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Recent trends in industrial microbiology

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The majority of current biotechnological applications are of microbial origin, and it is widely appreciated that the microbial world contains by far the greatest fraction of biodiversity in the biosphere. Because of their biotech impact, numerous efforts are being undertaken worldwide, with an ultimate goal to deliver new usable substances of microbial origin to the marketplace. However, the direct isolation of microbes always revealed that the majority are not amenable to be cultured and no representatives for many major microbial phyla have been thus far characterized. Therefore, the knowledge on new microbes and/or genomic information thereof, or from their communities, will pose an enormous potential to provide industry with novel products and processes based on the use of microbial resources, and contribute to and extend the basic mechanistic knowledge on the functioning of organisms. The present review highlights some examples and advances in the exploration of the genetic reservoir of (un)cultured microbes for industrial applications.

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Introduction

The diversity of microorganisms inhabiting the biosphere is the most abundant source of genes on our Planet. Microorganisms are responsible for global primary energy and element cycling and they represent the most abundant part of living organisms in terms of total biomass cell numbers (6×10^{30} bacteria, 1.3×10^{28} archaea, and 3.1×10^{29} eukarya), cell biomass (6×10^{18} kg bacteria, 1.3×10^{16} kg archaea and 3.1×10^{17} kg eukarya, assuming an average weight of 1 pg per cell) and species diversity [1,2•,3•]. In this context, the harvest and exploitation of new biological products and activities from microbial diversity for a wide range of applications is currently one of the major drivers of progress, and source of technical solutions to current and future problems of mankind. However, basic and industrial microbiology studies have been primarily devoted to about 6500 microbial species documented [4]. From those, 762 complete genomes (50% with evident biotech potential) have been published and, together with the 2773 ongoing sequencing project (89 archaeal, 1749 bacterial, and 935 eukaryotic), will constitute a valuable information about their genetic and enzymatic repertoire. More interestingly, successful approaches for implementing microbial activities, including the production of small-volume high-value chemicals, antibiotics, vitamins, pharma chemicals, sweeteners, and performance materials, to cite some, have been predominantly on the basis of just about hundred and fifty processes operating at industrial scale [5]. To expand the resources for biotechnology, new ways to obtain pure cultures or enrichments are being undertaken. Robotized systems in which combinations of multiple nutrients and enrichment procedures at once are tested have been successfully applied to isolate microencapsulated microbes under a variety of nutritional conditions and to find a set of microbes or consortia responsible for the conversion of the desired substance [6••]. Further improvements in cellular functions are required for pharmacological interventions or biotechnological process optimization, through first, a high-throughput cloning of particular genes to establish recombinant microbial factories to develop processes around them, workable but suboptimal, and then to optimize those to operate at desired conditions; second, a solid knowledge of the biosynthetic pathways and the quantitative dynamic understanding of their systematic and functional properties; and third, metabolic-engineering and systems biology strategies to understand key metabolic and regulatory parameters that control cellular responses generated during biotechnological applications [7•]. The anticipated rich enzymatic pickings from the uncultured microbial majority have stimulated the development of new genomics-based discovery tools, the so-called 'metagenomics' or 'environmental genomics' approaches to understand and establish new microbial platforms, that is, the study of genomic information, by activity principles or massive sequencing, from whole environmental communities [8•]. This

review focuses on the strategies used so far, to explore, improve and/or engineer and implement the biochemical versatility of microorganisms at industrial scale.

A-la-carte microbial conversions: options of choice

Pure cultures of microorganisms have successfully been used for over 100 years and their economic potential continues to be enormous: its annual added value to the chemical industry alone is about €10 billion and, in the next 10 years, 60% of synthetic fine chemicals will rely on microbial conversions [9]. For industrial applications, one should consider that industrial microbes are self-replicating catalysts, which in many cases may have low cell density and volumetric productivity (activity per fermentation volume) and high interfering enzymatic activities, and there is a very serious drawback in industrial production because the 'unwanted' biomass is generated and substrate is wasted [10]. Also, taken away from their natural environmental context, for example, solvent tolerance, high pressure, low or high temperature or salinity, microbes, or their enzymes may perform suboptimally. There are four distinct strategies to increase microbial performance, according to the primary goal. First, the use of wild-type microbes which can be further improved by systems biology approaches. Second, a rational design or protein engineering of the enzyme(s) of interest, which can be further hyper-produced in a well-characterized microbial host working properly under physical–chemical conditions relevant to the desired industrial process. Third, the combination of different genetic pathways into a single cell factory to establish an organism capable of producing quickly and cost-efficiently the products of interest. Fourth, the utilization of a systems biotechnology approach which considers cells as a whole, rather than only a sum of its parts. Here, the aim is to establish a systemic understanding of unicellular microorganisms with the aid of OMICs tools for the discovery of new principles of cellular regulation that allow prediction of the behavior of the systems under internal and external perturbations in order to control, adapt, and optimize cellular functions. *Escherichia coli* and *Saccharomyces cerevisiae* are, for these purposes, the best-characterized hosts in terms of genomic, metabolic and regulatory information available. Below we recapitulate the biotech potential that each of these alternatives set out to deliver through the description of few recent examples with a preview of major benefits and drawbacks.

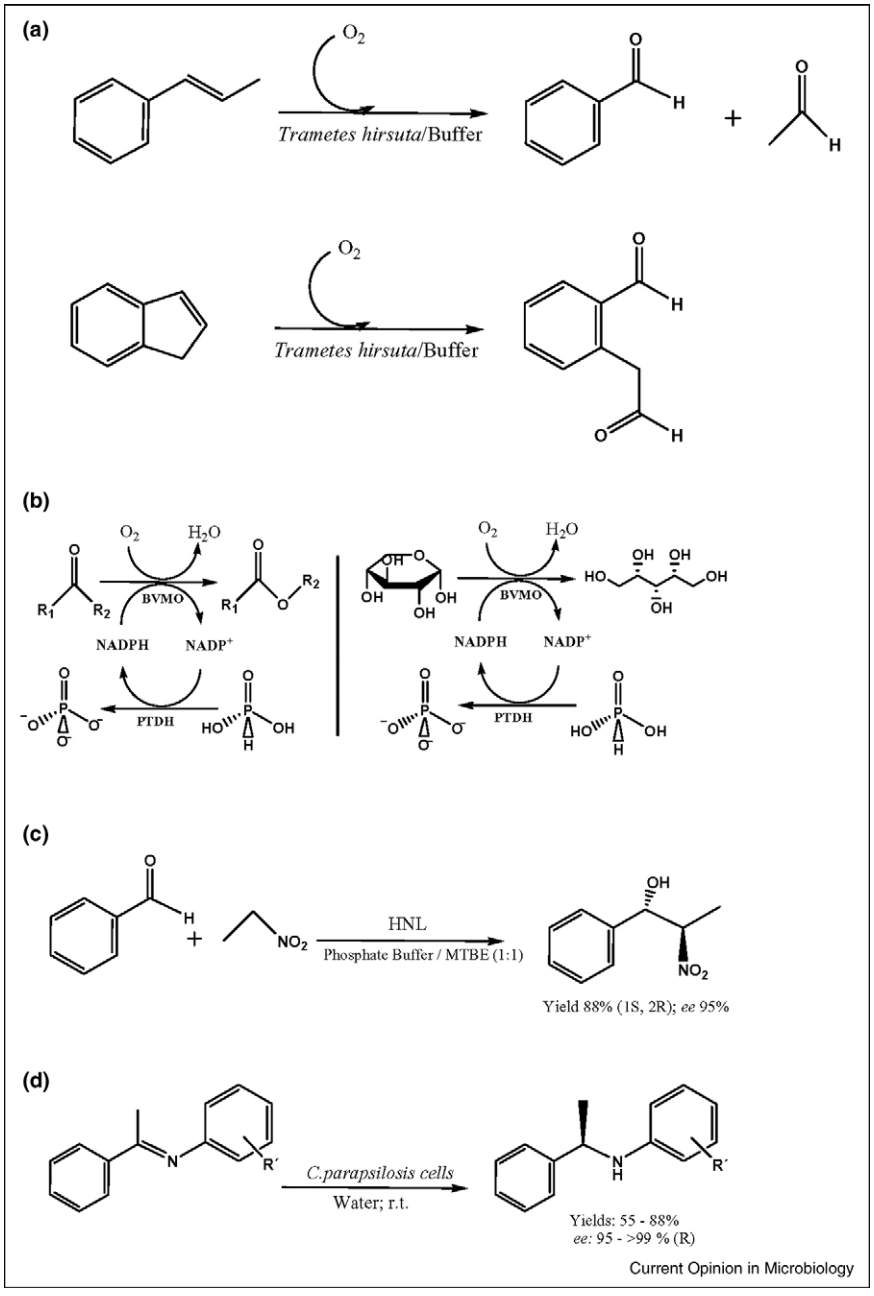
Recent biocatalytic strategies based on natural microbial and single enzymatic conversions

Oxido-reduction strategies

Elegant microbial routes to produce building blocks with a number of functional groups and chiral centers via a nonchiral or asymmetric synthesis, racemic and dynamic resolutions, and conversion of low-cost aromatic and nonaromatic substrates into valuable alcohols and reactive intermediates, have successfully been achieved at the industrial scale [11••]. Such conversions can be catalyzed by isolated enzymes; however, the complex nature and the prevalent cofactor dependency of oxido-reductases constrict the use of redox biocatalysis [12]. Thus, efforts towards the industrial application of redox conversions have mainly focused on whole resting cells. Natural biosynthetic industrial producers of building blocks include strains of *Rhodococcus rhodochrous* (acrylamide and nicotinamide), *Pseudomonas chlororaphis* (5-cyano-valeramide), *P. pseudocalcaligenes* (D-mandelic acid), *Serratia marcenis* (L-threonine), *Erwinia herbicola* (L-DOPA: 3,4-dihydroxy-L-phenylalanine), *Lactobacillus kefiri* ((2*R*,5*R*)-hexanediol), *Geotrichum candidum* ((*R*) and (*S*)-styrene oxides), *S. cerivisiae* (ephedrine and pseudoephedrine), *Nocardia coralline* (epoxidation of terminal and subterminal alkenes), *Candida rugosa* ((*R*)-β-hydroxy-isobutyric acid), *Alcaligenes* sp. (chiral epichlorohydrin), *Leuconostoc mesenteroides* ((*R*)-3-(4-fluorophenyl)-2-hydroxy propionic acid) and *Hevea brasiliensis* ((*S*)-cyanohydrins and (*S*)-hydroxycarboxylic acids), to cite some. The productivity of these strains ranges from 1 to 30 000 tons/year. We estimate that a commercially available strain should deliver yields of 10 g/l or higher, which also means that the natural producers are rarely applicable for industrial purposes.

A recent good example, which can also be regarded as an analogous reaction to the reductive ozonization, is alkene cleavage to yield aldehydes and ketones [13]. This application was found serendipitously while screening for microbes able to oxidize alkenes at the allylic position. Here, a fungus *Trametes hirsuta* GFCC047 was able to carry out the above enzymatic reaction in an aqueous buffer system by means of molecular oxygen as the sole reagent (Figure 1a). Novel enzymatic cofactor [NAD(P)H] regeneration systems, in which the target enzyme is cloned and efficiently over-expressed in *E. coli* as the heterologous host together with a phosphyte dehydrogenase (PTDH), have also been established [14,15]. In an elegant example, rational design and directed evolution were used to develop a PTDH-based NAD(P)H regeneration system for the bioconversion of trimethylpyruvate to L-*tert*-leucine and xylose to xylitol [15]. Here authors created a mutant PTDH with relaxed cofactor specificity with sixfold increased activity and $t_{1/2}$ at 45 °C by more than 7000-fold while maintaining its wild-type activity. This latter biocatalyst was able to selectively accept phosphite as a substrate, thus ruling out any possibility of by-product formation. Therefore, in this case the cofactor regeneration cycle is closed by means of (inexpensive) phosphite as a sacrificial electron donor, thus leading to a new, innovative and promising approach for enzymatic oxido-reduction procedures (Figure 1b).

Figure 1



Recent biocatalytic strategies. **(a)** Some examples of the 'biocatalytic-like ozonolysis'; **(b)** coupled Baeyer-Villiger-monooxygenases (BVMO) (left) and bioconversion of xylose to xylitol (right) with phosphite dehydrogenase (PTDH), to afford efficient enzymatic oxidations; **(c)** the biocatalytic Henry reaction, catalyzed by some hydroxynitrile lyases (HNLs); **(d)** biocatalyzed imine reduction catalyzed by cells of *Candida parapsilosis*.

Microbial promiscuity

Another relevant new source of biocatalytic applications is the enzyme promiscuity concept, which implies that enzymes can catalyze reactions different from the natural performance. The aspect is remarkably important in the case of hydrolases for which many practical promiscuous applications have been reported [16,17]. Following analogous strategies, very recently, the first example of the biocatalytic Henry reaction was made possible through the application of hydroxynitrile lyases [18]. The Henry or nitroaldol reaction constitutes a carboligation process of high synthetic value that furnishes vicinal nitroalcohols, which can easily be transformed to a series of valuable intermediates such as, for example, 1,2-aminoalcohols and hydroxycarboxylic acids. Actually, this *in vitro*-catalyzed reaction represents a '*double promiscuous*

application'. First, hydroxynitrile lyases (HNLs) catalyze *in vivo* the cleavage of cyanohydrins thought to play a defensive role in plants. Yet, HNLs are able to perform the reversible reaction as well, thus facilitating, by enzyme kinetic control, the production of a wide range of chiral cyanohydrins in good yields and reasonable-to-high enantiomeric excess. HNL from *Hevea brasiliensis* is additionally able to accept nitromethane and nitroethane as substrates instead of hydrogen cyanide (HCN). This is most likely to occur because of the similarity of those substrates in pK_a values, about 9. Notably, those HNLs of plant origin were used as cloned and overexpressed biocatalysts in *E. coli* cells, thus showing again how modern biocatalysis can have a huge benefit of the developments in the field of molecular biology: enzymes from plant origin could easily be produced and further improved by means of directed evolution techniques [19] (Figure 1c). Also, as a promiscuous application, the capability of reductases from *Candida parapsilosis* in the asymmetric imine reduction was recently reported [20]. The reductases were able to reduce not only the C=O bonds but also the C=N bonds (Figure 1d). These findings obviously open up some new avenues in research and applications.

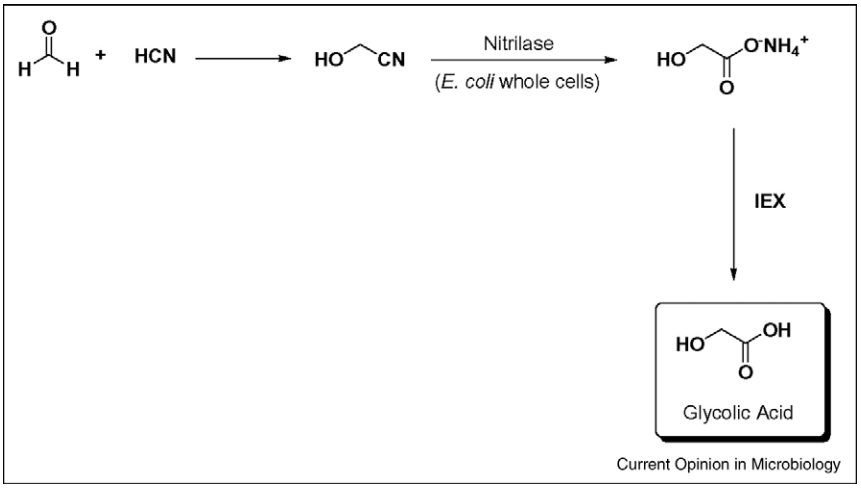
Enzyme discovery and engineering

The rational combination of enzymatic catalysis and modern techniques of molecular biology is boosting industrial biocatalysis. New powerful natural enzymes can be rapidly found through metagenomics (see below), tailored for specific applications via directed evolution [21] and produced in suitable heterologous hosts to allow large quantities in a reproducible manner. In this area, a new chemo-enzymatic process for the production of glycolic acid has recently been established [22]. Overall, the disclosed process encompasses two chemical steps and one biocatalytic reaction. First, formaldehyde and HCN are chemically combined to yield glycolonitrile. Subsequently, the latter is converted into ammonium glycolate by means of nitrilases. Finally, glycolic acid is obtained by using an ion-exchange column. To develop the biocatalytic step, a nitrilase from *Acidovorax facilis* 72W was selected as a promising candidate by a large screening of soil samples and further subjected to several directed evolution cycles, to achieve a final specific activity towards glycolonitrile 33-fold higher compared to the wild-type enzyme. To further improve the economical efficiency of the process the enzyme was cloned and efficiently over-expressed in *E. coli* cells that were immobilized in carrageenan to become re-usable over few rounds of application. Overall, an impressive biocatalyst productivity of >1 kg/g dry cell weight was achieved [22] (Figure 2). This approach of over-expressing engineered enzymes in recombinant whole-cell 'designer bugs' has also successfully been applied for the production of chiral alcohols [23,24]. To this end, two alcohol dehydrogenases, one *R*-specific from *Lactobacillus kefir* and one *S*-specific from *Rhodococcus erythropolis* were coexpressed with glucose dehydrogenase from *Bacillus subtilis* and *Thermoplasma acidophilumin*, to create *R*-selective and *S*-selective whole-cell catalysts. Substrate concentrations, yields, and enantiomeric excess higher than 100 g/l, 91% and 96%, respectively, were achieved during the reduction of ketones such as 4-phenoxyacetophenone and α-halogenated acetophenones [24].

Creating a microbial single-cell factory

The clear disadvantage of using single enzymatic conversions described above is its full reliance on the wild type or evolved enzyme that generates the product of

Figure 2



The chemo-enzymatic synthesis of glycolic acid. Here, formaldehyde and hydrogen cyanide are first reacted to prepare an aqueous solution of glycolonitrile and further converted to ammonium glycolate using an immobilized and evolved microbial nitrilase, and the product mixture is directly converted to glycolic acid using ion exchange chromatography (IEX).

interest after being hyper-produced in a recombinant host. This approach has its limitations especially when trying to build up a new chemical assembly line and to produce high-value products from cheap raw materials, such as sugars, where multiple reactions are required. An elegant alternative is the utilization of genetic assembly lines in a single cell. The final goal of this approach is to establish microbial factories with entire gene systems capable of producing any kind of chemical. Three elegant recent examples illustrate the potential of single-cell factories: first, the production of the antimalaria drug, artemisinic acid; second, the biosynthesis of carotenoids built from backbones not known in nature; and third, the combinatorial synthesis of ubiquinones. Although, the total synthesis of these types of products is established in some cases, the process is difficult and costly and their isolation from natural sources is, often, not feasible. In the first example, Ro *et al.*, combined genes from three separate organisms in *S. cerevisiae*: the productivity of a recombinant strain expressing an engineered mevalonate pathway, amorphadiene synthase and a novel cytochrome P450 monooxygenase, was 100 mg artemisinic acid per liter [25••]. This drug is one of the most promising next-generation antimalarials because of its effectiveness against strains of the malaria parasite that became resistant to the existing front-line drugs. Although, this production is far away from the optimal industrial yield (approx. 10 g/l), it represents an interesting example on how single-cell strategies may be a good starting point for practical applications. The second example is the application of combinatorial and evolutionary design strategies for the creation of novel biosynthetic carotenoid and flavonoid pathways in nonproducing organisms, such as *E. coli*. The groups of Arnold and Schmidt-Dannert produced a variety of structurally novel carotenoids by mixing, matching and mutating carotenoid biosynthetic enzymes and further screening these 'evolved' pathways for the emergence of new carotenoid products [26•,27•]. The very same strategy has been used to perform the combinatorial biosynthesis of ubiquinone with wide chemical structures and strain productivities ranging from 30 to 130 mg/l [28]. Single cell factories have also been employed for the replacement of chemical by microbial processes, to substitute expensive chemicals by cheaper sugars and chemical solvents by water. An interesting example is the production of the petroleum-based feedstock *p*-hydroxy-styrene from glucose by introducing genes from other organisms and uncoupling central metabolism of *E. coli* and generation of biomass [29]. The ability to produce high-value chemicals in a simple organism like *E. coli* opens up a whole realm of possible molecular backbones that can later be functionalized to make drugs or reduce chemical loading by using inexpensive agroindustry-produced plant oils as feedstocks. Other interesting example include the production of value-added products starting from glycerols, a byproduct of biodiesel production [30–32].

Systems biotechnology for strain improvement

The clear disadvantages of recombinant whole-cell transformations are first, its full dependence on expression machineries and reliance on given surrogate hosts to deliver the desired gene products and second, the low performance of the host after gene expression. To cope with this, a number of systems microbiology efforts were initiated, whose ultimate goal is to obtain and exploit the systems understanding of gene expression, cell metabolism, cofactor availability, oxygen transfer, membrane permeabilization, and cell stability, to cite some, to increase the productivity of a bioprocess and to develop robust strains which can be used in whole cell conversions [10,12]. This is of practical importance since biotechnological applications invariably impose unnatural, sometimes severe, stresses on the cell that reduce or cease their performance. In this context, the tremendous developments of expression systems and OMICs tools in the past decade greatly improved our ability to access and understand the complex metabolic networks. However, as knowledge of genome sequencing increases, complexity has arisen for the understanding of metabolic networks, and thus the implementation of microbial activities into a whole range of industrial processes requires several optimization strategies.

Following the above observations novel microbial expression hosts, including the Gram-negative bacterial genera *Burkholderia* and *Rhodobacter*, are being evaluated for their potential to produce at concentrations over 10% (w/w) 'difficult-to-express' proteins, including membrane-associated and cofactor-dependent enzymes; however, such levels of production may influence the host metabolism [33,34]. A prominent example for the versatility of expression solutions is the membrane display system where the outer membrane of cells are genetically modified to expose short peptides or specific chaperones that are capable of a tight and specific binding of the enzyme of interest, resembling the well-known phage display systems [35]. Recent developments in the systems biology approaches based on global information analysis [36,37], have been achieved in recent years for a number of organisms of biotechnological relevance, such as *Corynebacterium glutamicum*, *Clostridum acetobutylicum*, *E. coli*, *S. cerevisiae*, *Aspergillus terreus* and *A. niger* for the production of high-value chemicals such as methionine, L-lysine, IGF-I fusion protein, poly-hydroxyalkanoates, human leptin, riboflavin, L-threonine and lovastatin, to cite some, at concentration up to dozens of grams per liter [38–40]. A good example is the production of L-threonine (82.4 g/l versus 7.4 g/l in fed-bach culture) by an engineered *E. coli* strain in which feedback inhibitions of aspartokinase I and III (*thr*A and *lys*C) genes and their transcriptional attenuation were removed by applying transcriptome profiling combined with *in silico* flux response analysis to detect pathways for Thr degradation

[40]. Beyond application in yeast, integration of metabolome data with metabolic networks have been used to reveal reported reactions in wild-type and redox *S. cerevisiae* strains [41]. Here, authors capture a number of significantly affected reactions due to a NADPH-dependent glutamate dehydrogenase mutation and use this information to manipulate the oxygen availability and redox metabolism for fuel ethanol production. An interesting example of the systems/synthetic biology approach is the design of strains with minimal genomes by deleting 'unnecessary' genes while retaining the essential ones that most effectively use metabolic functions for cell survival and production of specific bioproducts [7]. However, the concept of generating a producing strain with the 'minimized genome' should be taken cautiously, since the function of 40–60% of the genes per genome is not known and their deletion could lead to less robustness and metabolic plasticity, or compromised survivability of the strain.

Although, individual or combined OMICs approaches have been successfully used, they do exhibit certain limitations hindering the establishment of systems biology framework for industrial strain development. First, considering that most cellular metabolic activities are directly or indirectly mediated by proteins, proteome profiling takes us one step further towards understanding cellular metabolic status. Nonetheless, although proteome analysis identifies protein spots in 2D gels that show altered intensities under protein hyper-secretion or key stresses, its analysis is limited by specific protein resolution, is time-consuming and difficult to automate. High-throughput quantitative analysis of metabolites during the conversion of interest may be analyzed by modern chromatography tools, and a comparative analysis of metabolite profiles under perturbations may help to analyze the physiological states of the cells. However, only accumulated and dead-end low molecular mass metabolites at concentrations enough to be detected and the fluxes of substrate uptake and product excretion rates are obtained [37]. Transcriptomics delivers the data on mRNA levels and is based on nonfunctional hybridization and often does do not reflect the protein levels and does not *per se* help to predict the cellular functions of upregulated or downregulated genes [38]. Finally, it should be noted the basal information for systems biology relies on genome sequencing and annotation which limits the prediction of cellular function to those from the sequenced organisms and, since *in silico* function predictions rely on homology, often the genome annotation data may cross-reference the erroneous annotations [38].

New industrial applications based on microbial metagenomics

The well-known dilemma of microbes, that the majority are recalcitrant to culturing, limits application of the traditional means of microbial discovery. This situation has spurred the development of metagenomics, a culture-independent approach to sample and characterize microbial genomes and the enormous genetic pool of 10^{33} putative genes in the environment [42]. There are two distinct strategies which should be considered in metagenomics, according to the primary goal. First, a large-scale sequencing of bulk DNA to capture the largest amount of the available genetic resources for its further data mining, mostly homology-based with the aid of powerful assembler computer programs. Actually 123 mega-sequencing projects are prospecting microbial communities for new genetic information and genes (http://www.genomesonline.org). Elegant examples of the biotech applications of this tool are the *in silico* and functional analysis of hindgut microbiota of a wood-feeding higher termite [43•] and worms [44], which provide potential sources of biochemical catalysts for converting wood into biofuels. Another sequencing analysis carried out in biological phosphorus removal sludge communities provided an insight into the new ways to generate biopolymers (poly-hydroxyalkanoates) through the hydrolysis of internally stored polyphosphate granules [45•]. Unfortunately, the generation of vast metagenome sequencing data and the plethora of genes deposited in databases is not followed, at the same extent, by the discovery of new activities, since up to 40% of those are related to the hypothetical or conserved hypothetical proteins. To cope with this, a number of structural genomics initiatives were established worldwide [46], whose ultimate goal is to obtain the structures for at least one protein representing each of the 10 000–15 000 existing protein families. However, the majority of the protein structures do not suggest the function of these proteins and the reasonable answer to this challenge will be to combine the *en masse* activity characterization of the proteins with elucidation of their structures and protein engineering approaches to consequently channel these activities towards new single-optimized microbial factories. In this respect, the screening of expression libraries may be a more direct alternative to discover new enzymes [47]. In the past two years, 140 metagenome-derived enzymes have been described, some of which may have biotech potential. For example, recent work by Riaz *et al.* [48] confirmed the ability of *E. coli* to degrade hexanoyl- (C6-), heptanoyl- (C7-), octanoyl- (C8-), and decanoyl-homoserine lactone (C10-HSL), as well as the hydroxy-substituted or oxo-substituted derivatives at carbon 3, such as OC6-HSL, OC8-HSL, and OHC8-HSL, after overproducing a gene, *glc*A, encoding a NHL-lactonase isolated from bacterial communities inhabiting pasture soils. Enzymatic disruption of lactones is a promising anti-infection therapeutic strategy. Other route has been developed by the group of Xu *et al.* [49]. Here, the terminal oxygenation of alkanes was achieved by strains of *P. fluorescens* and *P. putida* overproducing a few alkane hydroxylases from deep-sea sediments. A prominent example of the versatility of metagenome-based gene factories was the ability of *E.*

coli to cleave a wide range of aromatic compounds (from catechol to 2,3-dihydroxybiphenyl) when expressing 38 extradiol dioxygenases retrieved from coke plant wastewater [50]. Another biochemical route that starts from L-cysteine and that utilizes cheaply accessible decarboxylase from alkaline polluted soils has been developed by Jiang and Wu [51] to generate the therapeutically important drug, cysteamine. More recently, the group of Jaeger has constructed a broad range of different *E. coli* strains expressing industrially important enzymes such as benzaldehyde lyases, benzoylformate decarboxylases, hydroxynitrile lyases, and alcohol dehydrogenases, which all produced benzaldehyde by conversion of benzoin, benzoylformate, mandelonitrile, or benzoyl alcohol, respectively [52]. Although, these studies exemplified the potential of metagenome mining, some additional efforts should be undertaken to deliver metagenomic enzymes to the market, either as individual enzymes or as whole-cell catalysts. Till now, an esterase from uncultured microorganisms able to degrade terephtalate esters, important component of bio-plastics, is the only known example thus far [53] introduced at industrial scale, with, hopefully, many more to follow very soon. To achieve this objective, effort should be directed to design enzymes' or microbes'variants to fit industrially relevant transformations. In this line, very recently, numerous established shuffling processes attempted to recombine more or less related matagenome parental sequences (approx. 60% amino acid homology) to produce enzyme variants that fit against novel or poorly effective conversions. In a prominent example, which was developed by Boubakri *et al.* [54••], metagenomics and DNA shuffling were coupled to generate novel proteins capable of aromatic conversions. For that, metagenomic DNA extracted from nonpolluted soil was shuffled *in vitro* to create *linA* genes responsible for the first steps of lindane transformation. This demonstration might stimulate the attempts to mimic bacterial adaptation and to construct catalytic genes for novel compounds not yet attempted by the industry.

Conclusions

Industrial microbiology has more to offer than just providing a solution when the chemical synthesis does not work. The distinct strategies and examples in this review illustrate the enormous potential of microbial factories in fermentations and conversions catalyzed by resting cells combined with the new knowledge on microbial diversity, enzyme discovery, reactivity, and metabolic engineering. Environmental sampling hotspots to produce metagenomic resources for their further exploration, and microorganisms with recently sequenced genomes will cover the whole diversity of microbial life, which is of great interest to explore the new mechanisms determining industrial aspects. The ultimate goal of functional mining of the genomes of, and processes mediated in the environment by, communities of microorganisms as a whole, the so-called '*macro-organisms*' or '*microbial factories*', may become a reality which will allow the synthesis of myriad substrates into the desired products starting from cheap raw materials. This encompasses a potentially inexpensive and sustainable way of providing the chemical industry with high-value, customized, and functionalized materials.

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