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PERSPECTIVE

Systematics-guided bioprospecting for bioactive microbial natural products

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Abstract Advances in the taxonomic characterization of microorganisms have accelerated the rate at which new producers of natural products can be understood in relation to known organisms. Yet for many reasons, chemical efforts to characterize new compounds from new microbes have not kept pace with taxonomic advances. That there exists an ever-widening gap between the biological versus chemical characterization of new microorganisms creates tremendous opportunity for the discovery of novel natural products through the calculated selection and study of organisms from unique, untapped, ecological niches. A systematics-guided bioprospecting, including the construction of high quality libraries of marine microbes and their crude extracts, investigation of bioactive compounds, and increasing the active compounds by precision engineering, has become an efficient approach to drive drug leads discovery. This review outlines the recent advances in these issues and shares our experiences on anti-infectious drug discovery and improvement of avermectins production as well.

Keywords Microbial natural products library · High-throughput screening · Precision engineering

Introduction

Natural products occupy tremendous chemical structural space unmatched by any other small molecule families. They possess a range of biological activities thus remaining the best sources of drugs and drug leads, and serving as outstanding small molecule probes for dissecting fundamental biological processes. Many microbial natural products that have reached the market without any chemical modifications are a testimony to the remarkable ability of microorganisms to produce drug-like small molecules (Knight et al. 2003; Zhang et al. 2005, 2007; Bull and Stach 2007).

Historically, microorganisms have provided the source for the majority of the antibiotics in use today (Demain and Sanchez 2009). Among these, 45% are produced by actinomycetes, 38% by fungi and 17% by unicellular bacteria. Among the prokaryotes, actinomycetes particularly *Streptomyces* produce a rich source of 70–80% of currently characterized actinomycete natural compounds (Berdy 2005). *Actinobacteria* have made remarkable contributions to human

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life. More than 22,000 biologically active compounds were obtained from these microbes by the end of 2002. The class *Actinobacteria* is especially notable for containing organisms producing diverse natural products, with members of the order *Actinomycetales* alone accounting for ~10,000 such products (Bull and Stach 2007).

The marine environment covers more than two-thirds of the earth. It was once thought that the marine environment had unfavorable conditions for microbial production of natural products compared to terrestrial microbes that exist in a more crowded and competitive environment. It is now understood that this is untrue as many marine natural products have evolved to be chemical weapons that are highly potent inhibitors (Zhang et al. 2005). However, to date, the biodiversity of marine microbes and the versatility of their bioactive metabolites have not been fully explored. In the last 10 years research on marine microbes has accelerated in the search for novel compounds to fight infectious disease.

Research on marine microbial natural products attracts the interest of researchers based on four general aspects; the biodiversity of microorganisms, especially isolated from unexplored or extreme environments; structural diversity of secondary metabolites; broad spectrum of active compounds; and genetic engineering aimed at producing specific secondary metabolites and increasing the yields of important products. This review will discuss the new advances in systematics-guided bioprospecting natural products, specifically the approaches used in microbial natural product library construction, target and cell-based high-throughput screening (HTS) assays, synergistic drug discovery and increasing yields of avermectins by precision engineering (Fig. 1).

Microbial natural product library

The construction of a high-quality microbial natural product library includes high biodiversity of the microbes, structural diversity of small molecules and high capacity with for activities that will facilitate long-term usage for the drug discovery process. In order to increase the quality of a natural product library, the initial biodiversity of the strains used for the construction of the library is of ultimate importance to reduce the redundancy of chemical compounds. The secondary metabolites produced are predicted by biosynthetic gene cluster analysis and are confirmed based on their HPLC-UV and LC-MS analysis. Quality check with chemical de-replication and bioassay-guided isolation strongly assisted the construction of a high quality natural product library with high structural diverse and good functionality (Knight et al. 2003; Zhang et al. 2007).

Systematics-guided construction of a high quality library of marine microbes and their crude extracts

Normally, the biodiversity of microbes is based on their inhabiting environment. In order to collect microbes with high biodiversity, isolating the microbe strains from samples collected in different sites should be addressed. Species inhabiting unique environments with differing environmental conditions have been thought to be the best resources for novel compounds (Jensen and Fenical 1996; Bull et al. 1992). The maximum diversity in species exists in pristine environments compared to areas already explored or impacted by man (Cao et al. 2009). Conditions like extreme temperature (Zhu et al. 2009), pressure (Wagner et al. 2009) and pH (Bull and Stach 2007) offer unique, competitive environments favorable for the production of biologically interesting molecules (Stierle et al. 2004, 2006). Tools such as color-grouping, rep-PCR, single strand conformation polymorphism and analytical chemistry (FTIR, MALDI-TOF, pyrolysis mass spectrometry) have been developed to estimate diversity and dereplicate strains. It is crucial that a larger number of diverse microorganisms are brought into genome programs, based on their potential to produce a high number of compounds (Borresen et al. 2010). The actinomycete genome, for example, contains approximately 8,000 genes coding for 20–50 proteins from secondary metabolite synthetic gene clusters (Galm and Shen 2006). Bioinformatics technologies allow the rapid identification of known gene clusters encoding bioactive compounds and to make computer predictions of their chemical structure based on genetic sequence information (Zazopoulos et al. 2003; Farnet and Zazopoulos 2005). In a few cases, structure prediction has identified new chemical entities before they were then expressed using diverse cultivation approaches and chemically identification. As an example, our laboratory has constructed a marine

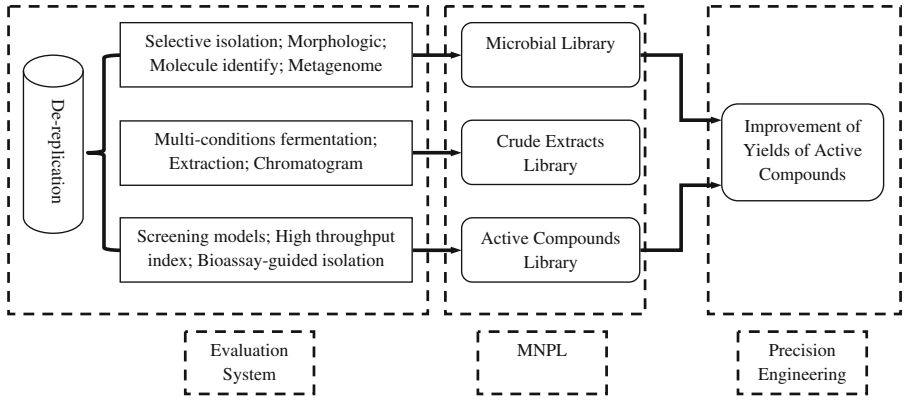

Fig. 1 The flow chart of systematics-guided bioprospecting for microbial natural products

microbe library, which contains 4,000 strains isolated from the sediment from South China Sea down below 4,000 m (data unpublished), based on comprehensive de-replication approaches. The criteria for strain selection include: (i) the uniqueness and richness of biodiversity of the ecological niches from which the strains are isolated, (ii) the bias towards actinomycetes for their proven track record as prolific natural product producers, (iii) morphological and taxonomical distinctiveness. Only 1% of the microbial community has been estimated to be cultivated in the laboratory, implying that the vast biodiversity of natural products in microorganisms remains to be exploited. Emerging new cultivating techniques, culture-independent methods by expressing gene clusters in model heterologous hosts, and diligent effort and innovative approaches in novel microbial strain collection, identification, and classification have started to permit access to these previously inaccessible natural product resources. We strive for isolation of novel strains on selective media, because novel microbes harbored novel genes and they are more likely to produce novel metabolites (Bian et al. 2009; Wang et al. 2009; Dai et al. 2009; Mao et al. 2010; Tang et al. 2011).

The structural diversity of secondary metabolites produced by microbes is the most important for construction of a high quality crude extract library. Whole genome sequencing has revealed far more biosynthetic gene clusters than actual metabolites currently known for a given organism, suggesting that the biosynthetic potential for natural products in microorganisms is greatly under-explored by traditional natural product discovery efforts. Among the *Streptomyces* whose genomes have been sequenced, every one of them has the potential of producing up to 30 natural products on average, and this optimism has already translated into the discovery of new natural products by fermentation optimization from strains that otherwise were not previously known as natural product producers. Maximum productivity can be achieved by manipulating the environment experienced by an organism leading to changes in chemical diversity; a method known as 'one strain, many active compounds' (Bode et al. 2002). Bode et al. used the systematic alteration of easily accessible cultivation parameters to increase the number of secondary metabolites from a single organism. Small changes in the cultivation conditions resulted in a complete shift in the metabolic profile of the microorganism. Tormo developed a method for the selection of production media for actinomycete strains based on their metabolite HPLC profiles, and three media types that yielded the highest metabolite diversity and least overlapping HPLC profiles were selected for large scale fermentation (Tormo et al. 2003). Researchers at Merck use eight different types of media for the cultivation of species to make new anti microbial compounds (Wang et al. 2006, 2007; Zhang et al. 2008, 2009; Llacer et al. 2009; Singh et al. 2009).

We used 10 different media (Table 1) for the cultivation of novel actinomycetes (Liu et al. 2010; Bian et al. 2010; Dai et al. 2009; Wang et al. 2009). The results were exceptional with a 70% higher positive hit rate compared to the historical hit rate (1%) with the same screening methods. Thirteen novel active compounds were isolated from the microbial product library including fungal products (You et al. 2010; Song et al. 2010).

Systematics-guided dereplication of hits from HTS: the challenge of avoiding rediscovery of known compounds

There are two primary approaches to the discovery of novel natural products from extracts: bioassay-guided fractionation and the singling out of agents possessing unique structural features and/or novelty as important representatives of different chemical classes. In both cases a molecule of interest must be produced in sufficiently large amounts so as to permit isolation, purification, characterization and dereplication on a reasonable timeframe. Though not always the case, prioritization of subpopulations of extract components is accomplished by a broad screening program in which crude extracts are assayed for bioactivity. The field of dereplication continues to evolve and new advances continue to center on hyphenated techniques, of which LC takes center stage. The combined use of LC, solid-phase extraction (SPE) and NMR spectroscopy (LC-SPE-NMR) has been developed and continues to undergo refinement via expansion of the types of NMR analysis one can perform on samples once they've been separated, removed from LC mobile phase and placed into deuterated solvents (Larsen et al. 2005; Bobzin et al. 2000; Wolfender et al. 2000; Gu et al. 2006; Konishi et al. 2007; Lambert et al. 2005).

LC-SPE-NMR permits the rapid and precise on-line identification of major and minor secondary metabolites present in natural product extracts (Lang et al. 2008). Very recent developments involving cryogenic flow and micro-coil NMR probes have allowed a dramatic increase in sensitivity to accomplish de novo structure elucidation of complex natural products with 10–50 μg quantities, and also makes the on-line NMR data acquisition possible (Russell et al. 2000). However, cost and effort requirements, for the foreseeable future, limit the availability of such techniques to many academic natural product laboratories. In such settings, dereplication efforts will likely continue to focus on the use of MS, UV–Vis and possibly, "second round" bioassays to narrow down those fractions of natural product extracts that warrant more focused structure elucidation efforts.

Once a crude extract library has been established it can be used for target-based and whole-cell high-throughput screens related to infectious disease for the identification of active natural products.

To screen a large numbers of compounds for growth/inhibition kinetics, simple HTS techniques are needed. Alamar Blue, validated against slow-growing tuberculosis (TB H37Rv) in 1997, is a rapid and inexpensive way to measure cell growth by the extent of pink fluorescence resulting from an oxidation/reduction reaction (Shawar et al. 1997). The

Table 1 The fingerprint characteristics of 10 culture media for strain MS098 (adapted from Liu et al. 2010)

Medium	Peaks	Area	DI (%)	QI (%)	D/Q (%)
9A	33	140427.7	6.42	1.39	2.65
NM2	53	446167.5	10.31	4.42	5.89
NM1	69	561396.1	13.42	5.56	7.52
MPG	87	1093319	16.93	10.82	12.35
M21	74	4980134	14.40	49.28	40.56
M12	51	445540.2	9.92	4.41	5.79
M004	48	253674.4	9.34	2.51	4.21
M001	33	1461252	6.42	14.46	12.45
GOT	40	79100.25	7.78	0.78	2.53
FR	77	644646.5	14.98	6.38	8.53
Total	514	10105658			

DI (*diversity index*) a ratio, expressed as a percentage, comparing the number of peaks present in the chromatogram for a single fermentation condition, *QI* (quantity index) a ratio, expressed as a percentage, comparing the area of the peaks present in a chromatogram for a given condition with the area of the peaks present in all the chromatograms obtained for a single strain, covering the whole panel of fermentation conditions, *D/Q* (*diversity/quantity ratio*) a ratio calculated to balance the influence of DI vs. QI from 100/0 to 0/100. For example, a balance of 75/25 indicates that the overall ranking percentage is determined by the formula: (DI × 0.75) + (QI × 0.25) (Tormo et al. 2003)

quantitative nature of the results makes it ideal for synergistic studies against target and non-target cells. Reporter genes expressed in *Mycobacterium tuberculosis* can also be used as a surrogate for growth in HTS. The green fluorescence protein (GFP) assay measures bacterial growth by direct readout of fluorescence. Recent improvements in GFP using an acetamidase promoter have increased the signal-to-background ratios, making it preferable over alternative reporters, including luciferase (Changsen et al. 2003; Pauli et al. 2005). Several other advantages of GFP include its intrinsic fluorescent nature, precluding the need for a substrate, and better biosafety (if the minimal bactericidal concentration is not being determined), as the microplate can remain closed after inoculation. Also, GFP measurements in intracellular environments preclude the need for host cell lysis, since a substrate is not required, allowing direct and repeated measurements of cell viability, thus offering easy kinetic monitoring and low cost.

We have used a GFP based assay to screen the effect of marine microbial natural products on the growth of *M. bovis* ATCC35743 (BCG) (a slow growing non-virulent strain closely related to *M. tuberculosis* H37Rv in terms of its drug susceptibility, profile, and genetic composition) and TB H37Rv, with throughput reaching 60,000 wells/day in an adapted 384-well format. Results from screening of an initial 5,000 extracts showed 80 as having >90% inhibition against logarithmically growing BCG, and 46 to have anti-TB activity, of which eight had activity at 1/16 dilution, and one at 1/128 dilution (Fig. 2).

In addition to our screen against BCG, our research group has also applied HTS to a multitude of pathogenic organisms including *Candida albicans* (CA; SCA: used for synergistic anti-fungal assay), and methicillin-resistant *Staphylococcus aureus* (MRSA), as well as specific targets within these organisms such as biotin protein ligase (BPL), which adds biotin to proteins involved in fatty acid synthesis and lysine synthesis, and 14-3-3 signaling protein (Table 2) (Liu et al. 2010). These data revealed a differentiation on hit rates from each assay, which revealed a potential diversity of microbial products in the constructed crude extract library.

When a hit is determined against a whole cell, it is an extremely laborious and time-consuming task to identify the target of the small molecule. Nevertheless, whole cell screening also has a role as many active compounds identified in target based screens may have limited efficacy against whole cells due to their inability to translocate through the membrane to reach their intended target (Dhiman et al. 2005). Therefore, a combination of primary and secondary screens incorporating these two approaches is worthwhile. Many assays themselves can be performed in a high throughput format using fluorescence and absorbance indicators to quickly screen out interesting compounds in amongst the thousands of possible hits.

Bioactive compounds identification from the natural product library

Bioassay-guided isolation and purification of anti-TB compounds

During the course of therapeutic drug discovery from a natural product library, bioassay-guided isolation is usually employed. In order to avoid known active compounds, several approaches could be applied. The first way is to generate antibiotic resistance markers. Cubist Pharmaceutics screened microbial crude extracts against a multi-drug-resistant *Escherichia coli* strain that carries resistance markers to 17 of the most frequently produced antibiotics by microorganisms and allowed rapid de-prioritizing of extracts (Baltz 2007). The second way to de-replicate known compounds is chemical profiling analysis based on HPLC-UV, LC-MS and LC-NMR techniques (Wolfender et al. 2000; Bobzin et al. 2000; Corcoran and Spraul 2003). Applying these two methods in our search for new anti-TB compounds from marine microbes, we constructed several specific antibiotic-resistant *Mycobacterium smegmatis* strains. Those crude extracts which demonstrate activity against a susceptible strain, but not the resistant strain, are presumed to the respective antibiotics resistance marker. This allows us to null out streptomycins or rifamycins (Ashforth et al. 2010). A series of anti-TB compounds have been found based on this approach (data not published).

Synergistic drugs discovery and their molecular mechanisms

Many therapeutics suffer from having serious side effects and lengthy treatment times. High throughput synergy screening uses quantitative data to discover

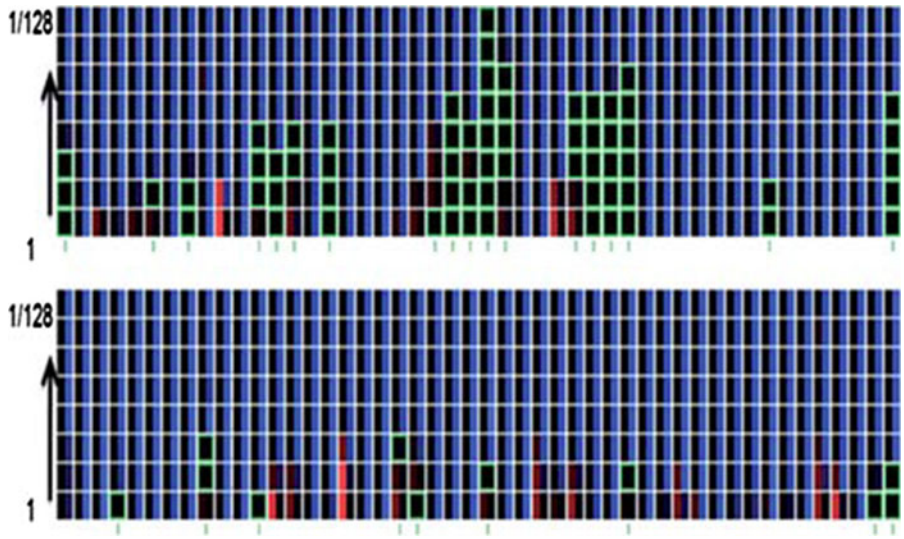

Fig. 2 Comparison of the activity of natural product extracts in vitro growth of *M. tuberculosis* (MTB), and on erythrocyte (RBC) lysis. Each column represents a twofold dilution series of each extract, ranging from 1 to 1/128. The *left side* of the column represents the lysis of RBC (*red*) and the *right side* represents inhibition of tuberculosis (TB H37Rv) growth (*blue*). Quantitative results are indicated by intensity of the color. At concentrations, where RBC lysis is less than 10% and growth inhibition is greater than 80%, the cells are *green*, highlighting concentrations with good anti-TB activity and no RBC toxicity. (Color figure online)

Table 2 Hits from our crude extract library using HTS models (Liu et al. 2010)

Assays	CA	SCA	BCG	BPL	MRSA	14-3-3
Extracts	14800	8000	10800	4000	12800	10000
Hits	84	39	97	42	100	35
Hit rates (%)	0.32	0.48	0.89	1.05	0.78	0.35

CA Candida albicans, *SCA* synergistic anti-fungal assay against *C. albicans*, *BCG Mycobacterium bovis*, *BPL* biotin protein ligase, *MRSA* methicillin-resistant *Staphylococcus aureus*, *14-3-3* 14-3-3 signaling protein

those compounds that are effective under non-toxic concentrations when combined with other classes of drugs. There is currently some discussion as to the definition of synergy but for simplicity it is where the effect of combining two drugs is far greater than the combined effect of drugs used individually. For example, a combination of 2 μg/ml of beauvericin (BEA) and 0.01 μg/ml Ketoconazole (KTC) showed a greater inhibition of *Candida parasilosis* ATCC22019 than 1 μg/ml KTC alone (Zhang et al. 2007; Mueller and Sen 2009). The flavonolignan 5′-methoxyhydnocarpin and berberine alkaloids showed synergistic inhibition of multi-drug resistance pump (Stermitz et al. 2000). Table 3 lists some synergistic anti-fungal compounds found in our laboratory.

KTC, initially synthesized in the laboratories of Janssen Pharmaceuticals, inhibits lanosterol 14-demethylase, which is critical for sterol synthesis in fungi and mammals. It is commonly used to treat *Candida* and mold infections. However, at clinical doses, KTC is associated with toxic side effects, including hepatitis, and resistant strains often emerge during long-term or prophylactic treatment as a result of using high concentrations of the drug.

BEA has been previously identified as a potentiator of antifungal miconozole activity in vitro (Fukuda et al. 2004, 2006), and was found to have synergistic activity with a low dosage of KTC in cell-based assays. The antifungal activities of KTC and BEA combinations reported here greatly exceeded those seen with either drug alone in vitro and in an immunocompromised mouse model. BEA not only improved the efficacy of a much-reduced dosage of KTC but also broadened its spectrum on drug-resistant strains and reduced its side effects. BEA had little antifungal activity, but the synergistic activity of KTC and BEA resulted in fungicidal, in contrast to fungistatic action of KTC. Furthermore, the combination did not significantly affect the proliferation of primary human liver HepG2 cells, indicating that the

Table 3 Synergistic anti-fungal compounds found in our laboratory (Zhang et al. 2007)

Hits identified	M formula	M weight	Producer
Lovastatin	$C_{24}H_{36}O_5$	405	*Aspergillus terreus*
Cyclosporin A	$C_{62}H_{11}N_{11}O_{12}$	1203	*Tolypocladium inflatum*
Beauvericin	$C_{45}H_{57}N_3O_9$	784	*Fusarium proliferatum*
Radicicol	$C_{18}H_{17}ClO_6$	365	*Diheterospora chlamydosporia*
Geldanamycin	$C_{29}H_{40}N_2O_9$	561	*Streptomyces hygroscopicus*
Berberine	$C_{20}H_{18}NO_4$	336	*Berberis fremontii*

combination is selective for fungal pathogens relative to mammalian cells. The synergy mechanism of BEA and KTC is still under investigation but our preliminary observations suggest that an efflux pump in the fungal cell membrane might be a target (Xu et al. 2006).

A serial of new polyketides (1–5) (Fig. 3) were found from a pilot screen of 5,000 crude extracts from library based on this approach (Song et al. 2010).

Other non-antifungal compounds have also been found to enhance the activity of conventional antifungal agents (Afeltra and Verweij 2003), and recent discoveries indicate that Hsp90 and calcineurin inhibitors potentiate fungistatic agents in drug-resistant fungal pathogens (Heitman 2005); the flavonolignan 5′-methoxyhydnocarpin and berberine alkaloids showed synergistic inhibition of multi-drug resistance pump (Stermitz et al. 2000). None of these observations had preclinical backup. BEA and other synergistic compounds isolated from microbial extracts will enable the existing drug KTC to be more effective and will contribute to a better understanding of multiple pathways to cure fungal infections.

Increasing yields of active natural products by precision engineering

When an active compound with therapeutic significance is identified, a large amount of this compound is needed for further testing in the drug discovery program and more, if the compound is suitable for commercial application. Several approaches can be used for large-scale production of a compound, such as chemical synthesis. However, in many cases, production of compounds directly by microbial fermentation is much more economical.

As an empirical method with a long history in strain improvement, random mutagenesis has been widely applied in industry (Elander 2003; Hermann 2003), which successfully generates a series of microbes capable of over-producing metabolites. More recently, a new approach, precision engineering, has been investigated to enable us to optimize an existing biotechnology process and hence to improve the desirable cell properties (Patnaik 2008). The strategy of precision engineering could be developed as shown in Fig. 4.

This new approach has been applied in many aspects, including: extension of substrate range (Gao et al. 2010a; Ostergaard et al. 2000; Sichwart et al. 2011), manipulation of pathway (Moon et al. 2009; Zhuo et al. 2010; Yuan et al. 2011), improvement of productivity (Leonard et al. 2010), and improvements

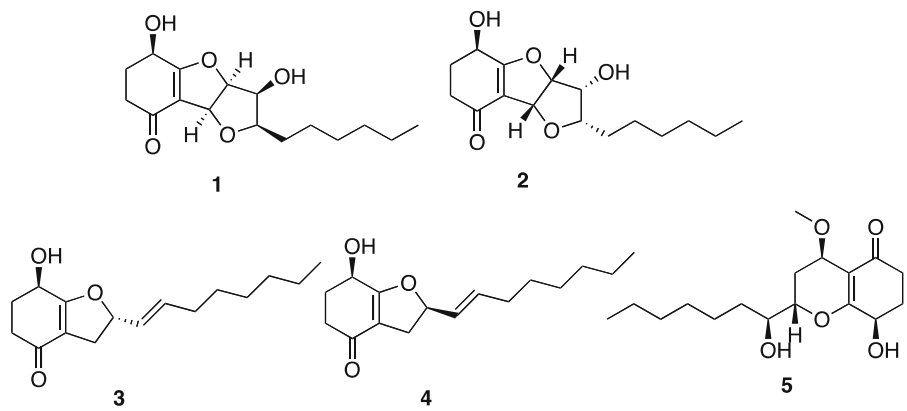

Fig. 3 Active compounds from marine fungus *Trichoderma koningii*

of cellular properties (Sanda et al. 2011; Carvalho et al. 2011; Runquist et al. 2010). Askenazi et al. described an approach to decipher the complex interrelationships between metabolite production trends and gene expression events in order to yield improved production strains. Genomic fragment microarrays were constructed for the *Aspergillus terreus* genome, and transcriptional profiles were generated from strains engineered to produce varying amounts of the medically significant natural product lovastatin (Askenazi et al. 2003).

Precision engineering in rational strain improvement

The application of precision engineering has been limited because of the complicated regulation systems in the cell. Molecular biology used as a rapid developing tool has driven the evolution of precision engineering. These advancements include: transcriptional profiling by DNA microarrays, proteome profiling by two-dimensional electrophoresis, and metabolite profiling by HPLC. Data from these sources enable us to more accurately identify key genetic targets and pathways for improving strains. The applications and examples of precision engineering in rational strain improvement have been well documented (Gao et al. 2010a).

Improving the yield of avermectins by precision engineering

Avermectins, produced by *Streptomyces avermitilis*, are potent against a broad spectrum of nematode and arthropod parasites (Burg et al. 1979). They have few side effects for host organisms and are widely used as anathematic and insecticidal agents (Ikeda and Omura 1995). Avermectins feature a 16-membered pent acyclic with a disaccharide of methylated deoxysugar L-oleandrose polyketides (Zimmermann et al. 2003). Our research group has developed a HTS strategy for efficient identification of avermectin high-yield strains (Gao et al. 2010b). The production protocol was miniaturized in 96 deep-well microplates and UV absorbance at 245 nm was used to monitor avermectin production. A good correlation between fermentation results in both 96 deep-well microplates and conventional Erlenmeyer flasks was observed. With this protocol, the production of avermectins was determined in less than 10 min for a full plate without compromising accuracy. The best high-yield strains selected through this protocol were assessed in 360 m^3 batch fermentations and showed a 1.6-fold improvement in production.

The investigation on the mechanism of high-yield avermectin of industrial overproducer strain *S. avermitilis* was undertaken (Zhuo et al. 2010). Transcriptional levels of the wild type strain and industrial overproducer in production cultures were monitored using microarray analysis. The avermectin biosynthetic genes, especially the pathway-specific regulatory gene, *aveR*, were up-regulated in the high-producing strain. The upstream promoter region of *aveR* was predicted and proved to be directly recognized by σ^{hrdB} in vitro. A mutant library of *hrdB* gene was constructed by error-prone PCR and selected by HTS (Fig. 5). As a result of evolved *hrdB* expressed in the modified avermectin high-producing strain,

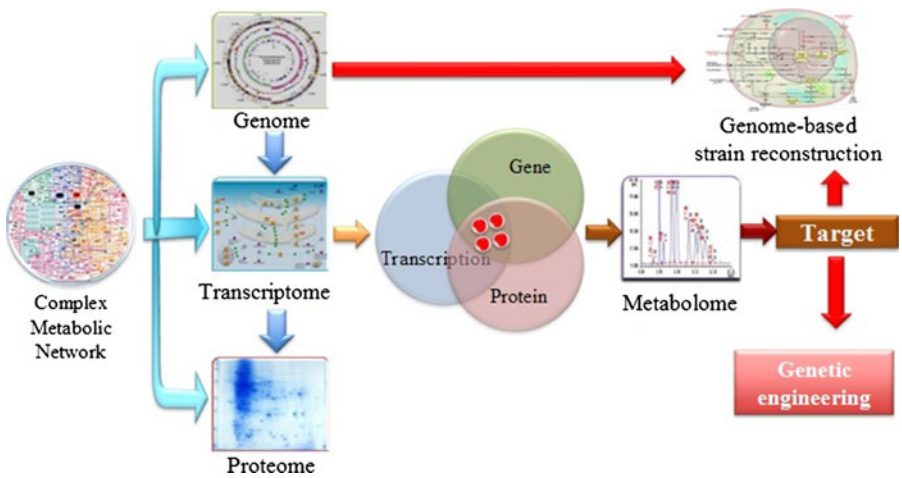

Fig. 4 Schematic of Precision Engineering: genome, transcriptome and proteome data are extracted from the complex metabolic network. Compare the dataset and search the key target/targets in the metabolic network. Then genetic engineering technique is applied for the target/targets and the optimized strain will be obtained

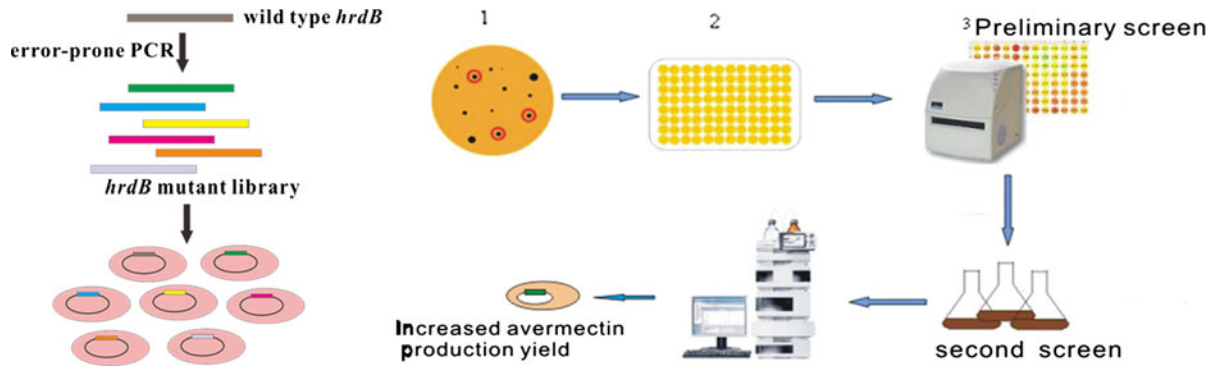

Fig. 5 HTS of a *hrdB* mutant library

6.38 g/l of avermectin B1a was produced with over 50% yield improvement, in which the transcription level of *aveR* was significantly increased. The relevant residues were identified to center in the conserved regions. Engineering of the *hrdB* gene not only elicit the overexpression of *aveR* but also allows for simultaneous transcription of many other genes. This example indicates that manipulating the key genes revealed by precision engineering can effectively improve the yield of the target metabolites, providing a route to optimize production in these complex regulatory systems.

Future perspectives

Natural products and their derivatives have historically been invaluable as a source of therapeutic agents. However, in the past decade, natural products research in the pharmaceutical industry has declined, owing to issues such as the lack of compatibility of traditional natural-product extract libraries with HTS. Furthermore, bioactive natural products identified with HTS have been delayed from progressing further in many cases due to lack of the required purity, availability and productivity of the novel strains and pure compounds (Mishra et al. 2008). As discussed in this review, recent technological advances that help to address these issues, coupled with unrealized expectations from current lead-generation strategies, have led to a renewed interest in natural products in drug discovery.

Microbes that are morphologically distinctive, taxonomically new, or isolated from ecologically unique sources/regions and that have not been subjected to prior screens for natural products are more likely to produce novel natural products. HTS employing a combination of cell and target-based assays is capable of detecting most representative natural products with new chemotypes. HPLC fractionation-guided bioassays and LC-MS follow-up for active fractions can permit rapid dereplication of known natural products from new ones in the extracts, thereby improving the odds of finding novel natural products and allowing prioritization of the strains for subsequent isolation and structural elucidation. Quantities of the natural products can be produced by scale-up of microbial fermentations. Based on precision engineering, the production of drugable secondary metabolites could be significantly improved.

As for the microbial natural products studies in the future, application of molecular biological techniques is increasing the availability of novel compounds that can be conveniently produced in microbes, and combinatorial chemistry approaches are being based on natural product scaffolds to create screening libraries that closely resemble drug-like compounds. Various screening approaches could be developed to improve the ease with which natural products can be used in drug discovery campaigns, and data mining and virtual screening techniques are also being applied to databases of natural products. It is expected that the more efficient and effective application of microbial natural products will improve the drug discovery process.

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