The evolution of biotransformation technologies

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Biotransformation is a broad and growing field of biotechnology and encompasses both enzymatic and microbial biocatalysis. Progress has been made in research on the key drivers of biotransformations, including the isolation and characterization of microbes and their enzymes from, and their utilization in, extreme environments, the manipulation, alteration, and augmentation of metabolic pathways, and the use of combinatorial biosynthesis and biocatalytic methodologies for new compound development.

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Abbreviation
PKS polyketide synthase

Introduction

When compared to their chemical counterparts, biocatalysts are exquisitely selective and highly reactive over a broad range of operating conditions. Moreover, whole microbial cells (primarily bacterial and fungal) and their catalytic machinery (e.g. enzymes and metabolic pathways) can accept a wide array of complex molecules as substrates, yielding products with unparalleled chiral (enantio-), positional (regio-), and chemical (chemo-) selectivities. Such high selectivity affords efficient reactions with few byproducts, thereby making biocatalysts an environmentally friendly alternative to conventional chemical catalysts. As a result of high selectivity, biocatalysts can be used in both simple and complex transformations without the need for tedious blocking and deblocking steps that are commonplace in enantioselective and regioselective organic synthesis.

History is repleat with examples of biotransformations. Ancient civilizations relied upon animal, plant, and microbial biocatalysts for the preparation of meat and dairy products. Today, biocatalysts are routinely used in the food industry and are increasingly becoming commonplace in the pharmaceutical and chemical industries for the synthesis of fine and specialty chemicals and intermediates. The tremendous synthetic potential of biocatalysts is most poignantly demonstrated in the complex structures of natural products. These compounds consist of an intricate array of stereochemistries coupled with a multitude of functionalities placed at critical locations throughout the molecule. A large number of natural products are microbial in origin and a powerful arsenal of enzymes and metabolic pathways are employed in their synthesis.

Advances in biotransformations during the past year continue to highlight the tremendous potential of biocatalysis in the synthesis of compounds of interest to the chemical and pharmaceutical industries. The aim of this review is not to provide a compendium of recent biotransformations — these exist elsewhere [1]. Rather, key technologies that are quickly shaping the future of biotransformations will be discussed. These technologies along with others form a major focus of this *Current Opinion in Microbiology* issue (see Hutchinson, pp 319–329; Nielsen pp 330–336; Horikoshi, pp 291–295), and their present and future impact on biotransformation technologies will be discussed.

Key biotransformation technologies of the future

Until recently, scientists wishing to utilize biotransformations needed to rely upon the availability (via traditional screening efforts) of a particular organism or isolated enzyme to catalyze a desired reaction or synthesis of a particular product. Moreover, it was necessary to utilize reaction conditions that were predominantly aqueous in character. Although such approaches remain critical today, new technologies have made an impact on our ability to utilize, tailor, and develop new biotransformations. These technologies include the isolation, characterization, and utilization of microbes and their enzymes from extreme environments; the manipulation, alteration, and augmentation of metabolic pathways, and the use of combinatorial biosynthesis and biocatalytic methodologies for new compound development. These key technologies and the recent progress made in these areas are further described below.

New biocatalyst development

Extremophiles and their enzymes

The biocatalyst lies at the heart of a biotransformation. It is not surprising, therefore, to note a great interest during the past year in the development of new and potentially useful biocatalysts either as whole cells (along with their metabolic pathways) or as isolated enzymes. One of the most promising research directions involves the isolation, characterization, and utilization of organisms that grow in harsh environments (so called 'extremophiles') [2•]. Table 1 lists several of the more important classes of extremophiles along with their unique growth requirements. Because many of these environments are similar to those found in chemical processes, the potential of biotechnology employing extremophiles has attracted

the attention of microbiologists, biochemists, chemical engineers, and synthetic chemists due to the ability of extremophiles to perform biotransformations under harsh conditions. Moreover, enzymes isolated from these organisms may show particular promise as biocatalysts for chemical transformations, some of which may be used to replace existing chemical routes that suffer from multiple reaction steps, formation of undesirable (and often polluting) byproducts, and costly processing.

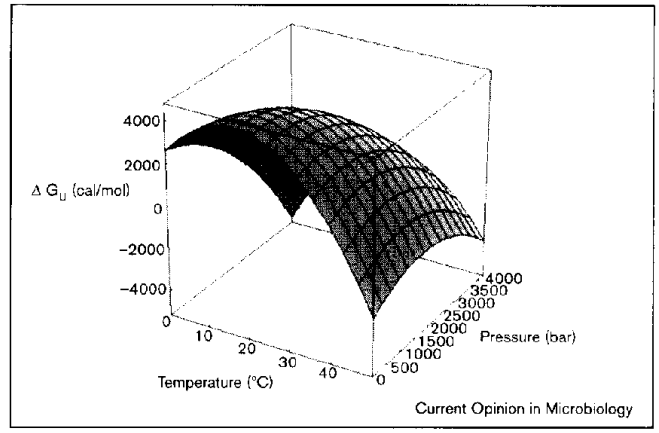
Table 1

Extremophiles and their potential applications.

Extremophilic class	Applications
Hyperthermophiles (growth ≥110°C)	PCR technology Starch and protein hydrolysis Pulp and paper processing Enhanced oil and gas recovery Textile processing Precious metal recovery Coal and oil desulfurization Bioremediation Biosensors
Psychrophiles (growth <0°C)	Waste treatment
Extreme halophiles (growth in saturated NaCl)	Nonaqueous enzymology Bioremediation of brines Enhanced oil recovery
Barophiles (growth ≥250 atm)	Enhanced thermophily Chemical processing Enhanced oil and gas recovery
Acidophiles and alkalophiles (growth down to pH 1 and up to pH 12)	Detergent additives Stonewashing of denim Additives for animal feeds

Particularly interesting are the archaeal hyperthermophiles; the upper temperature limit at which they can grow has still not been established. These organisms, and their isolated enzymes, show potential use in enhanced oil recovery, coal and oil desulfurization, recovery of precious metals, bleaching of paper pulp, starch and protein hydrolysis, and DNA amplification in PCR technologies [3]. Many hyperthermophiles are found in superheated water under pressure. Thus, associated barophily is a common trait exhibited by these organisms and their enzymes. Moreover, recent work has shown that high pressures (>250 atm) help to stabilize enzymes from hyperthermophiles [4••,5]. While the connection between high pressures and high thermostability has yet to be firmly established, there is little question that the conventional notion, that microbial and enzymatic catalysts evolved to function optimally at ambient temperatures and pressures, is quickly being eroded. Indeed, even the conventional mesophilic protein chymotrypsinogen does not show optimal thermostability at 1 atm, even though the protein is mammalian. As shown in Figure 1, the calculated maximum stability of chymotrypsinogen lies well above atmospheric pressure in pressure–temperature space [5].

Figure 1



Calculated pressure–temperature phase diagram for bovine chymotrypsinogen demonstrates that the maximum stability occurs at a pressure and temperature different from the ambient (atmospheric pressure, 25°C). Indeed, maximum stability (most positive ΔG_U) occurs at ≈17°C and 271 atm. Results from M Ru and DS Clark (personal communication), based on the data and equation of state reported by Hawley [38] and elaborated further by Michels *et al.* [5].

Nonaqueous conditions represent a commercially important and fundamentally interesting extreme environment. Such media may appear man-made; however, nature provides examples of extremophiles that exist in low-water environments. Specifically, extreme halophiles (from the Archaea) require nearly saturating NaCl concentrations for optimal growth, and their enzymes quickly denature at salt concentrations below 4 M. While these enzymes require high salt concentrations, presumably to salt-out and hence stabilize the weakly hydrophobic interiors of these proteins, the low-water activity of halophilic environments appears to make these enzymes highly functional in the presence of organic solvents [6]. Indeed, the salting-out capability of NaCl can be largely replaced by salting-out organic solvents such as dimethylformamide and ethylene glycol. Thus, halophilic enzymes may be particularly well suited for biotransformations in organic media. In addition to Archaea, eubacterial microbes have been isolated that possess high tolerance for organic solvents. For example, a variety of *Pseudomonas* sp., *Flavobacterium* sp., *Bacillus* sp., *Arthrobacter* sp., and yeast have been isolated that survive in the presence of up to 50% (v/v) hydrocarbon solvents [7].

Nonaqueous enzyme technology

From a biotechnological perspective there are many potential advantages of employing enzymes in organic as opposed to aqueous media, including higher substrate solubility, reversal of hydrolytic reactions, modified enzyme specificity and improved thermostability. Nonaqueous conditions enable one to tap into novel enzyme activities and selectivities heretofore only possible using genetic modifications or complex multistep pathways. Moreover,

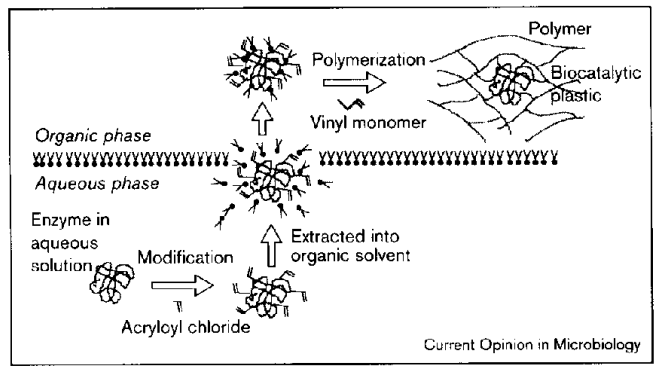
the process improvements attainable in a nonaqueous environment provide critical advantages to biotransformations performed on a large scale. By far the most significant advances have been made with isolated enzymes, where efforts are underway to understand the influence of organic solvent on enzyme structure [8•,9] and to activate and stabilize enzymes in dehydrated media for use in organic synthesis.

One recent advance in nonaqueous enzymology is the development of active and stable homogeneous (soluble) biocatalysts (native enzymes are insoluble in nearly all organic solvents). Solubilization of enzymes in organic solvents, particularly hydrophobic media, requires the covalent or noncovalent modification of the native enzyme. Covalent techniques are well described in the literature (e.g. attachment of polyethylene glycol chains to enzymes [10]). Noncovalent modifications are much less common; however, they are capable of providing highly active and soluble enzyme forms that can be studied spectroscopically in the nonaqueous milieu. For example, protein–lipid complexes soluble in organic solvent have been prepared using non-ionic lipids [11•].

A related technique involves the ion pairing of biocatalysts in the presence of low concentrations of ionic surfactants (too low to favor the formation of reversed micelles—aggregates of surfactant molecules which form spontaneously in organic solvents and are capable of solubilizing aqueous solutions of enzymes or other hydrophilic solutes) [12•]. Importantly, ion-paired enzymes are remarkably active in nonpolar organic solvents; activities of ion-paired subtilisin Carlsberg and α-chymotrypsin have been observed that are over 1000-fold higher than their native suspended counterparts in organic solvents for the synthesis of dipeptides and tripeptides [13]. This indicates that ion pairing results in powerful homogeneous biocatalysts in organic solvents. The high catalytic activity of ion-paired enzymes in organic solvents has paved the way for their incorporation into plastic materials for the preparation of highly active and selective heterogeneous catalysts [14••]. For example, incorporating α-chymotrypsin into a poly(methyl methacrylate) matrix (CT-pMMA) (Figure 2) resulted in a biocatalyst over 230-fold more reactive than native α-chymotrypsin suspended in hexane.

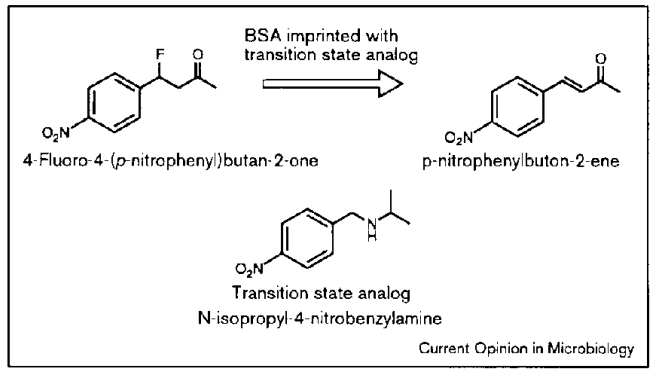
Enzymes used in organic solvents are most often prepared via lyophilizing from a suitable aqueous solution. The addition of small molecules to the freeze drying process (i.e. excipients) often results in improved catalytic activity and in some cases altered substrate specificities as compared to the lyophilized enzymes prepared in the absence of excipients. Following the study by Khmelnitsky *et al.* [15], whereby subtilisin Carlsberg was lyophilized in the presence of KCl to produce a biocatalyst with nearly 4000-fold improvement in the value of k_{cat}/K_m for transesterification of *N*-acetyl-L-phenylalanine ethyl

Figure 2



Synthesis of biocatalytic plastics. Incorporation of α-chymotrypsin into poly(methyl methacrylate) via solubilization of enzymes and *in situ* copolymerization in organic solvents [14••]. Enzyme is first chemically modified with acryloyl chloride and then extracted into a suitable organic solvent (e.g. isooctane) where it undergoes copolymerization with a vinyl monomer (e.g. methyl methacrylate) to give the biocatalytic plastic.

Figure 3

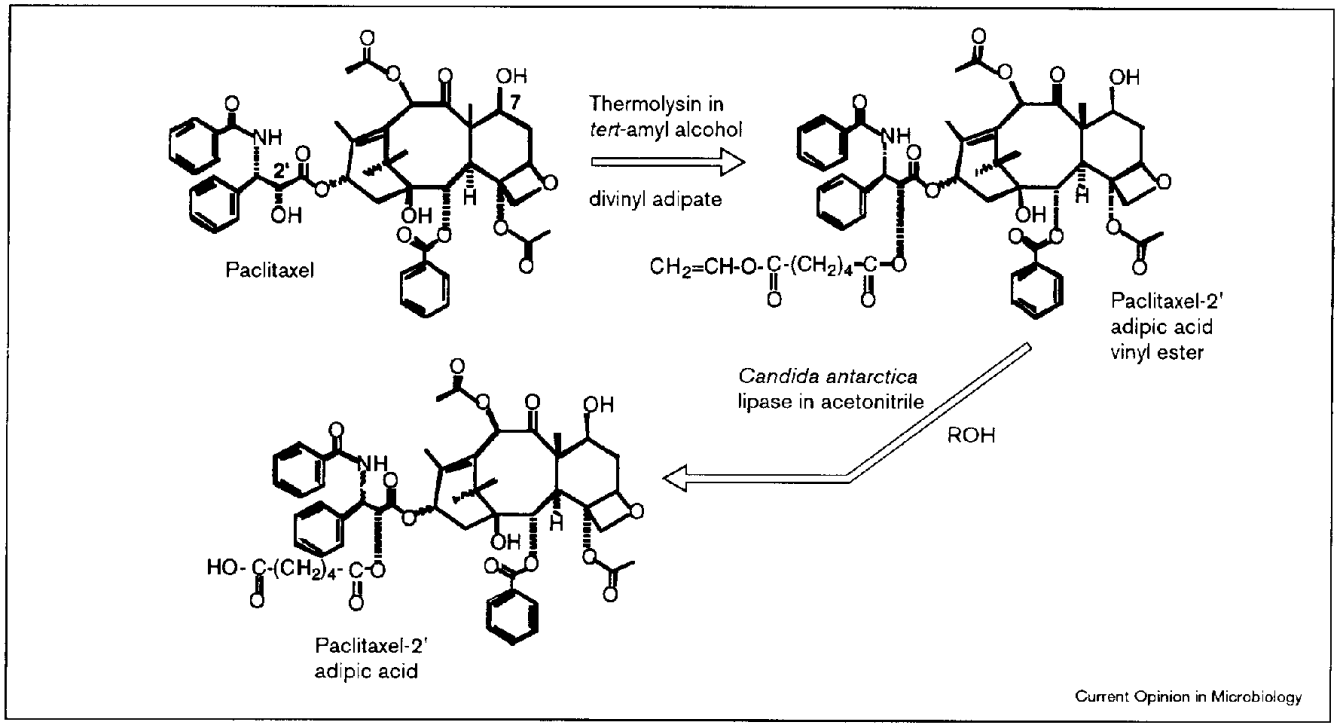


Molecular imprinting of bovine serum albumin (BSA) with N-isopropyl-4-nitrobenzylamine for the dehydrofluorination of 4-fluoro-4-(*p*-nitrophenyl)butan-2-one. As a result of the imprinting, BSA acquired the ability to catalyze this β-elimination reaction with a k_{cat}/K_m of 5.5×10^4 at pH 7.0 [21•].

ester (N-Ac-L-Phe-OEt), Bedell *et al.* [16] showed that the same result could be extended to the nonserine protease thermolysin. Indeed, this same study demonstrated unequivocally that such activation was intrinsic to the enzymes tested rather than a result of relaxed diffusional limitations on the suspended, lyophilized enzyme particles. Activation has also been achieved by the addition of crown ethers [17]. For example, 18-crown-6 has been used as the excipient during lyophilization of α-chymotrypsin, resulting in >600-fold activation over that of the enzyme lyophilized in the absence of the crown ether. Activation of tyrosinase by a similar procedure has also been reported [18].

Substrate specificity can be altered via lyophilization in the presence of substrates as excipients, using a technique

Figure 4



Two-step enzymatic modification of paclitaxel. In the first step paclitaxel was reacted with divinyl adipate (a bifunctional acylating agent) catalyzed by thermolysin to give paclitaxel-2′ adipic acid vinyl ester. The second step involved lipase-catalyzed hydrolysis in acetonitrile to give the 2′-adipic acid, which has a water solubility ≈1600-fold higher than native paclitaxel. In addition to divinyl adipate, a wide variety of ester and carbonate acyl donors were employed. Moreover, water could be replaced by several monosaccharides in the second step of the synthetic scheme to yield glycosylated ester derivatives of paclitaxel.

known as 'molecular imprinting' [19•]. Molecular imprinting combined with nonaqueous conditions provides an avenue for altering enzymic substrate specificity heretofore only attainable via protein engineering. In a related development, two groups in Japan and England [20,21•] independently reported the first example of successful imprinting of a non-enzymatic protein with molecules that resemble the transition state of reactions (e.g. transition state analogs) to convert it into an enzyme-like catalyst (Figure 3).

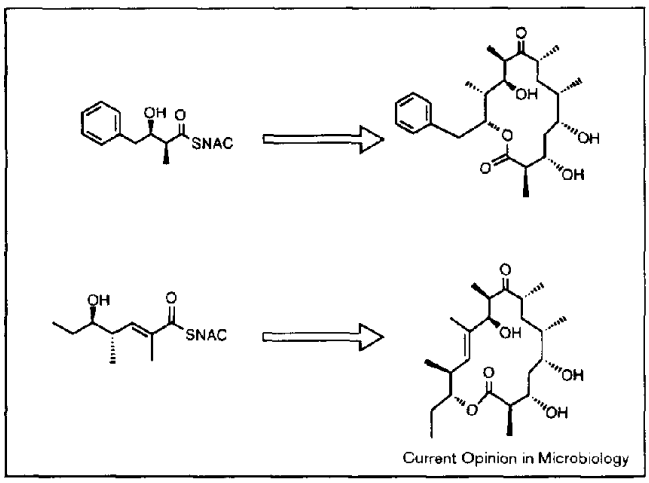
The development of 'solvent-free' systems has become of interest recently as a more environmentally benign technique for catalyzing reactions that cannot be performed in aqueous solutions. For example, enzymatic glycosylation reactions usually give low levels of conversion, mostly because of competing hydrolytic reactions. But, when glycosylation was performed via condensation of sugar and alcohol in the absence of water by solid enzyme adsorbed to mineral supports at elevated temperature, high conversion yields were achieved [22•].

Finally, the use of enzyme-catalyzed acylation reactions in nonaqueous media as a replacement for existing chemical syntheses has become more attractive due to two important developments: the availability and use of activated acyl donors, and the more general application of chemoenzymatic synthesis strategies. Vinyl esters are now used routinely as acylating agents to yield highly selective transformations. An example of such selectivity and reactivity is demonstrated in a unique two-step enzymatic derivatization of the complex natural compound paclitaxel (a member of the anticancer class of compounds) (Figure 4) [23••]. A number of new taxol derivatives with dramatically improved aqueous solubility properties were produced using this procedure. Chemoenzymatic synthesis takes advantage of the best of biology and chemistry — the high degree of selectivity of biocatalysts together with the reaction breadth of organic synthesis. An example of this type of combination is a chemoenzymatic Diels–Alder cycloaddition catalyzed partly by tyrosinase [24]. In the first step of this elegant one-pot process a phenolic substrate is oxidized by tyrosinase in chloroform to give the corresponding *o*-quinone, which then undergoes spontaneous Diels–Alder cycloaddition with a dienophile present in the reaction mixture. Final products of the reaction cascade are highly functionalized bicyclic 1,2-diketones in overall product yields of 55–82%.

Directed molecular evolution

The tools of molecular biology have been crucial in generating new enzyme catalysts. One particularly promising technique developed over the past few years is gene shuffling [25], in which multiple rounds of *in vitro*

Figure 5



Examples of unnatural diketide precursors as substrates of deoxyerythronolide B synthase (DEBS) for the production of novel erythromycin analogs [32••]. The upstream enzyme, ketosynthase KS1, is inactivated by mutation thereby eliminating the diketide substrate for DEBS. Replacement of DEBS substrates with unnatural synthetically derived diketides (two of which are shown on the left hand side of both reactions) results in novel polyketide structures, and demonstrates the broad substrate specificity of DEBS. SNAC, *N*-acetyl cysteamine.

recombination and mutation of pools of related gene sequences are performed, and selection of a desired enzymatic function is made *in vivo*. This process, also known as directed molecular evolution, can be a powerful technique for the generation of novel enzyme activities starting from a readily available enzyme activity. An example of this has been demonstrated for the conversion of a β-galactosidase into a β-fucosidase [26••]. Gene shuffling has been combined with random mutagenesis techniques to generate biocatalysts with improved catalytic activity. This strategy has been reviewed by Kuchner and Arnold [27], and has shown significant promise in the generation of enzymes with improved reactivity and tailored specificity for a variety of aqueous and nonaqueous conditions.

Metabolic pathway engineering and combinatorial biosynthesis

Similar to the rapid generation of improved enzymes, improved metabolic pathways can be obtained by directed molecular evolution. One striking example of this technique is the construction of an arsenate detoxification pathway [28••]. Using the natural arsenate resistance operon from *Staphylococcus aureus* plasmid pI258, three recursive cycles of DNA shuffling were performed and selection for increased resistance of the *Escherichia coli* host was carried out. Growth of *E. coli* containing the wild-type arsenate resistance plasmid was completely inhibited in the presence of 40 mM arsenate, whereas the *E. coli* containing the evolved arsenate resistance plasmid grew under conditions of up to 0.5 M arsenate.

The ability to transfer simultaneously large numbers of genes has been used to engineer 'unnatural natural products', and forms the basis of combinatorial biosynthesis, wherein the alteration of gene sequences and gene products can be made in a combinatorial

Table 2

A subset of biocatalytic transformations* available to combinatorial biocatalysis.

Introduction of functional groups	Modification of existing functionalities	Addition onto functional groups
Carbon–carbon bonds	Oxidation of alcohols to aldehydes/ketones	Acylation trihaloethyl esters oxime esters oxime carbonates bifunctional esters
Hydroxylation	Reduction of aldehydes/ketones to alcohols	
Halogenation	Oxidation of sulfides to sulfoxides	
Halohydrin formation	Oxidation of amino groups to nitro groups	Glycosylation glycosides aminoglycosides glycosidic acids
Cycloadditions	Oxidation of thiols to thioaldehydes	
Addition of amines	Hydrolysis of nitriles to amides and carboxylic acids	Amidation amides peptides hydazides
	Replacement of amino groups with hydroxyl groups	
	Lactonization	
	Isomerization	Phosphorylation phosphates phospholipids
	Epimerization	
	Dealkylation	
	Methyl transfer	

*These reactions can be divided into three general categories as shown in the table. The first two categories result in significant changes to the molecular scaffold of a given lead compound, while the third category gives rise to large numbers of derivatives.

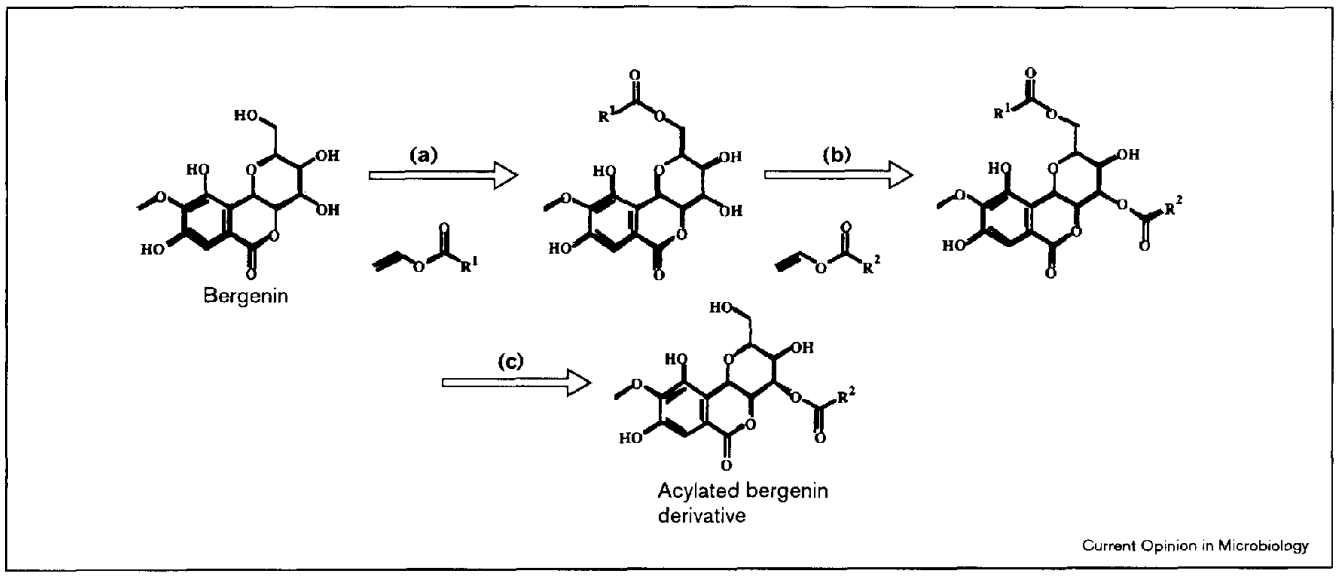
fashion to exploit the inherent diversity of nature. For example, the polyketide synthases (PKSs) are modular, multifunctional proteins that catalyze the synthesis of complex natural products. Mutations in the genes that control the organization of the precursor components of the polyketides results in their controlled incorporation into the resulting polyketides, thereby providing both aliphatic [29] and aromatic [30•] structures with unique and potentially novel pharmacological activity.

The ability to incorporate atypical enzyme activities into the PKS domains has also been demonstrated. Specifically, analogs of erythromycin, a well-known polyketide with antibacterial activity and having a structure similar to many other polyketides, containing unsaturated functionalities were prepared by replacing the β-ketoreductase domain of erythromycin PKS with β-ketoreductase and dehydratase domains from the rapamycin PKS [31••]. Finally, directional synthesis of unnatural polyketides has been performed by a combination of mutagenesis and cellular uptake of unnatural PKS substrates. For example, Jacobsen *et al.* [32••] introduced a genetic block into the initial condensation step of the 6-deoxyerythronolide B biosynthesis pathway. The biosynthetic block was then circumvented by the addition of cell-permeable diketide precursors (Figure 5) which were incorporated into a continuation of the polyketide biosynthetic pathway. Polyketides are not unique in being amenable to pathway engineering using promiscuous enzyme architectures. Indeed, the modular structure of peptide synthetases may also be ideal for such a strategy, and this opens up the possibility of generating large numbers of unique cyclic peptides and glycopeptides that may extend their usefulness as antibacterial and antifungal agents [33].

Combinatorial biocatalysis

Nature's most potent molecules are produced by enzyme-catalyzed reactions coupled with natural selection of those products with optimal biological activity. Combinatorial biocatalysis harnesses the natural diversity of enzymatic reactions for the iterative synthesis of organic compound libraries [34•,35•]. Such a technique has shown considerable potential for derivatization of natural products, wherein complex blocking and deblocking strategies and/or the use of harsh chemical conditions are often required. Combinatorial biocatalysis employs iterative reactions catalyzed by isolated enzymes or whole cells, in natural and unnatural environments, on substrates in solution or on a solid phase. A subset of these reactions are summarized in Table 2. Using just a few of these biotransformations, it is possible to generate a diverse library of unique chemical structures starting with a fairly simple lead compound. For example, the use of lipases alone has led to the generation of a library of derivatives of dibenzyl 1,2-phenylenedioxydiacetate [36•]. Lipases and proteases have been used in concert in nonaqueous media to generate a library of acylated flavonoid derivatives (Figure 6) [37••]. Expansion of the flavonoid nucleus by a variety of automated and iterative enzymatic and microbial transformations led to the synthesis of a 500-member library [35•].

Figure 6



Synthetic strategy for production of acylated bergenin derivatives by three-step regioselective enzymatic acylation/hydrolysis [37••]. **(a)** Lipase catalysis in the presence of various acyl donors; **(b)** subtilisin catalysis in the presence of various acyl donors; **(c)** lipase-catalyzed hydrolysis. The lipase catalyst was prepared by mixing equal weight parts of immobilized Chirazyme L-2, Chirazyme L-9, lipase PS30, and lipase FAP-15. A wide range of ester and carbonate acyl moieties were employed to give rise to the 167-compound bergenin-derivative library.

Conclusions

The broad field of biotransformations is truly at the interface between biology and chemistry. Advances in both fields, whether through improved techniques in molecular biology or the development of chemobiocatalytic synthetic strategies, are already having a significant impact on the chemical and pharmaceutical industries. Additional opportunities are likely to result from further developments in catalytic antibodies and biomimetic catalysts (which are beyond the scope of this review). The increased awareness of chemists of the synthetic power of nature, and the increased interest in synthetic chemistry from biologists and biochemical engineers, will surely maintain the importance of biotransformations in years to come.

Acknowledgements

The authors are grateful to Douglas S Clark and Michael Ru for supplying Figure 1.

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- •• of outstanding interest

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A protease from *Methanococcus jannaschii* was partially purified and found to show increased enzyme activity up to 116°C, with activity still measurable at 130°C, which gives this enzyme one of the highest temperatures for activity reported. In addition, the activity and stability of the enzyme at high temperatures was increased upon increasing the pressure: raising the pressure to 500 atm at 125°C resulted in a 3.4- and 2.7-fold increase in activity and stability, respectively. Electron paramagnetic resonance spectroscopy of a suitably spin-labeled active-site serine indicated that the active-site geometry of the *M. jannaschii* protease is not substantially different from that of related mesophilic proteases; however, the active-site structure may be relatively rigid at moderate temperatures.

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The X-ray structure of subtilisin Carlsberg was determined to 2.6 Å resolution following replacement of water (the solvent used in crystallization) with anhydrous dioxane. Seven enzyme-bound dioxane molecules could be detected, and the locations of these molecules were distinct from enzyme-bound solvent in water or acetonitrile. Nevertheless, the overall structure of subtilisin in dioxane was essentially the same as in water or acetonitrile, indicating that the enzyme retains its bulk conformation even in vastly different media.

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Complexes formed via precipitation of proteins with lipids in an aqueous solution have been prepared with lipases, β-galactosidase, and catalytic antibodies for solubilization in benzene, ethyl acetate, isooctane, isopropyl ether, dimethyl sulfoxide, and ethanol. In all cases, the native structure is presumed to exist and this gives rise to relatively high activity when the biocatalysts are compared to their insoluble nonderivatized counterparts in the aforementioned solvents.

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Subtilisin BPN soluble in organic solvent retained both native secondary and tertiary structure in octane and other nonpolar solvents. Conversely, rapid denaturation occurred in tetrahydrofuran (THF) and other polar organic solvents. Interestingly, partially denatured subtilisin in THF could be renatured in anhydrous octane, thereby providing the first demonstration of enzyme renaturation in a bulk organic solvent.

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Enzyme-containing polymeric materials have been prepared that show high levels of activity and stability in both aqueous and organic media. These 'biocatalytic plastics', containing α-chymotrypsin and subtilisin Carlsberg, consist of up to ≈50% (w/w) total protein in plastic materials such as poly(methyl methacrylate, polystyrene, poly(vinyl acetate), and poly(ethyl vinyl ether). One significant attribute of these biocatalytic plastics is their high level of stability in polar organic solvents, which in turn is at least partly manifested in higher observed activity. For example, rate enhancements of over four orders of magnitude over native containing α-chymotrypsin have been observed for the synthesis of a tyrosine–leucine dipeptide derivative. Other polar solvents such as ethyl acetate and acetonitrile also support peptide synthesis to a far greater degree than native α-chymotrypsin.

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The activity and substrate specificity of subtilisin-catalyzed acylation of nucleosides in organic solvents can be controlled by lyophilizing the enzyme from an aqueous solution containing the substrate. This molecular imprinting technique was examined using thymidine as a model nucleoside, and the resulting subtilisin preparation was up to 50-fold more reactive toward thymidine acylation in nearly anhydrous tetrahydrofuran than subtilisin lyophilized from aqueous buffer in the absence of the nucleoside. Furthermore, it was possible to alter the substrate selectivity of subtilisin by lyophilizing the enzyme in the presence of a different nucleophilic substrate. For example, imprinting made possible the discrimination between structurally different nucleophiles. Imprinting was performed with thymidine and sucrose separately. The two imprinted enzyme preparations were then examined for both sucrose and thymidine acylation. For sucrose acylation, the sucrose-activated and thymidine-activated enzymes were 40-fold and fourfold more active, respectively, than the native enzyme. The trend was reversed, however, for thymidine acylation where the sucrose-enzyme and the thymidine-enzyme were ninefold and 52-fold more active, respectively, than the native enzyme.

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β-Glucosidase from almonds and *Rhizopus* amyloglucosidase were used as catalysts at 80°C. Reaction yields were dependent on the type of the mineral support and were significantly higher than in standard water-containing systems.

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Thermolysin was used as a catalyst for acylation of paclitaxel. This is the first reported enzymatic transformation of a taxane in a completely organic medium, and the first report of a thermolysin-catalyzed transesterification reaction.

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Combinatorial manipulation of the genes for polyketide synthesis has led to the generation of a wide variety of aliphatic and aromatic polyketide derivatives. The success of this approach is aided by the broad substrate tolerance of the polyketide synthases polyketide synthase (PKSs) and the ability of PKS modules to be heterologously expressed in different hosts.

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Unnatural enzyme activities were introduced into the multistep polyketide synthase catalyzed pathway to generate a number of novel polyketides with unique functionalities that may improve pharmacological properties.

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This powerful technique combines pathway engineering with synthetic chemistry and has the potential to generate novel polyketides that could then be available for further chemical or biocatalytic modifications.

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The integration of biocatalysis with high-speed robotics represents a new avenue of biotechnology for the discovery of new molecules and biotransformation schemes. The versatility of this approach was demonstrated for the synthesis of diverse libraries from small organic precursors. For example, the iterative combinatorial biocatalytic derivatization of (±)-(2-endo, 3-exo)-bicyclo[2.2.2]oct-5-ene-2,3-dimethanol resulted in a library of >1200 compounds.

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The reactions applied to the flavonoid bergenin can be generally classified as those that introduce new functional groups to this lead molecule (e.g. hydroxylation and halogenation), those that modify existing functionalities (e.g. oxidations/reductions), and those that add new groups on to existing functionalities (e.g. acylation, glycosylation, and phosphorylation). The resulting library was screened for biological activity against several targets, including xanthine oxidase (a potential target for treatment of gout and multiple sclerosis) and urokinase (an anticancer target). Inhibitors with potency improved by approximately two orders of magnitude compared with bergenin were identified.

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Significant control over the regioselectivity of acylation was achieved enzymatically, in contrast to what could be achieved using chemical acylation. Moreover, the enzymatic reactions were performed on a robotic workstation for the rapid synthesis of 167 distinct selectively acylated bergenin derivatives.

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