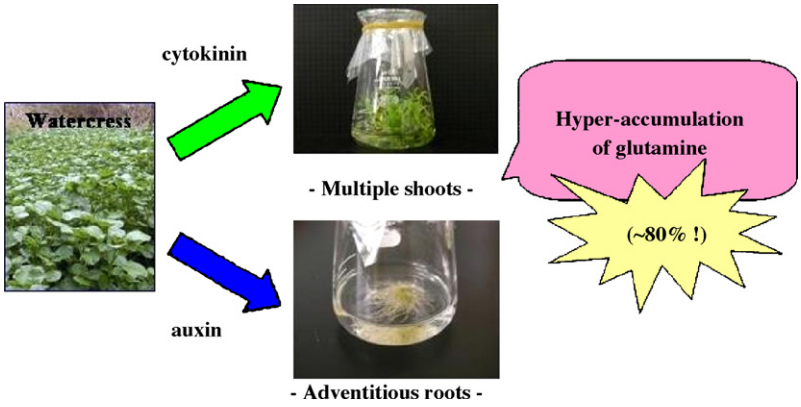


Fig. 1. Hyper-accumulation of glutamine in tissue cultured watercress.

Chiral alcohol production by enantioselective reduction with immobilized recombinant *E. coli* cells expressing PAR and LSADH

Masatoshi Nakamura, Kousuke Inoue, Yoshihide Makino, Tohru Dairi, Nobuya Itoh*
Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan.
E-mail: itoh@pu-toyama.ac.jp

E. coli cells expressing the mutated phenylacetaldehyde reductase (Sar268) or *Leifsonia* alcohol dehydrogenase (LSADH) were immobilized with glutaraldehyde and 1,6-dianimohexane. Immobilized *E. coli* cells packed in a column indicated sufficient activity and stability in aqueous 2-propanol solution (10–20%), and were able to be used for continuously producing (*R*)-1,3-butanediol for more than 600 h (Fig. 2).

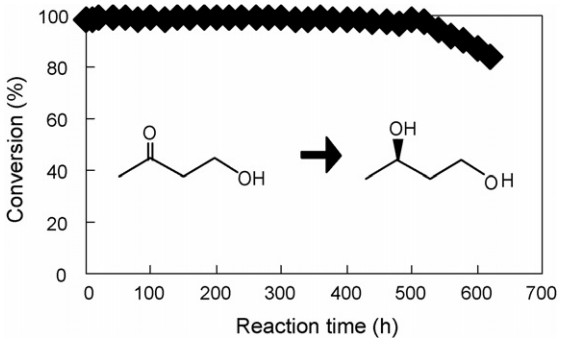


Fig. 2. Continuous production of (*R*)-1,3-butanediol (5%, v/v, 99% yield, 96% e.e.) by immobilized LSADH.

Further improvement of engineered phenylacetaldehyde reductase (PAR) on activity under concentrated 2-propanol

Yoshihide Makino*, Tohru Dairi, Nobuya Itoh
Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan.
E-mail: makino@pu-toyama.ac.jp

Asymmetric reduction of several ketones, such as *m*-chlorophenacyl chloride (*m*-CPC), with concentrated 2-propanol was further improved by engineering of phenylacetaldehyde reductase mutant (Table 1).

Table 1
Percent *m*-CPC conversion by 30 mg resting *E. coli* cells in 500 μl reaction

Plasmid	*m*-CPC added per reaction (mg)				
	100	125	150	175	200
pSar268 (original mutant)	97.1	90.7	84.8	75.6	71.5
pHAR1 (this study)	100.0	100.0	96.8	91.1	87.4

Lipase-catalyzed resolution of primary alcohol and application of chiral alcohol for synthesis of natural product

Masashi Kawasaki[a,*], Yuko Hayashi[a], Hiroko Kakuda[b], Naoki Toyooka[b], Akira Tanaka[a], Michimasa Goto[c], Tadashi Kometani[c]
[a]Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan
[b]University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[c]Toyama National College of Technology, 13 Hongo, Toyama 939-8630, Japan.
E-mail: kawasaki@pu-toyama.ac.jp

The highly enantioselective kinetic resolution of a racemic primary alcohol by lipase-catalyzed transesterification with vinyl 3-(4-trifluoromethylphenyl)propanoate afforded the optically pure primary alcohol (*R*)-**3** which was used for the asymmetric synthesis of 5,6-dehydrosenedigitalene (*R*)-**1** (Fig. 3).

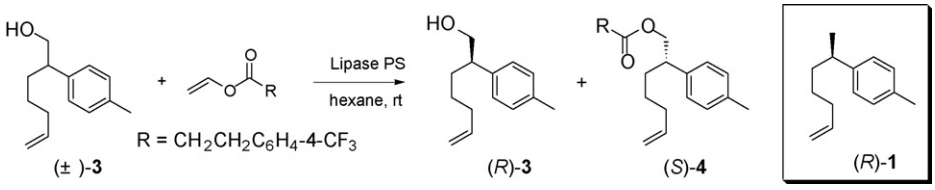


Fig. 3. Lipase-catalyzed transesterification with vinyl 3-(4-trifluoromethylphenyl)propanoate.

Lipase-catalyzed acylation of phenolic hydroxyls

Toshifumi Miyazawa*, Manabu Hamada, 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

Lipase-catalyzed direct acylation of phenolic hydroxyls has been investigated using hydroquinones having several substituents and vinyl propanoate as substrates and *Candida antarctica* lipase B as well as *Burkholderia cepacia* lipase as biocatalysts (Fig. 4).

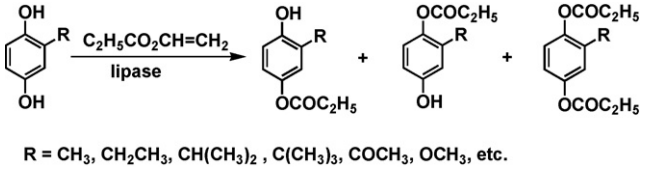


Fig. 4. Lipase-catalyzed regioselective acylation of hydroquinones.

Papain-catalyzed peptide synthesis through segment condensation: Application to the preparation of partial sequences of bioactive peptides

Toshifumi Miyazawa*, Takao Horimoto, 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

Papain-catalyzed peptide synthesis utilizing the carbamoylmethyl ester as acyl donor has been applied to the preparation of partial sequences of some bioactive peptides, e.g., substance P (7–11)-pentapeptide, through segment condensation (Fig. 5).

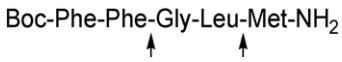


Fig. 5. Papain-catalyzed segment synthesis of substance P (7–11)-pentapeptide.

The chemoenzymatic synthesis of optically active γ-octenolide

Mikio Fujii[a], Motonori Fukumura[a], Kanako Tanifuji[a], Yumiko Hori[a], Yasuaki Hirai[a], Kazuo Toriizuka[a], Kaoru Nakamaru[b], Yoshiteru Ida[a,*]

[a]School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

[b]Institute for Chemical Research, Kyoto University, Uji, Kyoto 610-0011, Japan.

E-mail: ida@pharm.showa-u.ac.jp

Optically active γ-oct-2-enolide was prepared from hept-1-en-3-ol by lipase-catalyzed transesterification followed by ring-closing metathesis by Grubbs' catalyst (Fig. 6).

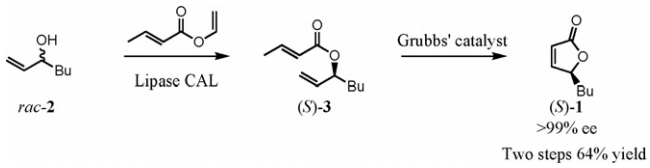


Fig. 6. Two steps synthesis of optically active γ-octenolide.

Light-mediated asymmetric reduction of ketones by photosynthetic organisms

Junya Horitsune[a,*], Rio Yamnaka[b], Shin-ichi Ueji[a], Kaoru Nakamura[b]

[a]Graduate School of Cultural Studies and Human Science, Kobe University, 3-11, Tsurukabuto, Nada-ku, Kobe, Hyogo 657-8501, Japan

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

E-mail: 045f720f@y04.kobe-u.ac.jp

Ketones were reduced with photosynthetic organisms under illumination of fluorescent light. Effect of growth and concentrations of the photosynthetic organisms to chemical yields and enantioselectivies of product was investigated (Fig. 7).

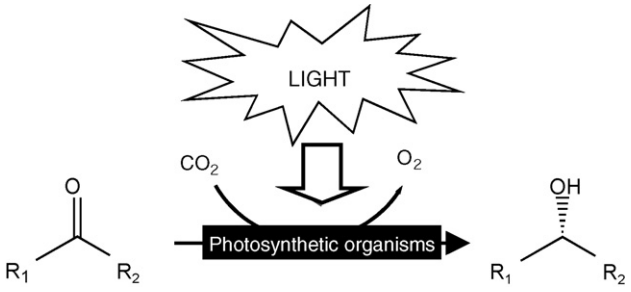


Fig. 7. Light-mediated asymmetric reduction of ketones by photosynthetic organisms.

Enzymatic glucose-O_2 biofuel cells

Akio Ishii*, Seiya Tsujimura, Kenji Kano

Division of Applied Life Science, Graduated School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.

E-mail: a_ishii@kais.kyoto-u.ac.jp

The properties of prototype of glucose-O_2 biofuel cells that consist of bioanode containing NAD- or PQQ-dependent glucose dehydrogenase and biocathode containing bilirubin oxidase were investigated (Fig. 8).

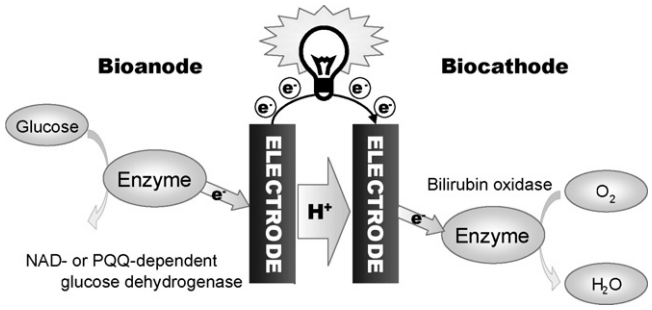


Fig. 8. Constitution of prototype of glucose-O_2 biofuel cells.

Redox behavior of histamine dehydrogenase by separator-less one-compartment bulk electrolysis

Maiko Tsutsumi*, Nobutaka Fujieda, Kenji Kano
Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.
E-mail: maikot@kais.kyoto-u.ac.jp

Each redox potential of cofactors contained in histamine dehydrogenase from *Nocardioides simplex* has been measured by means of UV–vis spectroelectrochemistry, understanding the redox behavior during reduction with substrate (Fig. 9).

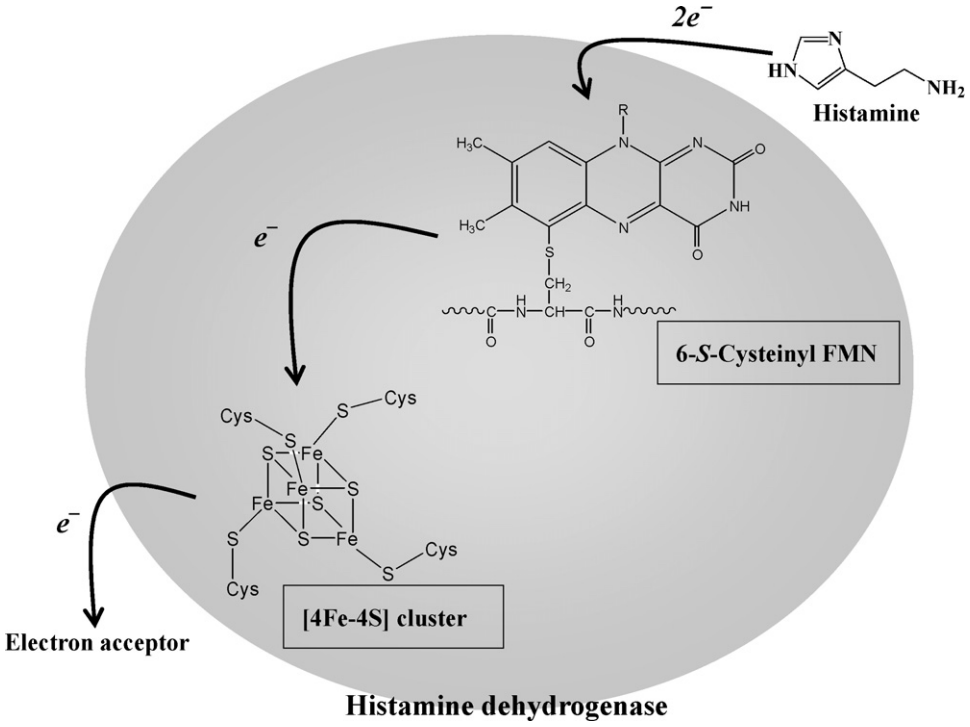


Fig. 9. Reaction scheme of histamine dehydrogenase.

Production of D-pseudoephedrine using amino-alcohol dehydrogenase from *Rhodococcus erythropolis* MAK154

Nobuyuki Urano[a,*], Takeru Ishige[a], Satoko Fukui[a], Michihiko Kataoka[a], Shinji Kita[b], Keiji Sakamoto[b], Sakayu Shimizu[a]
[a]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
[b]Daiichi Fine Chemical Co., Takaoka, Toyama 933-8511, Japan.
E-mail: uranob@kais.kyoto-u.ac.jp

We found that amino-alcohol dehydrogenase from *Rhodococcus erythropolis* MAK 154 catalyzed the asymetric reduction of L-1-phenyl-1-keto-2-methylaminopropane (L-MAK) to D-pseudoephedrine. Using recombinant *E. coli* cells which expressed this enzyme and be introduced coenzyme regeneration system, we could convert DL-MAK to D-pseudoephedrine with high molar reaction yield (Fig. 10).

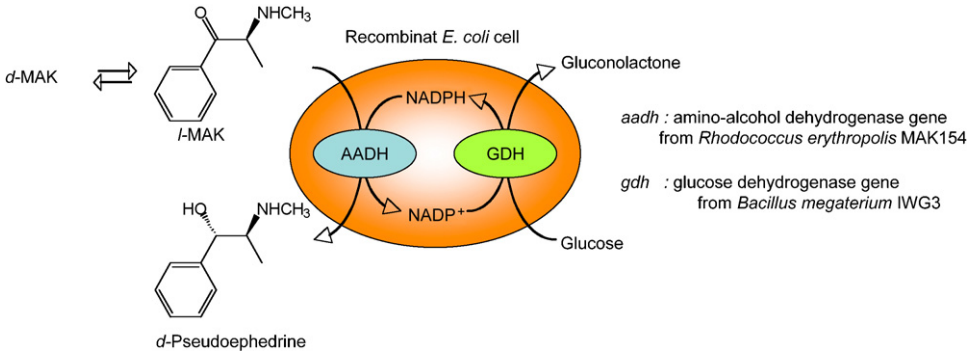


Fig. 10. The conversion of DL-MAK to D-pseudoephedrine using recombinant *E. coli*.

Screening and biochemical analysis of biocatalysts useful for chiral β-amino acid synthesis

Junichi Mano, Jun Ogawa*, Sakayu Shimizu
Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.
E-mail: ogawa@kais.kyoto-u.ac.jp

Chiral 3-amino-3-phenylpropionic acid (β-phenylalanine) production was performed through kinetic resolution of racemic β-phenylalanine by microbial stereoselective degradation and the biochemical properties of enzymes involved in stereospecific degradation were analyzed (Fig. 11).

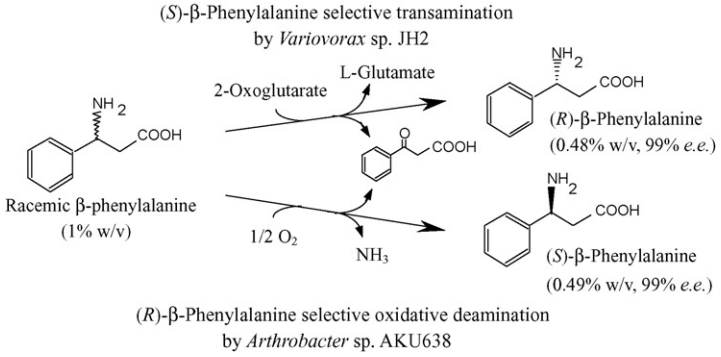


Fig. 11. Chiral β-phenylalanine production by microbial stereoselective degradation.

Thermal stability of immobilized lipase in *n*-alcohol

Yayoi Yoshida, Yukitaka Kimura, Shuji Adachi*
Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.
E-mail: adachi@kais.kyoto-u.ac.jp

The inactivation process of immobilized lipase from *Candida antarctica* (Chirazyme® L-2, C2) in *n*-alcohols did not obey the first-order kinetics but was well expressed using a model in which the heterogeneity of enzyme stability was assumed as shown by the solid curve (Fig. 12).

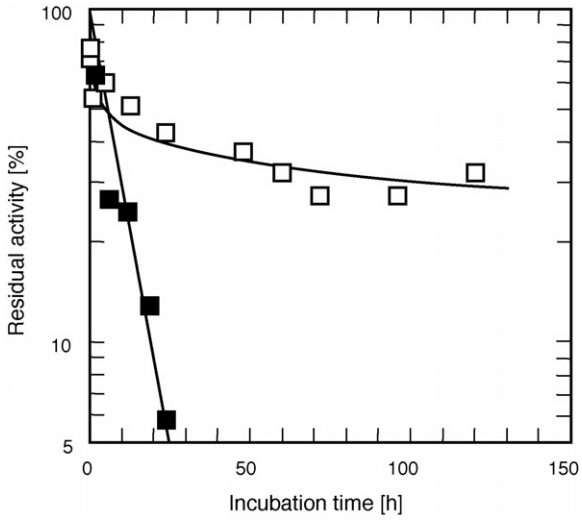


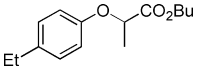
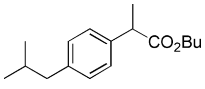
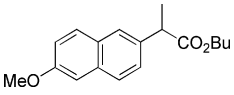
Fig. 12. Inactivation processes of free (■) and immobilized (□) lipases in *n*-pentanol at 90 °C.

A greatly improvement of the enantioselectivity of lipase-catalyzed hydrolysis using sodium dodecyl sulfate as an additive

Shuichi Mori, Tomohiro Nishigaki, Shin-ichi Ueji*
The Graduate School of Science and Technology, Kobe University, 3-11 Tsurukabuto, Nada-ku, Kobe 657-8501, Japan.
E-mail: ueji@kobe-u.ac.jp

The addition of sodium dodecyl sulfate (SDS) resulted in a dramatic improvement of the enantioselectivity of the lipase-catalyzed hydrolysis of several racemic butyl 2-substituted propanoate (Table 2).

Table 2
The enhancement of the enantioselectivity caused by the addition of SDS for the hydrolysis reaction of three different racemic esters

Ester	Additive	Time (min)	Conversion (%)	% e.e.	*E*
	None	8	36	50	14
	100 mM SDS	18	45	98	225
	None	1080	25	86	18
	1 mM SDS	1080	23	100	–
	None	660	42	92	49
	1 mM SDS	720	44	98	254

A new method to improve the enantioselectivity of lipase based on the chemical modification of lipase

Naoto Okada, Miki Tachibana, Shuichi Mori, Shin-ichi Ueji*
Graduate School of Cultural Science and Human Science, Kobe University, 3-11 Tsurukabuto, Nada-ku, Kobe 657-8501, Japan.
E-mail: Ueji@kobe-u.ac.jp

We tried to fluorinate the lipase-surface by introducing several fluoro-groups to lysine residues of *Candida rugosa* lipase. For the esterification of 2-(4-ethyl phenoxy)propanoic acid or the hydrolysis of corresponding butyl ester, the enantioselectivity observed for these fluoro-lipases is found to be very sensitive to the change of the solvents, compared to non-fluoro-lipases (Fig. 13).

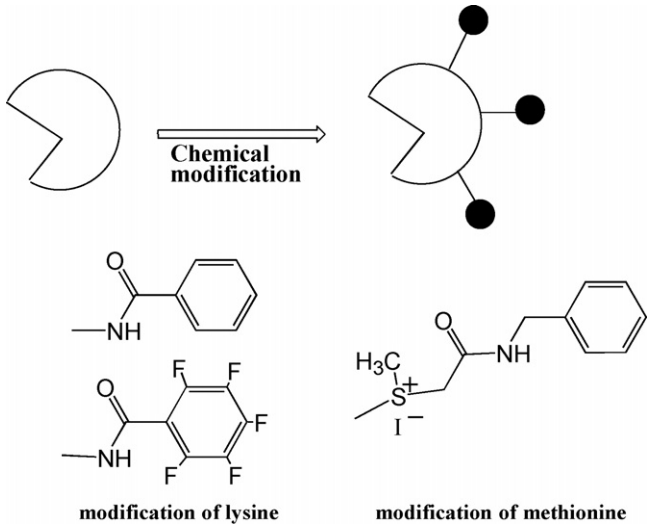


Fig. 13. The chemical modification to lysine and methionine residues of lipase.

Enzyme expression in periplasmic space of *E. coli* and its application

Katsuyuki Nagashige, Yasuyuki Ueda, Satoko Matsuo, Shokichi Ohuchi*
Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan.
E-mail: ohuchi@bio.kyutech.ac.jp

Phosphotriesterase was expressed in *E. coli*'s periplasmic space and it was demonstrated as a biocatalyst for the esterification in organic solvent (Fig. 14).

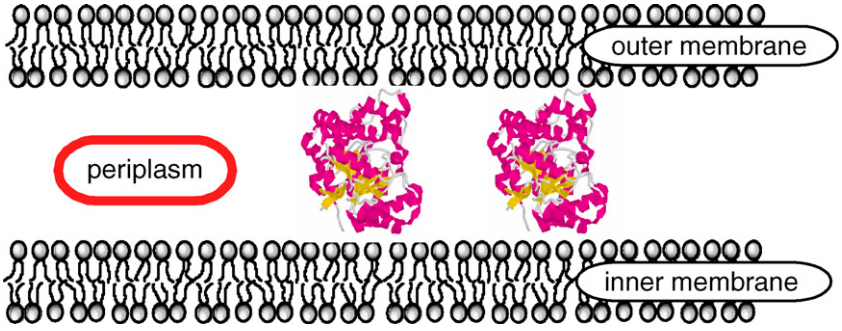


Fig. 14. Phosphotriesterase expression in periplasmic space of *E. coli*.

Chiral resolution of phosphorous compound by *E. coil* immobilized phosphotriesterase

Kensuke Chikuda, Satoko Matsuo, Katsuyuki Nagashige, Shokichi Ohuchi*
Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan.
E-mail: ohuchi@bio.kyutech.ac.jp

Phosphotriesterase immobilized on the surface of *E. coil* was applied to the chiral resolution of organophosphorous compounds (Fig. 15).

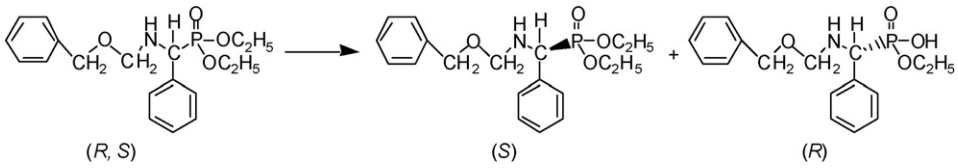


Fig. 15. Amino alkyl phosphonate was resolved by the cell surface immobilized phosphotriesterase.

Expression and characterization of β-structural phosphotriesterase

Seiji Kurisu, Satoko Matsuo, Hiroya Osoegawa, Shokichi Ohuchi*
Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan.
E-mail: ohuchi@bio.kyutech.ac.jp

Phosphotriesterase formed by β propeller structure was prepared in *E. coli* expression system and it was applied for transesterification in organic solvent (Fig. 16).

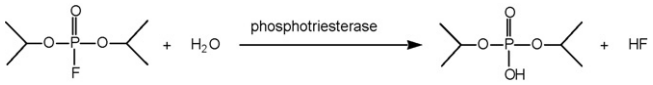


Fig. 16. Hydrolytic reaction by phoshotriesterase.

Assisted utilization of microwave for *Flavobacterium* biocatalyst

Satoko Matsuo, Yasuhiko Ohtsuka, Keiichi Uchibayashi, Katsuyuki Nagashige, Shokichi Ohuchi*
Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan.
E-mail: ohuchi@bio.kyutech.ac.jp

Flavobacterium sp. containing phosphotriesterase was carried out as a biocatalyst of optical resolution of chiral organophosphorous compounds under the microwave irradiation (Fig. 17).

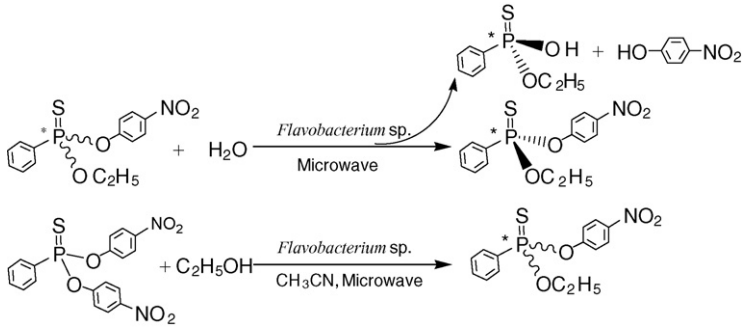


Fig. 17. *Flavobacterium* sp. catalyzed optical resolution.

Enzymatic reaction by use of non-thermal effect of microwave

Yasuhiko Ohtsuka, Keiichi Uchibayashi, Shokichi Ohuchi*
Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan.
E-mail: ohuchi@bio.kyutech.ac.jp

To demonstrate the non-thermal effect of microwave reaction, lipase was demonstrated with microwave irradiation under the controlled temperature (Fig. 18).

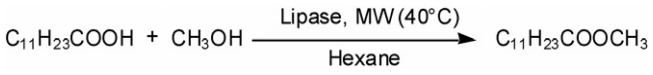
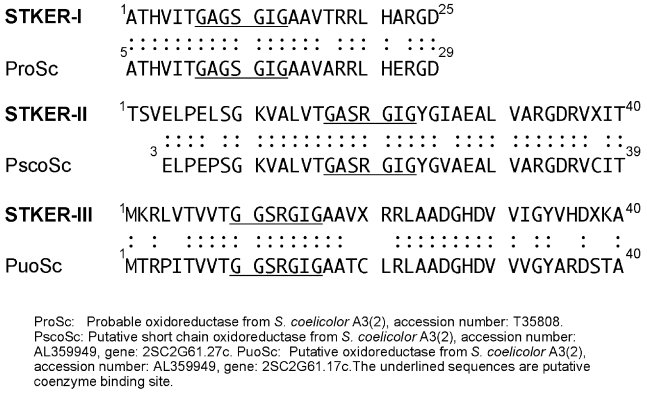


Fig. 18. Microwave irradiated enzymatic esterification under controlled temperature.

Purification and characterization of α-keto ester reductases from actinomycete: An approach based on protein chemistry and bioinformatics

Kohji Ishihara[a,*], Chiaki Kato[a], Hitomi Yamaguchi[b], Hiroki Hamada[a], Nobuyoshi Nakajima[c]
[a]Department of Life Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan
[b]Research and Development Center, Nagase & Co., Ltd., Nishi-ku, Kobe 651-2241, Japan
[c]Department of Health and Welfare Science, Okayama Prefectural University, 111 Kuboki, Soja, Okayama 719-1197, Japan.
E-mail: ishihara@dls.ous.ac.jp

We have achieved the purification of an α-keto ester reductase (SCKER) from *Streptomyces coelicolor* A3(2) whole cells. Non of three hypothetical proteins of *S. coelicolor* A3(2) having a high homology sequence with those of already purified α-keto ester reductases from *S. thermocyaneoviolaceus* [Biosci. Biotechnol. Biochem. 66 (2002) 588–597] was identical with that of SCKER (Fig. 19).



ProSc: Probable oxidoreductase from *S. coelicolor* A3(2), accession number: T35808. PscoSc: Putative short chain oxidoreductase from *S. coelicolor* A3(2), accession number: AL359949, gene: 2SC2G61.27c. PuoSc: Putative oxidoreductase from *S. coelicolor* A3(2), accession number: AL359949, gene: 2SC2G61.17c.The underlined sequences are putative coenzyme binding site.

Fig. 19. Comparison of N-terminal amino acid sequences.

Biotransformation of cinnamic acid derivatives by plant cultured cells

Masatomo Hirabayasi, Akiko Miyata, Hiroki Hamada*, Tsutomu Furuya
Graduate School of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan.
E-mail: hamada@das.ous.ac.jp

We investigated the biotransformation of cinnamic acid derivatives by plant cultured cells and it was found that the plant cells glycosylated hydroxyl groups and carboxyl groups of their derivatives (Fig. 20).

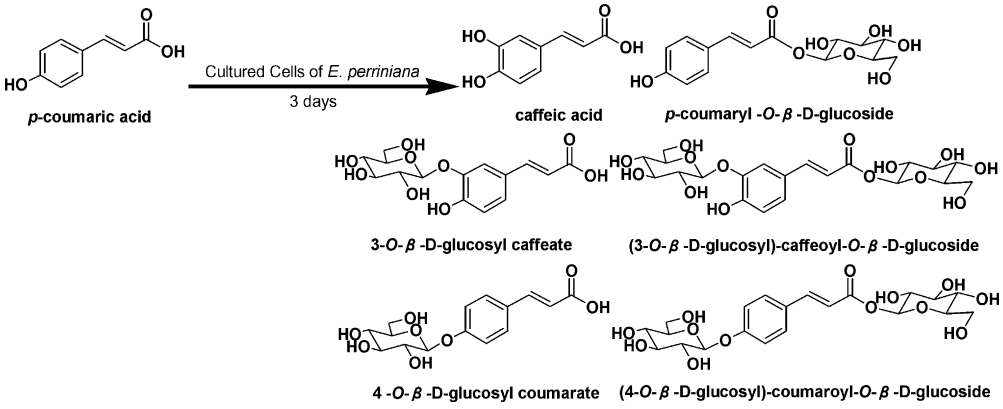


Fig. 20. Biotransformation of *p*-courmaric acid by *E. perriniana*.

Preparation of (−)-perillyl alcohol oligosaccharides

Naoko Yonemoto[a], Sou Sakamoto[a], Kohji Ishihara[b], Nobuyoshi Nakajima[c], Tsutomu Furuya[a], Hiroki Hamada[a,*]
[a]Department of Applied Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan
[b]Department 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

(−)-Perillyl alcohol was transformed into (−)-perillyl 7-*O*-β-D-glucoside, (−)-perillyl 7-*O*-β-D-gentiobioside by cultured cells of *Eucalyptus perriniana* and perillyl alcohol oligosaccharides was obtained using cyclodextrin glucanotransferase (CGTase) (Fig. 21).

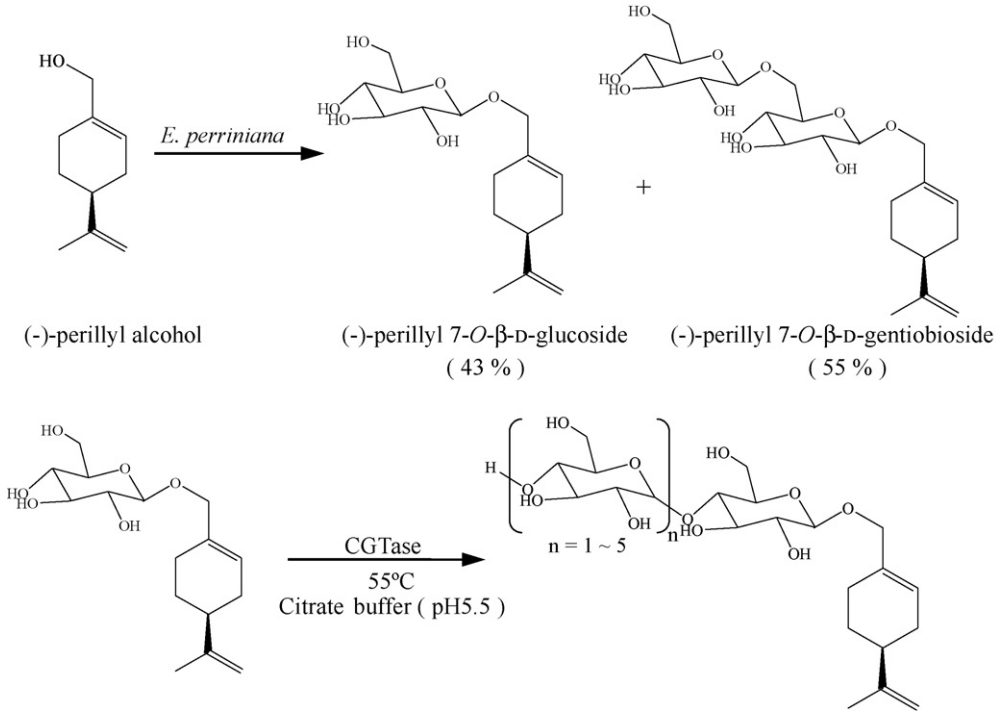


Fig. 21. Biotransformation of (−)-perillyl alcohol by the cultured cells of *E. perriniana* and enzymatic synthesis of (−)-perillyl alcohol oligosaccharides by CGTase.

Biotransformation of acyclic monoterpenes by biocatalysts of plant cultured cells and *cyanobacterium*

Ayako Matsuki[a], Junichi Takimura[a], Kohji Ishihara[b], Nobuyosi Nakajima[c], Fumiko Kasai[a], Hiroki Hamada[b,*]
[a]Department of Applied Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan
[b]Department 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

The cells of *Catharanthus roseus* hydroxylated the allylic position of the acyclic monoterpene. Cyanobacterium cells reduced the double bond and oxidized in a hydroxyl group of the monoterpene (Fig. 22).

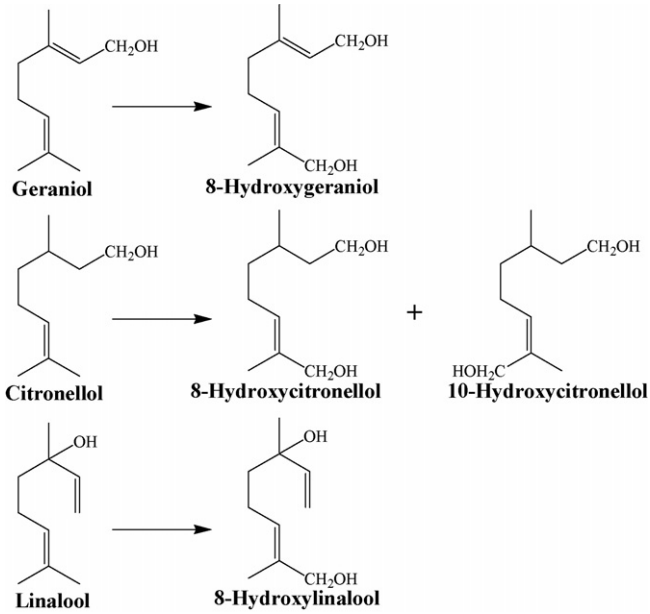


Fig. 22. Biotransformation of the acyclic monoterpene by the cells of *C. roseus*.

Cloning and expression of cyclo(Leu-Phe) oxidase gene from an actinomycete *Streptomyces albulus* KO23

Machiko Nagao[a], Atsushi Morimoto[a], Takashi Tamura[a], Tohru Dairi[b], Hiroshi Kanzaki[a,*]
[a]The Graduate School of Natural Science and Technology, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan
[b]Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 939-0398, Japan.
E-mail: hkanzaki@cc.okayama-u.ac.jp

An *Escherichia coli* transformant with a plasmid containing *alb*A and *alb*B of *Streptomyces albulus* KO23 exhibits the activity converting cyclo(Leu-Phe) to albonoursin, indicating that *albA* or *albB* are required for expression of cyclo(Leu-Phe) oxidase (Fig. 23).

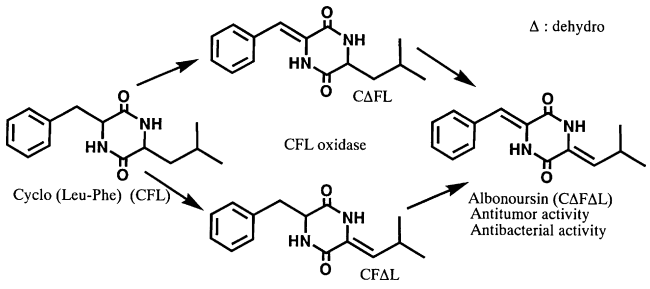


Fig. 23. Albonoursin biosynthetic pathway by CFL oxidase.

Ficin and asparagus juice catalyzed peptide synthesis

San Zhou, Tomoyori Fuchise, Kiyoshi Horita, Yukari Noguchi, Eiko Toyata, Haruo Sekizaki*
Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan.
E-mail: sekizaki@hoku-iryo-u.ac.jp

Ficin and asparagus juice were effective as catalyst for the peptide bond formation using Boc-amino acid phenyl and naphthyl esters as acyl donor components, respectively (Fig. 24).

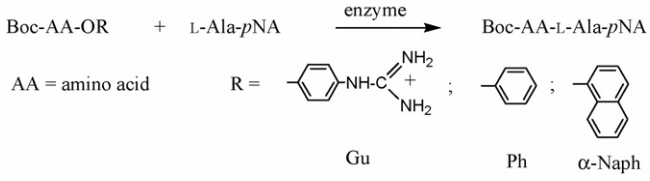


Fig. 24. Ficin and asparagus juice catalyzed peptide synthesis.

Microorganisms with homocysteine thiolactone hydrolase: Screening and stereospecificity

Shozo Honda, 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

Three bacterial and one fungal strains, which we found as homocysteine thiolactone (HCTL) hydrolase-producing microbes, can hydrolyze L-HCTL, but not D-HCTL, while L-pantoyl lactone (PL) and D-PL hydrolases were reported to be distributed in bacteria and fungi, respectively (Fig. 25).

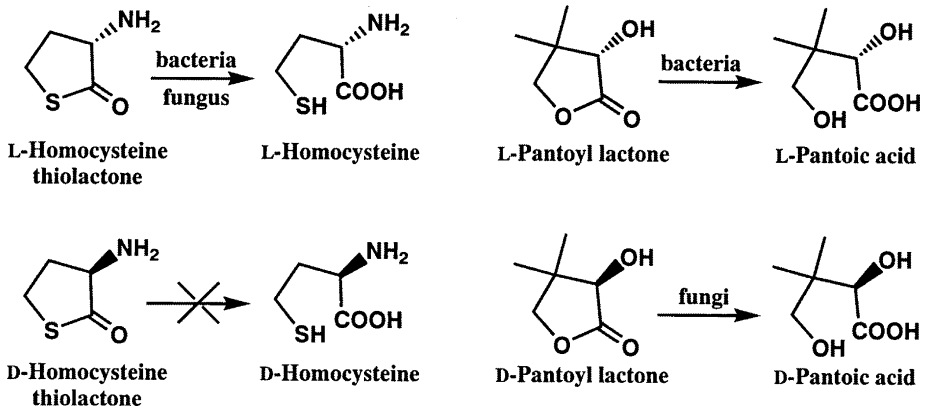


Fig. 25. Microbial hydrolysis of L-HCTL, L-PL and D-PL.

Theanine production by coupling with energy transfer employing yeast cells and *Pseudomonas taetrolens* Y-30 glutamine synthetase

Sachiko Yamamoto*, Mamoru Wakayama, Takashi Tachiki
Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, 1-1-1 Noji-higashi, Kusatsu, Shiga 525-8577, Japan.
E-mail: rb069954@se.ritsumei.ac.jp

Theanine was produced from glutamic acid and ethylamine by coupling the reaction of glutamine synthetase of *Pseudomonas taetrolens* Y-30 with the sugar fermentation of baker's yeast cells as an ATP-regeneration system (Fig. 26).

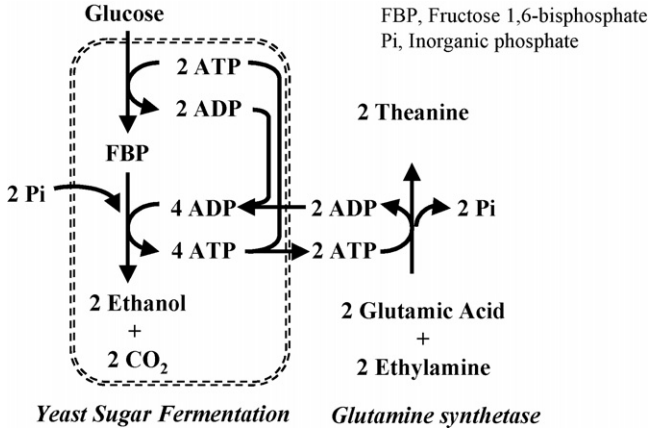


Fig. 26. Principle of theanine production by coupled fermentation with energy transfer.

Further proof for wide substrate specificity of cyclo(Leu-Phe) oxidase from an actinomycete: Enzymatic conversion of cyclic dipeptides containing non-aromatic amino acids

Rie Hirata, 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 cyclo(Leu-Phe) oxidase (CFL oxidase) can also catalyze the conversion of CDPs containing non-aromatic amino acids such as cyclo(His-His) and cyclo(Asp(OMe)-Asp(OMe)) to their dehydro derivatives (Fig. 27).

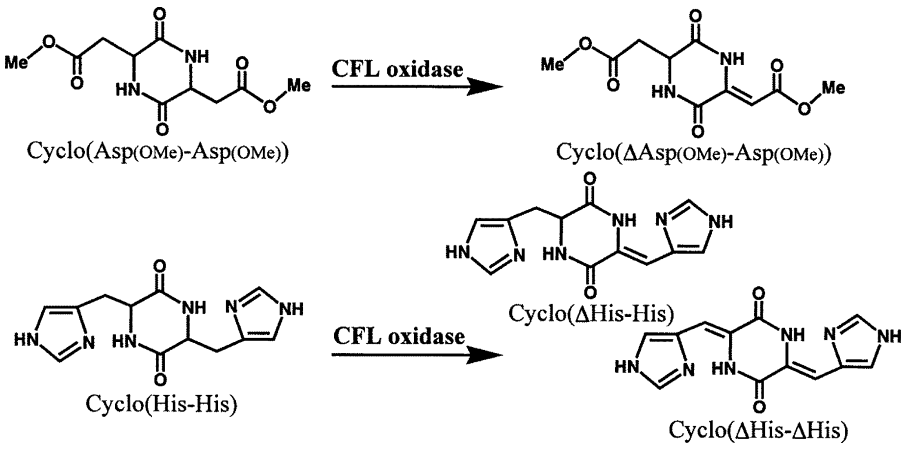


Fig. 27. Conversion of cyclo(His-His) and cyclo(Asp(OMe)-Asp(OMe)) by CFL oxidase.

Oxidative decarboxylation of β-hydroxy carboxylic acid using tropate-assimilating *Rhodococcus* sp.

Shinji Hirokawa, Kenji Miyamoto, Hiromichi Ohta*
Department of Biosciences and Informatics, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan.
E-mail: hohta@bio.keio.ac.jp

The incubation of racemic α-methyltropate with tropate-assimilating *Rhodococcus* sp. resulted in the formation of optically active α-phenylpropionate of which intermediate is supposed to be α-formyl-α-phenylpropionate (Fig. 28).

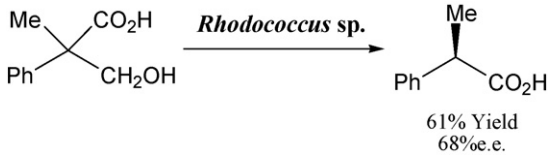


Fig. 28. Oxidative decarboxylation of α-methyltropate.

Aldol type reaction catalyzed by arylmalonate decarboxylase (AMDase)

Yousuke Terao, Kenji Miyamoto, Hiromichi Ohta*
Department of Biosciences and Informatics, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan.
E-mail: hohta@bio.keio.ac.jp

Arylmalonate decarboxylase (AMDase) exhibited a new enzymatic activity, aldolase-like activity, in addition to its original decarboxylase activity when an aldehyde group was introduced on the ortho-position of the substrate (Fig. 29).

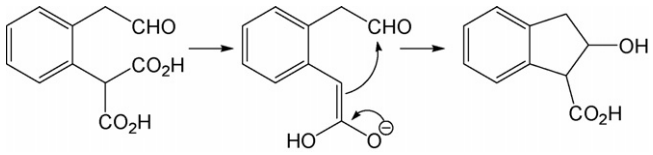


Fig. 29. The aldol type reaction catalyzed by AMDase.

Development of a clean and effective biocatalytic alcohol-oxidizing system

Jun-ichiro Hirano, Kenji Miyamoto, Hiromichi Ohta*
Department of Biosciences and Informatics, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan.
E-mail: hohta@bio.keio.ac.jp

Alcohol-oxidation system was developed by the combination of two dehydrogenases from *Brevibacterium* sp. and NADH oxidase from *Lactobacillus brevis* (Fig. 30).

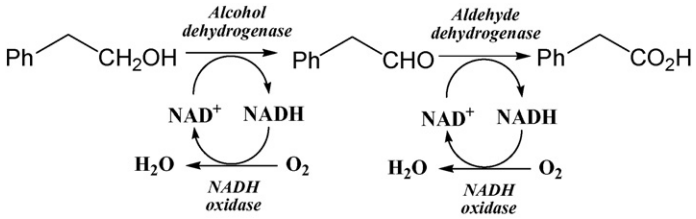


Fig. 30. Oxidation system using three oxidizing enzymes.

Microbial asymmetric reduction of sterically hindered carbonyl compound: Substrate specificity and stereoselectivity

Chihiro Hiraoka, Masaaki Matsuda, Yuya Suzuki, Shigeo Fujieda, Ken-ichi Fuhshuku, Shigeru Nishiyama, Takeshi Sugai*
Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan.
E-mail: sugai@chem.keio.ac.jp

Several yeast strains which can reduce sterically hindered ketone were found by screening, and the specificity and stereoselectivity on a wide range of substrates were examined (Fig. 31).

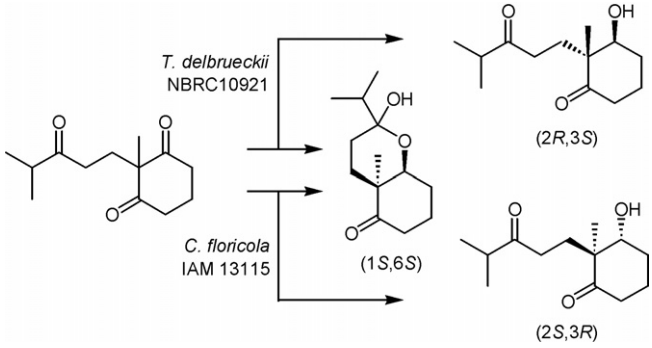


Fig. 31. Yeast-mediated reduction of sterically hindered ketone—change of selectivity.

Production of N^{α}-Z-DL-aminoadipic acid and N^{α}-Z-DL-aminoadipic-δ-semialdehyde with *Rhodococcus* sp. AIU Z-35-1

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

A new bacterial strain, *Rhodococcus* sp. AIU Z-35-1, which was isolated as a producer of N^{α}-Z-DL-aminoadipic acid, was also useful for the production of N^{α}-Z-DL-aminoadipic-δ-semialdehyde (Fig. 32).

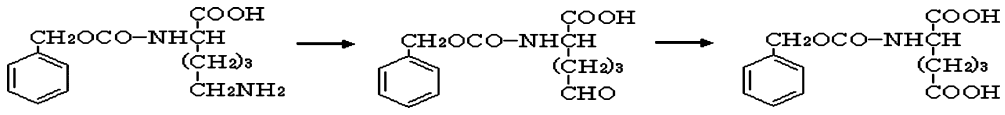


Fig. 32. Conversion of N^{α}-Z-DL-lysine into N^{α}-Z-DL-aminoadipic acid by *Rhodococcus* sp. AIU Z-35-1.

Kinetic analyses of the roles of amino acid residues near the active site of β-amylase on a 27 MHz quartz-crystal microbalance

Toshiaki Mori[a], Masayoshi Shibata[a], Takanori Nihira[a], Bunzo Mikami[b], Yoshio Okahata[a,*]
[a]Department of Biomolecular Engineering, Tokyo Institute of Technology, Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan
[b]Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan.
E-mail: tmori@bio.titech.ac.jp

A quartz-crystal microbalance (QCM) technique was applied to analyze effects of site-directed mutagenesis of a β-amylase on the hydrolysis mechanism of the substrate binding and the catalytic process, separately, by using an amylose-immobilized QCM in buffer solution. We could follow both the product and the ES complex formation as mass changes, and obtain kinetic parameters such as k_{on}, k_{off}, K_d, and k_{cat} values (Fig. 33).

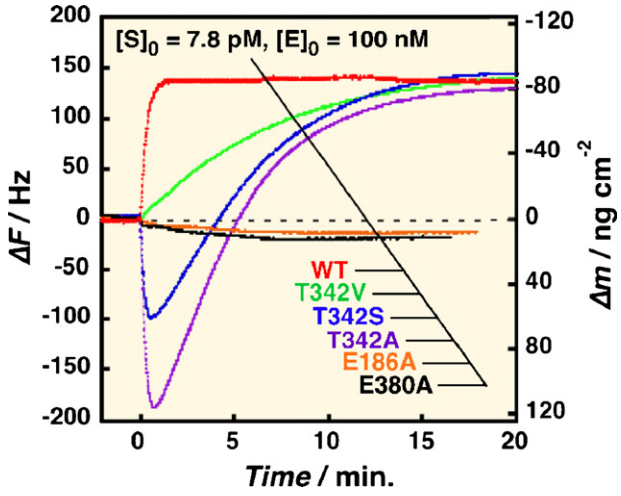


Fig. 33. Time-course of amylosehydrolysis on QCM by mutant β-amylase from soybean.

Rational control of enantioselectivity of lipase by means of site-directed mutagenesis based on the mechanism

Tadashi Ema*, Toshiyuki Fujii, Misa Ozaki, Toshinobu Korenaga, Takashi Sakai
Department of Applied Chemistry, Faculty of Engineering, Okayama University, 3-1-1 Tsushima-naka, Okayama 700-8530, Japan.
E-mail: ema@cc.okayama-u.ac.jp

The enantioselectivity of a *Burkholderia cepacia* lipase toward secondary alcohols could be both increased and decreased rationally by introducing only a single mutation on the basis of the mechanism proposed previously (Fig. 34).

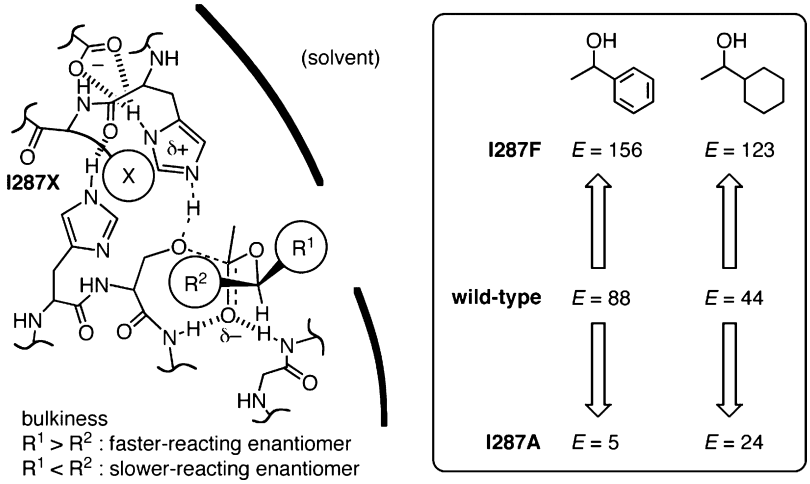


Fig. 34. Rational control of enantioselectivity of lipase by introducing a single mutation based on the transition-state model.

Lipase-catalyzed asymmetric transesterification using imidazolium highly fluorinated alkyl sulfate as a reaction medium

Yoshikazu Abe, Yuichi Matsushita, Masakazu Iwamoto, Motoi Kawatsura, Shuichi Hayase, Toshiyuki Itoh*
Department Materials Science, Faculty of Engineering, Tottori University, Tottori 680-8552, Japan.
E-mail: titoh@chem.tottori-u.ac.jp

Various types of differently fluorinated alkyl sulfate ionic liquids have been prepared; the hydrophobicity was dependent on the content ratio of the fluorine on the alkyl sulfate anion and 1-butyl-3-methylimidazolium 2,2,3,3,4,4,5,5-octafluoropentyl sulfate (Bm-C5F_8) showed hydrophobic properties. Efficient lipase-catalyzed transesterification was demonstrated using hydrophobic alkyl sulfate imidazolium salt (Bm-C5F_8) (Fig. 35).

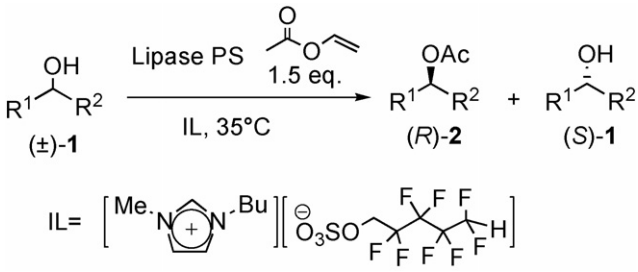


Fig. 35. Lipase-catalyzed asymmetric transesterification using imidazolium highly fluorinated alkyl sulfate as a reaction medium.

Reactivity of methyltransferase involved in biosynthesis of chlorophyllous pigments in photosynthetic green sulfur bacteria

Jiro Harada[a], Shigeaki Osumi[a], Yoshitaka Saga[b], Hirozo Oh-oka[c], Hitoshi Tamiaki[a,*]

[a]Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

[b]Department of Chemistry, School of Science and Engineering, Kinki University, Higashi-Osaka, Osaka 577-8502, Japan

[c]Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan.

E-mail: tamiaki@se.ritsumei.ac.jp

We observed in vitro enzymatic activity of BchU which catalyzed methylation at the C-20 of a chlorin moiety in biosynthetic pathway of bacteriochlorophyll *c*, and concluded that this enzyme had a broad substrate specificity (Fig. 36).

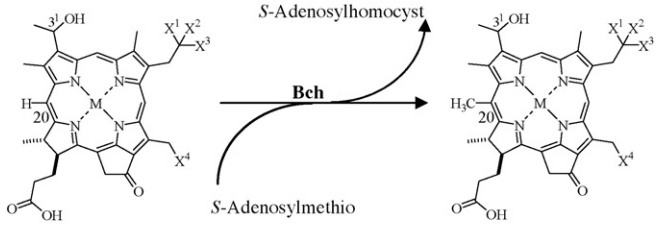


Fig. 36. BchU-catalyzed methylation of bacteriochlorophyllide-*d*.

Biotransformation of 4-oxoisophorone by cultured plant cells of *Marchantia polymorpha*

Mohamed-Elamir F. Hegazy[a], Yuya Sato[a], Miki Otsuka[a], Chika Kuwata[a], Toshihiko Iwasaki[a], Akihito Matsushima[b], Toshifumi Hirata[a,*]

[a]Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan

[b]Natural Science Center for Basic Research and Development, Hiroshima University, Kagamiyama, Higashi-Hiroshima 739-8526, Japan.

E-mail: thirata@sci.hiroshima-u.ac.jp

Biotransformation of 4-oxoisophorone by cultured cells of *Marchantia polymorpha* gave chiral ketones and alcohols (Fig. 37).

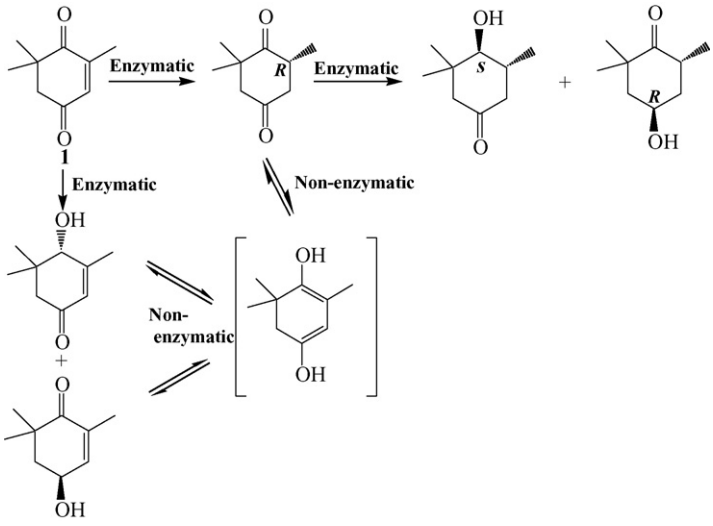


Fig. 37. Biotransformation of 4-oxoisophorone (**1**) by the cultured plant cells.

Microbial asymmetric oxidation of 2-butyl-1,3-propanediol

Koichi Mitsukura, Takanori Uno, Toyokazu Yoshida, Toru Nagasawa*
Department of Biomolecular Science, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan.
E-mail: tonagasa@biomol.gifu-u.ac.jp

Through oxidation of 2-butyl-1,3-propanediol by *Acetobacter pasteurianus* cells, an effective production of (*S*)-2-hydroxymethylhexanoic acid with high optical purity was achieved (Fig. 38).

Fig. 38. Oxidation of 2-butyl-1,3-propanediol by *A. pasteurianus* cells.

Dynamic kinetic resolution of racemic allyl alcohols by the combined use of lipases and $VO(OSiPh_3)_3$

Shuji Akai[a,*], Yukiko Kanao[b], Kouichi Tanimoto[b], Masahiro Egi[a], Tomoko Yamamoto[a], Yasuyuki Kita[b]
[a]School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[b]Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan.
E-mail: akai@u-shizuoka-ken.ac.jp

An unprecedented combination of lipases and $VO(OSiPh_3)_3$ **1** has achieved a new type of dynamic kinetic resolution of racemic allyl alcohols (±)-**2**. The vanadium compound **1** catalyzed the 1,3-transposition of **2** to give thermodynamic mixtures of two regioisomers (**2** and **4**) with racemization, of which the lipases carried out the enantio- and chemo-selective esterification to give (*R*)-allyl acetates **5** (78–99% e.e.) in 74–96% yields (Fig. 39).

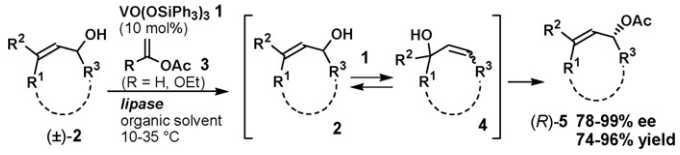


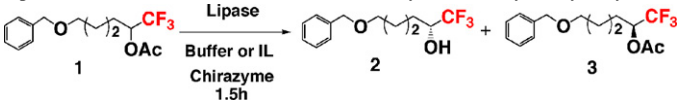
Fig. 39. Dynamic kinetic resolution of (±)-**2** using lipases and $VO(OSiPh_3)_3$ **1**.

Simple preparation of optically active fluorinated liquid crystal elements using lipase

Yumiko Takagi[a,*], Fumi Yamana[a], Tsutomu Ogawa[a], Toshiyuki Itoh[b]
[a]Department of Chemistry, Faculty of Education, Kagawa University, 1-1 Saiwai-cho, Kagawa 760-8522, Japan
[b]Department of Material Science, Faculty of Engineering, Tottori University, 4-101 Koyama-minami, Tottori 680-8552, Japan.
E-mail: ytakagi@ed.lkagawa-u.ac.jp

Preparation of optically pure bis(trifluoromethyl)alkanediols through lipase-catalyzed hydrolysis of corresponding diacetates has been demonstrated. Using optically active bis(trifluormethyl)-alkanediols as starting materials, synthesis of novel dimer type AFCLs has been progressed (Table 3).

Table 3
Optical resolution of 1,1,1-trifluoro-2-alkanol by CAL-catalyzed hydrolysis reaction



Entry	Solvent	Conversion (%)	% e.e. of **2**	% e.e. of **3**	*E*
1	Buffer pH 7.2	36	99	57	296
2	Buffer pH 6.5	30	99	59	427
3	Buffer pH 5.8	44	99	58	258
4	[bmim][PF_6] + H_2O (1.5 eq)	29	96	39	66
5	IL-4+ pH 7.2 (1.5 eq)	40	99.9	53	>2000

Quantitative structure–reactivity relationships of the reduction of acetophenone derivatives by rat liver 3α-HSD

Koji Uwai, Noboru Konno, Yuka Yasuta, Yukiko Nakashige, Mitsuhiro Takeshita*
Department of Pharmaceutics, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan.
E-mail: mtake@tohoku-pharm.ac.jp

This study demonstrated the importance of the electron-withdrawing effect in the determination of formation rate in enzymatic reduction of 4′-substituted acetophenone derivatives by rat liver 3α-HSD (Fig. 40).

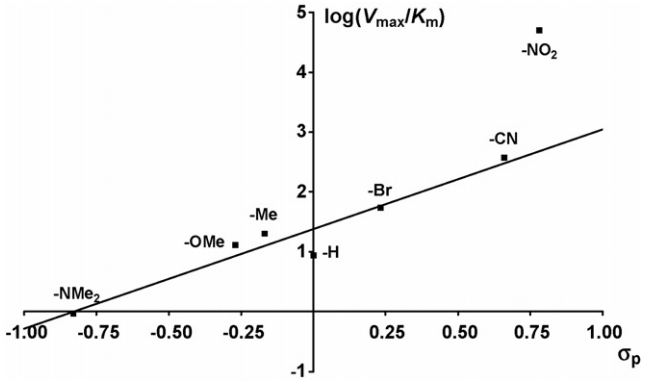


Fig. 40. Correlation between log V_{max}/K_m values and s_p in the reduction of 4′-substituted acetophenone derivatives by rat liver 3α-HSD.

Mechanism of action of *Burkholderia cepacia* lipase: Thermodynamic and proton inventory study in acylation step

Yuki Yoshimura, Tomoaki Yokota, Atsushi Tanikawa, Yoshinori Inoue, Hideo Hirohara*
Department of Materials Science, The University of Shiga Prefecture, 2500 Hassaka-cho, Hikone 522-8533, Japan.
E-mail: hirohara@mat.usp.ac.jp

We have examined kinetics, thermodynamics and solvent isotope effects for acylation step in *Burkholderia cepacia* lipase-catalysed hydrolysis (Fig. 41).

$$ {}^{n}k = {}^{D_2O}k \frac{\prod_{i}^{x} (1 - n + n\phi_i^T)}{\prod_{i}^{x} (1 - n + n\phi_i^R)} Z_k^{-n} $$

$$ \Downarrow $$

$$ {}^{n}k = {}^{D_2O}k \prod_{i}^{x} (1 - n + n\phi_i^T) Z_k^{-n} $$

Fig. 41. All proton inventory studies reflected on transition state.

Mechanism of action of *Candida antarctica* lipase B: Thermodynamics and proton inventory study of monochloroacetate

Nozomi Ito, Yasuyuki Shimomachi, Atsushi Tanikawa, Yoshinori Inoue, Hideo Hirohara*
Department of Materials Science, The University of Shiga Prefecture, 2500 Hassaka-cho, Hikone 522-8533, Japan.
E-mail: hirohara@mat.usp.ac.jp

We have studied the thermodynamics and solvent isotope effects of *Candida antarctica* lipase B (CALB)-catalyzed hydrolysis of monochloroacetic acid esters and compared with those of the corresponding acetic acid esters (Fig. 42).

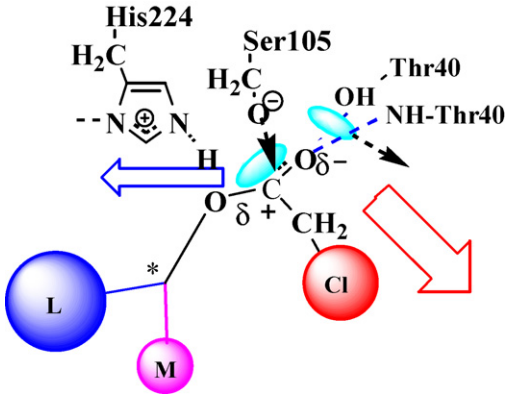


Fig. 42. Transition state of monochloroacetic acid ester.

Cell density dependent production and controlled molecular structure of poly(ε-L-lysine) in 10 novel producers of *Streptomyces* sp.

Masayuki Saimura*, Masahiro Miyamoto, Atsushi Ikezaki, Munenori Takehara, Hideo Hirohara
Department of Materials Science, The University of Shiga Prefecture, 2500 Hassaka-cho, Hikone 522-8533, Japan.
E-mail: hirohara@mat.usp.ac.jp

We found USE-11 is the most advantageous strain to isolate poly(ε-L-lysine) synthesizing enzymes and its encoding genes (Table 4).

Table 4
Production of poly(ε-L-lysine) by newly isolated *Streptomyces* strains

Strain	P_n	M_n	M_w/M_n	Yield (g/l)	Production of poly(ε-L-lysine)	
					Regulation	Rate-determining step
USE-11	15–35	3500 ± 60	1.03	4.0	Independent of quorum sensing regulation	Lys supply
USE-12	14–35	3500 ± 80	1.03	2.0		Lys supply
USE-13	14–36	3500 ± 20	1.03	2.5		Lys supply
USE-31	15–30	2840 ± 60	1.03	1.5		Polymerization
USE-32	10–29	2720 ± 70	1.03	1.0		
USE-54	10–24	2350 ± 50	1.03	0.3	Dependent upon quorum sensing regulation	Polymerization
USE-51	10–23	2150 ± 10	1.03	0.6		Polymerization
USE-52	19–24	2150 ± 30	1.03	0.4		Polymerization
USE-81	19–18	1740 ± 20	1.03	0.8		Lys supply
USE-82	19–18	1780 ± 20	1.03	4.0	Weakly dependent	Lys supply

Molecular cloning and characterization of a novel hydrolase of aromatic carboxylic acid esters from a *Bacillus* strain

Masahiro Miyamoto, Kaori Kinoshita, Yoshinori Kawasaki, Munenori Takehara, Hideo Hirohara*
Department of Materials Science, The University of Shiga Prefecture, 2500 Hassaka-cho, Hikone 522-8533, Japan.
E-mail: hirohara@mat.usp.ac.jp

The gene coding for a novel esterase which hydrolyzes not only aromatic carboxylic acid esters but also esters of short chain fatty acids was cloned from *Bacillus* sp. SP-04 and expressed in *Escherichia coli* (Table 5).

Table 5
Hydrolytic activity of the esterase purified from the strain SP-04

Substrate	Specific activity (μM min^{-1} mg^{-1})
Ethyl benzoate	60
Diethyl terephthalate	61
Ethyl acetate	49
Ethyl propionate	98
Ethyl *n*-butanoate	79
Ethyl *n*-hexanoate	52

4 mM carboxylate, 25 mM Tris–HCl (pH 8.0), 5% DMSO, 35 °C.