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Drug Discovery Based on Microbial Metabolism and Communities

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Natural products (NPs) are already recognized as a prominent source of compounds with pharmacological values, thus established as the best starting point in drug discovery process [1,2]. Generally these compounds are biosynthesized by plants and microorganisms through versatile metabolic pathways [3]. They are often termed as "secondary metabolites(SM)," since they are synthesized by producer organism but are not essential for their own metabolic processes [4]. These SM or their derivatives are commonly utilized as "drug leads" for the ailment of different disease conditions. Generally they exhibit diverse pharmacophores containing high degree of stereochemistry, but still there is demand of novel NPs with better activities or novel mechanism of action [5,6]. Recently, versatile approaches utilizing synthetic-biology tools, system-biology guided metabolic engineering techniques and enzymatic modifications are utilized for maximizing the applications of such NPs [5,7-9]. Furthermore, the maximal use of such microbial NPs can be improved with considerable efforts on precise screening, higher production, and defined structural variations based on structure-activity-relationship [10,11].

The fundamental process for attaining the bioactive compound from microbial source includes: i) isolation and characterization of microbe ii) establishing culture condition for microbe and production of target NPs iii) isolation, determination of structure and assessment of activity of isolated NPs. Hence, most of these approaches rely on pure culture or single isolate method. The culture dependent approaches provides platform for building the depth and details of microorganisms, and exploration of their microbial physiology and genetics. Hence, for long duration characterization of uncultured bacteria and their biochemical and metabolic potency were ignored [12]. The cultured microorganism cannot substantially represent the microbial world as observed in "great plate count anomaly"- the significant discrepancy between size of population estimated on dilution plating and microscopic observation [13]. Many microbial flora cannot be cultured in media due to their incapability to grow in common media. On the other hand, it is enormous labor to surge for suitable medium components. In some other cases, the growth requirements for optimal temperature, pH, humidity, salinity etc, hinders the proper propagation in the selected media. Only lesser than 2% of bacterial cells are reported to form colonies on conventional plate culture approaches, whereas major of them belong to "viable but not culturable" (VNBC) strains [14]. Recently, there has been substantial progress on the development of conditions mimicking natural environments in terms of pH, nutrient compositions, culture conditions etc. But, still these approaches seems inadequate to study the physiology, biochemistry, and metabolism of all the microbial resources residing on air, water and soil.

Moreover, the laborious culture dependent and microscopy dependent techniques are being replaced by techniques utilizing recent advances of genomics, proteomics and bioinformatics analyses [15]. Metagenomics is most prominent approach for gaining access to the physiology, genetics and metabolic capacity of uncultured organism. . These approaches provide analysis through study of complete microbial community. Hence, it is otherwise termed as community genomics, environmental genomics or population genomics which involves direct isolation of genomic DNA from environment cloning in suitable vector system. The vectors are then introduced to target host for characterization and maintenance which are selected through appropriate screening approaches [12]. Hence, these processes

utilizing direct isolation of genomic DNA from environmental samples prevents requirement of culturing the microbes under study. Recently these studies are conducted based on two important approaches viz sequence based metagenomics analysis or functional metagenomics. The sequence based analysis involves complete sequencing or random sequencing to anchor phylogenetic relationship of particular gene or gene clusters with previously characterized gene/gene clusters. Generally, the high sequence similarity and unwanted repetitions in biosynthetic domains cause the assembly of biosynthetic gene clusters from metagenomics sequences difficult and less successful. However, there are ample of successful examples of metagenomics connection between microbial resources and their biosynthetic gene cluster [16]. The functional metagenomics requires transcription and translation to identify/determine the function of gene/biosynthetic gene cluster based on product formations in screening processes. The strength of later approach is that there is not requirement of prior sequence analysis, thus possess opportunities for characterizing new gene/s functions. The functional approach aim to accelerate the rate of bioactive molecule discovery directly based on screening of the environmental DNA expression platforms for bioactivities of interest. But the major limitation to them is devising precise screening systems in high throughput manner which may be expensive and troublesome. Another constraint can be the availability of suitable cloning vehicle for large chunk of DNA and suitable host system for expression of diverse molecules of interest. By utilizing the conventional approaches and new advances introduced in the field of functional metagenomics, numerous NP biosynthetic gene cluster has been characterized [16]. Further approaches of strain optimizations, metabolic pathway designing and systematic modulations have been employed for easy access to bioactive molecules through the microbial community-based search of bioactive molecules [11].

Similar to metagenomics, concurrently other approaches as "metatranscriptomics" "metaproteomics" and "metabolomics" are popular strategies for studying the physiology, genetics and biochemistry of microbial communities. "Metatranscriptomics [17] involves sequencing of complete transcriptome of the microbial community. It provides functional annotations of expressed genes that are expressed by whole community under specific condition. The metaproteomics [18] provided the profile of functionally expressed genes under such conditions. The metabolomics [19] provides identification and quantification of metabolites released by the microbial community at tested location and condition [20]. Hence, the integration of all these multiple "–omics" data and assessment of all the analytical parameters can offer better access to discovery of novel bioactive microbial metabolites utilizing the complete microbial community at a particular location at certain condition.

Another popular approach utilizing microbial community for identifying new bioactive molecule is co-culture approach. The co-culture systems have been studied for unraveling the interaction between cell populations and fundamentals of cell-cell interactions. Recently, such co-culture approaches have been successfully employed for engineering the complex biosynthetic systems in microbial community [21]. The motivation for co-cultivation experiments has been the identification of new molecules by unlocking the cryptic biosynthetic pathway in the genome of microorganisms. This has been used for many microbial combinations as bacterium-fungus, bacterium-bacterium and fungus-fungus, plant bacterial, etc [21,22]. The development and study of such co-culture systems are feasible to be utilized for re-engineering the biosynthetic pathways in even difficult to manipulate or difficult to manipulate organisms. The co-culture studies are complex, usually requiring extensive optimizations for adjusting the culture and metabolite production parameters. Similarly, all the analyzed diversified metabolite profile may not be easily predicted from genome information. However, there have been ample examples illustrating the importance of co-culture systems in discovery of novel secondary metabolites. The NMR-based metabolomics has been a significant achievement for characterization of novel natural products derived from co-culture [23]. Further refinements on such robust analytical techniques are definite to revolutionize the microbial community driven drug discovery processes.

In conclusion, the drug discovery process has a paradigm shift from one-strain-major-compound (OSMM) to one-strain-many compound (OSMC) concept, which has introduced numerous chemical diversities. Further, the comprehensive approach of mining the bioactive NPs mediated by systematic analysis of entire microbial community has created larger chemical space than expectation [11,24].

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