Introduction
The symposium on the chemistry of biocatalysis was held on 11 and 12 December 2002 in Hokkaido. Approximately 98 researchers from industry and academic world and 51 students participated to the symposium. We had the following program:

Special lecture: Professor S. Kinoshita (Graduate School of Engineering, Hokkaido University, Japan); “Biological degradation of synthetic polymers,” Professor H. Sekizaki (Faculty of Pharmaceutical Science, Health Sciences University of Hokkaido, Japan); “Food waste utilization on drug preparations—biocatalysts investigation in food and industrial waste.”

Oral and poster presentation (9 presentations and 49 posters): We enjoyed oral and poster presentations as well as discussions.


Symposium organizer: Harumi Kaga
We thank assistant professor K. Ishihara (Okayama University of Science) for his help in summarizing this abstracts.

Yasuhisa Asano, Editor

Oral Presentations

Lipase-catalyzed reactions at extreme temperatures from −40 to 120 °C
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Fig. 1. Lipase-catalyzed reactions at extreme temperatures.

doi:10.1016/j.molcatb.2004.03.011
Biocatalytic reactions in supercritical carbon dioxide

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Asymmetric reduction of ketones using an alcohol dehydrogenase, kinetic resolution of an alcohol using a lipase, and carboxylation of pyrrole using a decarboxylase in supercritical carbon dioxide were investigated (Fig. 2).

Asymmetric reduction of ketones using an alcohol dehydrogenase, kinetic resolution of an alcohol using a lipase, and carboxylation of pyrrole using a decarboxylase in supercritical carbon dioxide were investigated (Fig. 2).

Poster Presentations

Ability of different biomaterials to enantioselectively catalyze oxidation and reduction reactions
Hiroyuki Nagaoka\textsuperscript{*}
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The pH of reaction medium can determine the direction of NAD(P)\textsuperscript{+}-dependent secondary alcohol dehydrogenase (NAD(P)-E) from immobilized water-soluble biomaterials (e.g. plant leaf, cereal tissues, and vegetables), toward enantioselectively catalyzed oxidation (pH $> 7.0$) or reduction reaction (pH $< 7.0$) (Fig. 3).

Production of N\textsuperscript{a}-Z-aminoadipate-\delta-semialdehyde from N\textsuperscript{a}-Z-lysine with amine oxidase from Aspergillus niger
Kimiyasu Isobe\textsuperscript{a*}, Keigo Tokuta\textsuperscript{a}, Yuuki Narita\textsuperscript{a}, Akira Matsuura\textsuperscript{b}, Takehiko Sakaguchi\textsuperscript{b}, Norio Wakao\textsuperscript{b}
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Production of N\textsuperscript{a}-Z-aminoadipate-\delta-semialdehyde from N\textsuperscript{a}-Z-lysine with amine oxidase from Aspergillus niger

Kimiyasu Isobe\textsuperscript{a*}, Keigo Tokuta\textsuperscript{a}, Yuuki Narita\textsuperscript{a}, Akira Matsuura\textsuperscript{b}, Takehiko Sakaguchi\textsuperscript{b}, Norio Wakao\textsuperscript{b}
\textsuperscript{a}Department of Agro-bioscience, Iwate University, 3 Ueda, Morioka 020-8550 Japan
\textsuperscript{b}Sanyo Fine Co., Ltd., 1 Hiranomachi, Chuo-ku, Osaka 541-0046, Japan. E-mail: kiso@iwate-u.ac.jp
More than 95% of \( \text{N}^2\text{-Z-l-lysine} \) and \( \text{N}^2\text{-Z-d-lysine} \) were oxidized by amine oxidase from *Aspergillus niger* to \( \text{N}^2\text{-Z-l-amino-adipate-δ-semialdehyde} (\text{N}^2\text{-Z-l-AASA}) \) and \( \text{N}^2\text{-Z-d-AASA} \), respectively, in the presence of catalase (Fig. 4).

![Fig. 4. Oxidation of \( \text{N}^2\text{-Z-l-lysine} \) and \( \text{N}^2\text{-Z-d-lysine} \) by amine oxidase from *Aspergillus niger*.](image)

**Novel serine protease from earthworm: catalytic functions and application (part IV)**

Nobuyoshi Nakajima\(^*\), Kohji Ishihara\(^*\)

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Novel serine proteases, purified from earthworm, were characterized for application of the catalytic functions. The enzymes, composed of six isozymes, acted on various proteins, peptides, ester compounds, and so on. They were very stable and strongly resistant to organic solvents and detergents (Fig. 5).

![Fig. 5. Sequence alignment of the earthworm proteases, isozyme A (A), isozyme C (C), bovine trypsin (BT), and porcine elastase (EL). The numberings shown above and across the sequence are based on those of the chymotrypsinogen A and the active earthworm proteases, respectively. The amino acid residues of the catalytic triad are represented by reversal letters. The primary substrate specificity determinant and the subsites, S1, S2, and S3 are indicated by asterisks.](image)

**Enzymatic conversion of bioactive compounds (part V): stabilization and functionalization of naturally occurring plant pigments**

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The regioselective acylation of flavonoid glucosides was achieved by lipase-catalyzed transesterification in dry organic solvent. The participation of the acyl group in flavonoid glucoside molecules resulted in increasing of the physiological function (thermostability and light-resistibility) of the acylated flavonoid glucosides (Fig. 6).

![Fig. 6. Lipase-catalyzed esterification of flavonoid glucoside.](image)
Stability of catalytic antibodies immobilized on mesoporous silicates
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Catalytic antibodies (38C2 and 84G3) were immobilized on various mesoporous silicates (FMS, PESO, SBA). These silicates had similar adsorption ratios to two antibodies (Fig. 7).

Fig. 7. Immobilization ratios of catalytic antibody 84G3 on mesoporous silicates.

Biocatalytic reduction of ketones by a semi-continuous flow process using supercritical carbon dioxide
Tomoko Matsuda a,∗, Kazunori Watanabe a, Takashi Kamitanaka a, Tadao Harada a, Kaoru Nakamura b
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The immobilized resting-cell of Geotrichum candidum was used as a catalyst for the reduction of a ketone in a semicontinuous flow process using supercritical carbon dioxide for the first time; it was also applied for the asymmetric reduction of a ketone and resulted in excellent enantioselectivity (>99% e.e.) and a higher space-time yield than that of the corresponding batch process (Fig. 8).

Fig. 8. Biocatalytic reduction of ketones by a semi-continuous flow process using supercritical CO2.

Lipase-catalyzed reaction in an ionic liquid solvent system
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1-Butyl-2,3-dimethylimidazolium tetrafluoroborate ([bdmim][BF4]) was found to be an excellent solvent to realize a lipase-recycling system using vinyl acetate as acyl donor; no accumulation of an acetaldehyde oligomer was observed in this solvent system and it was possible to use the lipase repeatedly ten times while still maintaining perfect enantioselectivity and high reactivity (Fig. 9).
Asymmetric hydrogenation of N-substituted maleimides by the cultured plant cells
Asuka Takarada\textsuperscript{a}, Yuya Sato\textsuperscript{a}, Toshifumi Hirata\textsuperscript{a,}\textsuperscript{∗}, Akihito Matsushima\textsuperscript{b}, Yoko Kondo\textsuperscript{a}, Hiroki Hamada\textsuperscript{a}
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\textsuperscript{b}Natural Science Center for Basic Research and Development, Hiroshima University, Kgamiyama, Higashi-Hiroshima, Hiroshima 739-8526, Japan

Hydrogenation of 2-methyl-N-phenylmaleimide by the cultured cells of \textit{Nicotiana tabacum} and \textit{Marchantia polymorpha} was highly enantiospecific to give (R)-2-methyl-N-phenyl succinimide (Fig. 10).

Construction of a functionalized oligosaccharide library by using a transition state analogue as a glycosyl donor
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Department of Biochemistry and Bioengineering, Tohoku University, Japan. E-mail: kohri@poly.che.tohoku.ac.jp

An efficient enzymatic synthesis of functionalized oligosaccharides (oligo-N-acetyl lactosamides) has been achieved by means of chitinase-catalyzed transglycosylation utilizing sugar oxazolines, a transition state analogue for chitinase (Fig. 11).
A correlation between conformational flexibility of enzymes and steric effects of substrates for subtilisin-catalyzed transesterification

Masato Date a, Ken Umemura a, Naomi Hiroshima a, Shin-ichi Ueji b,∗

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A correlation between optimum conformational flexibility of enzymes induced by the addition of dimethyl sulfoxide and Es parameter (the scale of steric effects of substrates) was found for subtilisin-catalyzed transesterification of the racemic esters in i-octane (Fig. 12).

![Fig. 12. A correlation between conformational flexibility of enzymes and Es parameter of substrates.](image)

A remarkable improvement of enantioselectivity of lipase in organic solvents by use of lipase coated with phosphates

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The enantioselectivity of lipase-catalyzed esterification of 2-(4-ethylphenoxy) propionic acid was found to be dramatically enhanced by using lipase coated with phosphates, which was prepared by lyophilizing lipase in the presence of phosphates (Table 1).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Time (h)</th>
<th>Conversion (%)</th>
<th>e.e. (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native lipase VII</td>
<td>29</td>
<td>37.6</td>
<td>84.8</td>
<td>20</td>
</tr>
<tr>
<td>Lipase VII coated with sodium phosphate dibasic</td>
<td>24</td>
<td>38.4</td>
<td>99.2</td>
<td>449</td>
</tr>
<tr>
<td>Lipase VII coated with sodium tripolyphosphate</td>
<td>24</td>
<td>37.5</td>
<td>97.0</td>
<td>170</td>
</tr>
<tr>
<td>Lipase VII coated with DNA sodium salt</td>
<td>117</td>
<td>37.4</td>
<td>91.6</td>
<td>40</td>
</tr>
</tbody>
</table>

Effect of metal cations on the enantioselectivity of crown ether modified lipase in organic media

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For the esterification of 2-(4-substituted phenoxy) propionic acids with 1-butanol catalyzed by the 18-crown-6 ether modified lipase in disopropyl ether, its enantioselectivity was found to be most significantly enhanced by addition of KCl among the other alkali metal ions used here (Fig. 13).
A new method to improve the functions of lipase, based on the combination of chemical modification and refolding of lipase

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A new method was tried to improve the functions of lipase, based on the combined effects of the chemical modification and the refolding of lipase, thus leading to the enhancement of the enantioselectivity of the lipase-catalyzed hydrolysis of butyl 2-(4-substituted phenoxy) propionates, the enantioselectivity enhancement of which can be attributed to the conformational change of the lipase estimated from the result of the CD and FT-IR spectra (Fig. 14).

Microbial deracemization of α-substituted carboxylic acids—optimization of reaction conditions and mechanistic investigations using cell-free extract

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Through the investigation of whole cell system and cell-free system including the study of inhibitors and the detection of intermediates, we could obtain the supporting evidences that deracemization is a competitive reaction against the fatty acid metabolism and proceeds via a part of β-oxidation pathway (Fig. 15).
Enzymatic decarboxylation of phenylmalonates with a hydrophilic α-substituent
Keisuke Tamura, Yosuke Terao, Kenji Miyamoto, Hiromichi Ohta∗
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Arylmalonate decarboxylase (AMDase) catalyzed asymmetric decarboxylation of α-phenylmalonates with a hydrophilic substituent (OH, NH₂) at α-position, and these substrates exhibited characteristic “pH-rate of reaction” profiles compared to that of phenylmalonate (Table 2).

Table 2
AMDase-catalyzed reaction profiles of phenylmalonates with an α-substituent (R=R)

<table>
<thead>
<tr>
<th>Substituent</th>
<th>HPLC yield (%)</th>
<th>Isolated yield (%)</th>
<th>e.e. (%)</th>
<th>Configuration</th>
<th>Optimum pH</th>
<th>Kₘ (mM)</th>
<th>kₗ (s⁻¹)</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8.5</td>
<td>11.9</td>
<td>353</td>
<td>100</td>
</tr>
<tr>
<td>Me</td>
<td>99</td>
<td>99</td>
<td>R</td>
<td>NT</td>
<td>25.5</td>
<td>29.8</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>OH</td>
<td>98</td>
<td>92</td>
<td>96</td>
<td>R</td>
<td>7.0</td>
<td>1.88</td>
<td>2.45</td>
<td>5.1</td>
</tr>
<tr>
<td>NH₂</td>
<td>97</td>
<td>71</td>
<td>96</td>
<td>R</td>
<td>9.5</td>
<td>31.2</td>
<td>1.73</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Production of chiral alcohols by enantioselective reduction with phenyl trifluoromethyl ketone reductase (PTKR) from Leifsonia sp. S749
Kousuke Inoue∗, Yoshihide Makino, Nobuya Itoh
Biotechnology Research Center, Faculty of Engineering, Toyama Prefectural University, Kurokawa 5180, Kosugi, Toyama 939-0398, Japan. E-mail: itoh@pu-toyama.ac.jp

Chiral alcohols were synthesized with high enantioselectivity and molar yield by using E. coli cells efficiently expressing the ptkr gene from Leifsonia sp. S749, and the reaction proceeded without the NADH-regeneration system because PTKR could reproduce NADH in the presence of 2-propanol (Fig. 16).
Analysis of polymerization and phosphorolysis of α-glucans by glycogen phosphorylases using quartz crystal microbalances

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We kinetically analyzed the reaction catalyzed by glycogen phosphorylases (GP), that is, the binding of GP to the substrates such as amylopectins and the resulting both polymerization and/or phosphorolysis, using quartz crystal microbalances (Fig. 17).

Fig. 17. Reaction scheme of glycogen phosphorylase.

Asymmetric synthesis of 2-substituted 4-chromanones: synthesis of chiral intermediates by lipase-catalyzed reactions

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(R)- and (S)-2,6-dimethyl-4-chromanone were prepared from the chiral intermediates which were obtained by lipase-catalyzed reactions (Fig. 18).

Fig. 18. Kinetic resolution of 3-(4-methylphenoxyl)butanoic acid.

Degradation of bisphenol A by plants and cyanobacteria

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The degradation of 2,2-bis(4-hydroxyphenol)propane (BPA, 1), a representative endocrine disruptor, was studied with cyanobacteria and plants as a biocatalyst (Table 3).
Table 3
Screening of biocatalysts on the degradation of BPA

<table>
<thead>
<tr>
<th>Biocatalysts</th>
<th>Time (days)</th>
<th>Bisphenol A Concentration (ppm)</th>
<th>Degradation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanobacteria</td>
<td>Synechococcus elongatus</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>Synechocystis sp.</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Plants</td>
<td>Alpinia zerumbet</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Plants</td>
<td>Canavaria gladiata</td>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td>Plants</td>
<td>Rhizophora stylosa</td>
<td>14</td>
<td>10</td>
</tr>
</tbody>
</table>

The degradation of di(2-ethylhexyl) phthalate by cultured plant cells and cyanobacterium

Masako Suzuki a, Wen Chai a, Nakahide Katoh a, Kaoru Nakamura a, C. Akira Horiuchi a,∗

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The di(2-ethylhexyl) phthalate (DEHP) disappeared in the solution after several days of incubation, but a few amount of DEHP remained in the cells of Caragana chamlagu and cyanobacterium (Fig. 19).

Fig. 19. Degradation of DEHP by C. chamlagu and Synechococcus elongatus PCC 7942.

Biotransformation of diketones by plant cultured-cells (part 4)

Wen Chai a, Yoshitomo Matsura a, Hiroshi Sakamaki a, C. Akira Horiuchi a,∗

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The oxidation of hydroxythymobenzoquinones using some kinds of plant cultured-cells was investigated, and it was found that the biotransformation of 3-hydroxythymo-1,4-benzoquinone (1) by M. polymorpha gave the γ-hydroxybutenolide (1a, 60%) as major products (Fig. 20).

Fig. 20. Biotransformation of hydroxythymobenzoquinones by plant cultured-cells.
Stereoselective oxidation of the sulfur compounds by rat liver S-9 fraction
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Department of Pharmaceutical Sciences, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan. E-mail: mtake@tohoku-pharm.ac.jp

The stereoselective oxidation of sulfide (1) and β-hydroxy sulfur compounds (3 and 5) by rat liver S-9 fraction were investigated (Fig. 21).

![Fig. 21. Enantioselective oxidation of sulfur compounds by rat liver S-9 fraction. Each step was started with racemic material.](image)

Syntheses of biologically active substances such as insect pheromones by use of a FPP synthase
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In order to develop the synthetic methods of such kinds of isoprenoid pheromones, we examined substrate specificity of FPP synthase from Bacillus steatothermophilus with respect to allylic substrate homologs with a hydrophilic group at w-position (Fig. 22).

![Fig. 22. A thermostable FPP synthase reaction and Danaus Chryippus sex insect pheromone.](image)

Pseudomonas cepacia lipase (PCL)-catalyzed hydrolysis of acetates of single enantiomers of secondary alcohols: origin of high enantioselectivity in rate-determining step
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Thermodynamic analysis of PCL-catalyzed hydrolysis of acetates of single enantiomers of secondary alcohols indicated a substrate having high $E$ value showed no enthalpy–entropy compensation in the transition state (Table 4).
Table 4
Thermodynamic parameters for $E$ value of PCL-catalyzed hydrolysis

<table>
<thead>
<tr>
<th>$\Delta H_{R,S}$</th>
<th>$T\Delta S_{R,S}$</th>
<th>$\Delta G_{R,S}$</th>
<th>$E$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$-4.9$</td>
<td>$0.4$</td>
<td>$-5.2$</td>
<td>High</td>
</tr>
<tr>
<td>$-6.5$</td>
<td>$-4.1$</td>
<td>$-2.4$</td>
<td>Low</td>
</tr>
</tbody>
</table>

Pseudomonas cepacia lipase (PCL)-catalyzed hydrolysis of acetates of single enantiomers of primary alcohols: enantioslectivity of primary alcohols in the acylation step

Tomoaki Yokota, Seiji Shinohara, Yoshinori Inoue, Hideo Hirohara*
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From the kinetics and thermodynamics of the acylation step of Pseudomonas cepacia lipase (PCL)-catalyzed hydrolysis of acetates of single enantiomers of primary alcohols, we discussed the difference of the mechanism of the enantioselectivity between the primary and the secondary alcohols (Fig. 23).

Mechanism of Candida antarctica lipase B (CALB)-catalyzed hydrolysis of acetates of primary alcohols: change of the rate-determining step by substituents

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

We have examined kinetics and thermodynamics of Candida antarctica lipase B (CALB)-catalyzed hydrolysis of the acetates of single enantiomers of primary alcohols to discuss the mechanism of action of the enzyme (Fig. 24).
Fungal chitinases coproduced with insect chitinase inhibitors
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Faculty of Agriculture, Okayama University, Okayama 700-8530, Japan. E-mail: nitoda@cc.okayama-u.ac.jp

A fungal strain TNPT116-Cz producing a chitinase inhibitor FPS-1 was found to express chitinase simultaneously, which could not be inhibited by FPS-1 (Fig. 25).

Fig. 25. The relationship between FPS-1 and chitinase coproduced by TNPT116-Cz.

Characterization of cyclo (Leu–Phe) dehydrogenase from an actinomycete Streptomyces albulus KO23
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Purified cyclo (Leu–Phe) dehydrogenase from Streptomyces albulus KO23 was characterized in detail, and was found to catalyze all reactions involved in the conversion of cyclo (Leu–Phe) to albonoursin (Fig. 26).

Fig. 26. Dehydrogenation by Streptomyces albulus KO23.

Lipase-catalyzed domino dynamic-kinetic-resolution of racemic alcohols/intramolecular Diels–Alder reaction
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A combined use of a lipase and a ruthenium catalyst (2a or b) achieved the first domino process that involved the dynamic kinetic resolution of (±)-1 with 3 and the intramolecular Diels–Alder reaction of the resultant 4 to directly provide polysubstituted decalines 5 with up to 95% e.e. in 81% isolated yield (Fig. 27).

Fig. 27. Lipase-catalyzed domino dynamic-kinetic-resolution of racemic alcohols/intramolecular Diels–Alder reaction.
Enzymatic hydrolysis of cyclic carbonates bearing a methyl group
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_Pseudomonas diminuta_ efficiently catalyzed the hydrolysis of the cyclic carbonates bearing a methyl group to obtain optically active diols (Fig. 28).

![Fig. 28. Enantioselective hydrolysis of cyclic carbonates by a bacterium.](image)

Phytoremediation of endocrine disturbing of plant cultured cells—biotransformation of BPA and BZP
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We have investigated the biotransformation of organic compound by plant suspension cells. In this study we study the biotransformation of bisphenol A and benzophenone by plant suspension cells and it was found that plant suspension cells glycosylate the hydroxyl group of bisphenol A and benzophenone (Fig. 29).

![Fig. 29. Biotransformation of BPA and BZP by the cultured cells of _Eucalyptus perriniana_.](image)

Development of functional foods by plant cultured cells: biotransformation of vanillin
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We have investigated the biotransformation of organic compound by plant cultured cells. In this study we study the biotransformation of vanillin by plant cultured cells. In the paper we report that the plant cultured cells have glycosylation and hydroxylation ability in the biotransformation of vanillin (Fig. 30).

![Fig. 30. Biotransformation of vanillin by _Eucalyptus perriniana_.](image)
Development of functional foods by cultured cells of Catharanthus roseus: biotransformation of curcumin
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We have investigated the biotransformation of organic compound by plant cultured cells. In this study we study the biotransformation of curcumin by the cultured cells of C. roseus and it was found that the cultured cells of C. roseus glycosylate the hydroxyl group of curcumin (Fig. 31).

Fig. 31. Biotransformation of curcumin by C. roseus.

Simple preparation of optically pure trifluoromethylalkanol through lipase catalyzed reaction
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We report the results of lipase-catalyzed hydrolysis reaction of diacetates of bis(trifluoromethyl)alkanediols and synthesis of novel liquid crystal molecules which possesses chiral bis(trifluoromethyl)alkanol moieties and aromatic core structure at the center of the molecular flame (Fig. 32).

Fig. 32. Enzymatic hydrolysis of PEG-tagged carbonates.

Enzyme-mediated enantioselective hydrolysis of PEG-tagged carbonates
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PEG-tagged substrates, which had some unique properties, were hydrolyzed by PPL with enantioselectivity. The resulting optically active sec-alcohols were easily separated from the substrates (Fig. 33).

Fig. 33. Optical resolution of 1,1,1-trifluoromethyl-2-alkanol by CAL-catalyzed enantioselective hydrolysis.

A process for producing l-arabinose-containing syrup by treating the beet pulp and the effect on the elevation of blood glucose in rats
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l-Arabinose-containing syrup could be economically produced by treating the beet pulp directly with an enzyme and the elevation of blood glucose after sucrose ingestion in rats was significantly suppressed by adding the syrup (Fig. 34).

Fig. 34. Blood glucose after sucrose ingestion in rats. (■) Sucrose; (●) sucrose + l-arabinose-containing syrup.