Hindawi Publishing Corporation
Chinese Journal of Biology
Volume 2014, Article ID 146030, 7 pages
http://dx.doi.org/10.1155/2014/146030

Review Article

Metagenomics: An Application Based Perspective

Yasir Bashir, Salam Pradeep Singh, and Bolin Kumar Konwar

Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur, Assam 784028, India

Correspondence should be addressed to Salam Pradeep Singh; salampradeep@gmail.com

Received 26 May 2014; Revised 14 August 2014; Accepted 15 August 2014; Published 21 August 2014

Academic Editor: Jingyuan Song

Metagenomics deals with the isolation of genetic material directly recovered from environmental samples. Metagenomics as an approach has emerged over the past two decades to elucidate a host of microbial communities inhabiting a specific niche with the goal of understanding their genetic diversity, population structure, and ecological role played by them. A number of new and novel molecules with significant functionalities and applications have been identified through this approach. In fact, many investigators are engaged in this field to unlock the untapped genetic resources with funding from governments sector. The sustainable economic future of modern industrialized societies requires the development of novel molecules, enzymes, processes, products, and applications. Metagenomics can also be applied to solve practical challenges in the field of medicine, agriculture, sustainability, and ecology. Metagenomics promises to provide new molecules and novel enzymes with diverse functions and enhanced features compared to the enzymes from the culturable microorganisms. Besides the application of metagenomics for unlocking novel biocatalysts from nature, it also has found applications in fields as diverse as bioremediation, personalized medicine, xenobiotic metabolism, and so forth.

1. Introduction

The term metagenomics, the genomic analysis of a population of microorganisms, was coined by Handelsman et al. with a notion to analyse a collection of similar but not identical items, as in the statistical concept of meta-analysis [1]. The idea that the whole environmental microbiome can be explored and analysed together has revolutionized our understanding of the ecology around us. It has opened new horizons in the development of biotechnology based on the exploitation of uncultivated microbial species. The vast majority of microorganisms being unculturable [2], metagenomics has resulted in discoveries that remained hidden from the traditional culturing techniques. Though a multifaceted approach, the crux of applied metagenomics is to express recovered genes in a cultivable heterologous host. A booming area of biotechnology is the industrial use of microorganisms to produce antibiotics, enzymes, and other bioactive compounds. The demand for the commercial production of enzymes that are used in large-scale industrial processes is growing rapidly. The industrial applications of metagenomics include identification of novel biocatalysts, discovery of new antibiotics, personalized medicine, and bioremediation. In addition, biosurfactant producing bacteria have been successfully used for the bioremediation of industrial, agricultural, and domestic wastes, resulting in a reduction of the environmental pollution. A wealth of information has been uncovered by metagenomics, such as microbial diversity, vast swathes of uncharacterized metabolism, and increased complexity of biogeochemical pathways and it promises to provide new enzymes and molecules with diverse applications [3]. In fact, the crystal structure of metagenomics derived RNase H1 has also been determined which indicated structure-based mutational shift at the active site of the motif [4]. López-López et al. also discovered metagenomic extremophilic esterases having a different active site compared to the esterases produced by culturable microorganisms [5]. Schallmey and coworkers also harvested novel metagenomics polyhydroxyalkanoate (PHA) synthase encoding genes [6]. The DNA sequence analysis of the studied clones indicates that the complementing genes are homologous but substantially different from the known polyhydroxyalkanoate synthase-encoding genes. Such genes are of great potential for the industrial production of bioplastics [6]. Additionally, a metagenomic library constructed from crude oil-contaminated soil encoding polyhydroxyalkanoate

Table 1: Biocatalysts screened from metagenomic libraries by function-based approach.

Function	Habitat	Library type	Substrate	Reference
Alkaline pectate lyase	Soil	Plasmid	Pectin	[41]
Amylase	Soil	Cosmid	Starch	[43]
α-amylase	Soil	Fosmid	Starch	[44]
Cellulase	Soil	Plasmid	Carboxymethyl-cellulose	[15]
Cellulase	Soil	Fosmid	Hydroxyethyl cellulose	[14]
Endocellulase	Soil	Plasmid	Carboxymethyl-cellulose	[13]
Endoglucanase	Rice straw compost	λ phage	Carboxymethyl-cellulose	[12]
Lipase	Marine sediment	Plasmid	p-Nitrophenyl (pNP) esters	[22]
Lipase	Marine sponge (*Haliclonasimulans*)	Fosmid	Tributyrin agar	[25]
Lipase		Fosmid	p-Nitrophenyl (pNP) esters	[26]
Lipase	Soil	Fosmid	Tributyrin agar	[27]
Lipase	Sediment	Fosmid	Triolein	[29]
Pectinase	Lagoon	Plasmid	Polygalacturonic acid	[40]
Protease	Soil	Plasmid	Azocasein	[36]
Serine proteases	Soil	Plasmid	Skim milk agar	[66]
Xylanase/endoglucanase	Sand Yak rumen	Cosmid	Xylan/CMC	[30]
Xylanase		Fosmid	Birch wood xylan	[31]
Xylanase	Holstein cattle	Fosmid	Xylan	[32]
Xylanase	rumen	Plasmid	Xylan	[23]
Xylanase	Compost soil Rumen	BAC	Oat spelt xylan	[34]

synthase showed 76% identity with the synthase of *Alcaligenes* sp. [7]. Moreover, revealing the importance of metagenomics and unculturable microorganisms, microbiome project, such as the Human Microbiome Project and Gut Microbiome Projects, has been initiated. The ultimate goal of the project is to understand the changes in the human microbiome which are associated with human health or disease. The present review mostly highlights the recent applications of metagenomics to microbial ecology and industrial biotechnology.

2. Industrial Enzymes

There is ample demand for novel enzymes and biocatalysts, and metagenomics is currently thought to be one of the most likely technologies to provide the required candidate molecules [8, 9]. Cellulases, lipases, xylanases, amylases, proteases, and various other industrially important enzymes have been produced through metagenomics. The following are some of the main enzymes that have been unlocked from genetically untapped resources.

2.1. Cellulases. Cellulases catalyze the hydrolysis of cellulose, the most abundant biopolymer on earth. They are use in paper recycling, cotton processing, juice extraction, detergent enzymes, animal feed additives, and so forth. Thus, cellulose is the third largest industrial enzymes worldwide, by dollar volume [10]. Metagenomics has been used to unlock novel cellulases from various natural environments, namely, compost soils, soil from cold regions, rumen samples, and so forth (Table 1) by constructing the metagenomic libraries followed by screening of the biologically active clones. These biocatalysts have gained considerable importance owing to their potential candidature for the bioconversion of biomass into renewable liquid fuels. In fact there is a major funding from Department of Energy, USA, for three bioenergy research centers: one led by the Berkeley Argonne National Laboratory, one led by Oak Ridge National Laboratory, and one led by the University of Wisconsin that will study all aspects of liquid biofuel production from biomass sugars (http://genomicscience.energy.gov/centers/). A breakthrough study in this regard was carried out by Hess et al. and identified 27,755 candidate genes with a significant match to at least one relevant catalytic domain or carbohydrate-binding module [11]. They generated tremendous data in order to demonstrate the potential of deep sequencing of a complex community to accurately reveal cellulolytic genes at a massive scale and to generate draft genomes of uncultured novel organisms involved in biomass deconstruction. Other studies focusing on unlocking novel cellulases of industrial importance from nature using metagenomics technique have met a lot of success [12–14]. In addition, Alvarez et al. isolated and characterized a novel cellulase from a sugarcane soil metagenome [15], while Duan et al. isolated and characterized metagenomic gene encoding acidic cellulases from buffalo rumen metagenome [16] and Voget et al. also characterized a metagenome-derived halotolerant cellulase which is highly stable revealing the importance of metagenomics cellulases.

The metagenome-derived cellulase is ideal for industrial applications [17]. Its unconventional characteristics render it as a potential candidature to be employed in biotechnological processes that require the more tolerant and alkaline cellulases. Given the amount of research that is underway in order to unlock novel cellulases from nature, it is expected that the vast gene mining for cellulase enzymes will become literally possible in the near future.

2.2. Lipases. Lipases are triacylglycerol hydrolases that catalyze the hydrolysis and synthesis of long chain acyl glycerol with trioleyl glycerol being standard substrate [18]. The use of lipases in detergents, food industry, pulp and paper industry, agrochemicals, cosmetics and flavours, diagnostics, therapeutics, leather industry, biodiesel production, and biopolymer synthesis has been well reviewed [19, 20]. The hunt for novel lipases continues unabated as evidenced by the discovery of new families of microbial lipases mostly by metagenomic approaches [21]. Among the hundreds of sequences encoding lipases that have been identified through recent metagenomic studies, it is notable that novel sequences are frequently reported. Peng et al. isolated a novel alkaline-stable lipase from a metagenomic library constructed from marine sediments and concluded that this novel lipase may be used to impart a distinctive and desirable flavour and odour in milk fat flavor production [22]. Lee et al. isolated and characterized a novel metagenomic lipase from tidal flat sediments which evidence a new family of bacterial lipase [23]. Hårdeman and Sjöling also isolated a novel low-temperature active lipase from uncultured bacteria of marine sediment. The conserved regions, including the putative active site and catalytic triad, were found to be similar to the culturable lipases [24]. A novel halotolerant lipase was isolated following a functional screening of a marine sponge fosmid metagenomic library by Selvin et al. [25]. The stability and activity over a wide range of salinity, pH, and temperature and in the presence of organic solvent and metal ions suggest a utility for this enzyme in a variety of industrial applications. In the recent past, lipases isolated and characterized by Ngo et al. [26], Fu et al. [27], Chow et al. [28], and Glogauer et al. [29] from various metagenomic libraries showed novel characteristics, namely, thermal stability, alkaline stability, organic solvent tolerance, cold active nature, and so forth, making them potential candidates for industrial use. The vast mining of genetically untapped sources for lipases of certain unique and desired features like substrate specificity, enantioselectivity, extreme temperature, pH, tolerance, and so forth using culture-independent metagenomic approach has proved it to be a promising approach for biotechnological advancement.

2.3. Xylanases. Hemicellulose, the second most abundant renewable polymer in lingo cellulosic material after cellulose, consists of a complex matrix of polysaccharides constructed from xylan (β-1,4-linked xylose) and mannan (β-1,4-linked mannose). The principle enzyme in the progressive breakdown of xylan is endo-β-1, 4-xylanase which attacks the nonhydrolyzed polymer. In order to efficiently utilize plant biomass for biofuel production, lignocellulose degrading enzymes need to be widely developed and utilized. The accessibility of hemicellulose, apart from cellulose, for biofuel production is limited by crosslinking between lignin, cellulose, and hemicellulose via ester and ether linkages. To overcome this problem, the need for xylanases has triggered massive scientific endeavour to unlock novel xylanases from nature using metagenomic approach that may be used to hydrolyze these linkages. The properties like thermostability and tolerance to extreme pH conditions are inevitable for such purposes. The studies of Chang et al. [30], Cheng et al. [31], and Jeong et al. [32] yielded significant results with xylanases having immense biotechnological applications for lignocellulosic deconstruction and bioethanol production. Moreover, Verma et al. also isolated a novel metagenomic xylanase from compost-soil metagenome that shows alkali stability and thermostability, thus bearing a potential application in paper and pulp industry in pulp bleaching [33]. In another interesting fact, the rumen is the most potent fiber fermentation system. Microbes play an essential role in degrading lignocellulosic materials of plants in the rumen, which represents a potentially important resource of lignocellulose degrading enzymes. Gong et al. isolated and characterized a GH10 family xylanase from uncultured microorganisms in cow rumen through functional screening of a metagenomic BAC library [34]. The industrial application of this enzyme is manifested by its properties including a broad pH profile, high pH stability, particularly at basic pH, and high substrate specificity including a lack of cellulase activity.

2.4. Proteases. Proteases represent one of the three largest groups of industrial enzymes, and most of them are of bacterial origin [35]. The applications of proteases span a wide range of use including protein stain removal, fuel, food preparation, biofilm removal, and leather preparation. Several proteases have been discovered using metagenomic approach in the recent times. Biver et al. isolated an alkaline protease that displayed oxidant stability suggesting its possible applications in the detergent and bleaching industries [36]. Two serine proteases from surface sand of deserts were found to be relatively resistant to detergent, making them interesting for possible industrial applications [29]. A novel protease belonging to chymotrypsin-like S1 serine proteases was isolated by Niehaus et al. [37]. Neveu et al. also isolated two serine proteases from metagenomic libraries of the Gobi and Death Valley deserts [38], while Pushpam et al. identified and characterized metagenomic alkaline serine protease from the metagenome of goat skin surface [39]. This class of enzymes had not been described earlier for use in laundry and cleaning applications. Proteases also find use in diagnostic industries and thus a lot of effort is needed to uncover novel proteases with better characteristics with special emphasis on metalloproteases and serine proteases from different habitats including extreme environments.

2.5. Pectinases. Pectin is an important component of the middle lamella and primary cell wall in plants and fungi. It consists of a backbone of galacturonic acid residues linked

by α-1, 4 glycosidic linkages. Pectic polymers are degraded by the action of pectinolytic enzymes. Pectinases play an important role in fruit ripening, fruit abscission, and plant diseases. Novel enzymes showing thermostability and other characteristics are constantly being sought to using metagenomics. Singh et al. discovered a novel pectinase from the soil metagenome that was found to be thermostable and thermoactive [40]. The study warranted the importance of this enzyme in industrial processes and for studies to elucidate structure/function relationships and to design inhibitors. A metagenome-derived alkaline pectate lyase showed diverse properties including high specific activity, alkaline pH specificity, thermo- and alkali stability at operating conditions (for pH 9.8 and 45°C), and broad substrate specificity, suggesting that this enzyme can be used in many industries [41].

2.6. Amylases. Amylases are among the most important hydrolytic enzymes belonging to family 13 (GH-13) of the glycoside hydrolase group of enzymes [42]. It is a widespread group of enzymes which hydrolyze starch molecules to give diverse products including dextrins and progressively smaller polymers composed of glucose units. These enzymes are of great significance in biotechnology with applications in all starch-based industries ranging from food, fermentation, textile, and paper industries to alcohol production in breweries. With the advent of new frontiers in biotechnology, the spectrum of amylase applications has expanded into many other fields, such as clinical, medicinal, and analytical chemistry. Sharma et al. discovered a novel amylase from a soil metagenome that retained 90% of activity even at low temperature suggesting its potential candidature for possible industrial applications [43]. Vidya et al. isolated a thermostable and calcium-dependant amylase from a soil metagenome and suggested its applications in baking and destarching [44]. The need for thermostable amylases for starch liquefaction, which needs a high temperature (above 100°C), is imperative and metagenomics has proved to be inevitable in unlocking the same from nature. In addition, Sharma et al. cloned and characterized a metagenomic amylase from the soil derived from Northwestern Himalayas which comprises 909 amino acids encoding the enzyme amylase [43].

3. Bioactive Compounds and Antibiotics

When Gillespie et al. isolated turbomycins A and B from a metagenomic library, it demonstrated the feasibility of metagenomic approaches for searching novel antimicrobial compounds [45]. Chang and Brady reported a gene cluster *bor* from soil that encodes indolotryptoline based compounds, a small and relatively rare family of natural products that exhibit potent activity against certain tumor cell lines [46]. These discoveries have triggered extensive search operations for the quest of novel and biomedical important drugs as evidenced by various studies [47–52]. The search for bioactive molecules using a metagenomic approach has generally been conducted using either homology-based methods or functional screening. Novel sequences found in homology-based screens can be examined for the ability to encode the biosynthesis of novel small molecules in heterologous expression experiments [52]. Development of expression systems with new selective, specific, and sensitive testers plus high-throughput platforms is needed to provide options that fully exploit the potential within metagenomic libraries. An interesting system, designated METREX, has been designed which consists of an expression host carrying a GFP reporter sensitive to compounds that induce quorum sensing; expression of metagenomic DNA library clones encoding genes for synthesis of bioactive small molecules that induced the reporter could then be isolated by fluorescence [53]. Although only a small number of compounds have been characterized till date using culture-independent methods, these initial studies indicate that as-yet-uncultured bacteria are likely to be a rich source of novel bioactive molecules. Table 2 shows the list of antibiotics that have been discovered using the metagenomic approach.

Table 2: Antibiotics discovered by functional screening of metagenomic clones.

Antibiotics	Habitat	Library type	Reference
Beta-lactamases	Soil	Fosmid	[47]
Fasamycin A and B	Soil	Cosmid	[49]
Indirubin	Soil	Fosmid	[51]
Terragine	Soil	Cosmid	[50]
Turbomycins A and B	Soil	BAC	[45]
Violacein	Soil	Cosmid	[49]

4. Xenobiotic Degradation and Personalized Medicine

The study of the action of xenobiotics, particularly, the antibiotics, on human gut microbiota is indispensable given the need to study the mechanism of drug resistance and the gene responsible for it in order to counter the menace of ever increasing drug resistance and to come up with drugs that are much effective and less likely to be countered by the invading pathogens. Elucidating the underlying mechanisms for xenobiotic resistance and metabolism in the active human gut microbiome not only will provide insight into host-microbial interactions and biochemistry but also may provide indications for unexplained patient-to-patient variations in drug efficacy and toxicity. This issue is, in part, being addressed by metagenomics that has enabled the analysis of the aggregate genomes of this microbial community, the gut microbiome [54]. Maurice et al. characterized how the gene expression and metabolic activity of a distinctive set of active gut microbes are rapidly affected by host-targeted drugs and antibiotics [55]. These findings highlighted the unintended consequences of xenobiotics and indicated the microbiota as another factor to consider in developing personalized medicines. The integrated characterization of the host, microbial, and environmental factors governing the response of the gut microbiome to xenobiotics could

ultimately be used for the design of diagnostic tests predicting drug pharmacokinetics or therapeutic interventions.

5. Biosurfactant Mediated Bioremediation

Chemical surfactants are currently employed in the treatment of petroleum hydrocarbons in oil spills, allowing their emulsification, thereby increasing their solubility and facilitating subsequent degradation by oil-degrading microbes. However, the use of these chemical surfactants in bioremediation is barred by their toxicity and low biodegradability [56]. Thus, the use of biosurfactants may represent an environmentally friendly alternative to help overcome the toxicity of these synthetic surfactants [57].

Biosurfactants are amphipathic molecules with both hydrophilic and hydrophobic moieties that partition preferentially at the interface between fluid phases that have different degrees of polarity and hydrogen bonding, such as oil and water or air and water interfaces [58]. Metagenomics has been used to make DNA libraries of the petroleum-contaminated samples (soil, water, etc.) followed by the screening of biosurfactant producing clones. A number of screening methods have been designed to screen the metagenomic clones for the production of biosurfactant, namely, atomized oil assay, wherein a fine mist of oil droplets is applied to the surface of agar plates and biosurfactant production is observed instantaneously as halos around metagenomics clones [59]; oil-coated agar plate overlay approach, with biosurfactant producers being identified by the appearance of an emulsified halo [60]; screening for haemolytic activity using nutrient agar supplemented with 5% fresh whole blood, with blood cell lysis being indicative of biosurfactant production [61]; and the blue agar method with mineral salts agar supplemented with a 2% carbon source, CTAB (0.0005%), and methylene blue (0.002%), with dark blue halos indicating biosurfactant production [62]. Other novel function-based approaches which have been developed include substrate-induced gene expression (SIGEX) for screening of metagenomic libraries [63]. Thus, by employing the above described screening methods, it is likely that novel gene clusters involved in the biosurfactant production could be identified thereby accelerating the development of bioremediation technologies involving biosurfactants. In addition, the metagenomics oriented bioremediation research has already been initiated. In fact, there are reports of metagenomics alkane hydroxylase enzyme degrading hydrocarbon that has been reported very recently [64].

6. Conclusion

Metagenomics provides a window into a world of unseen microbial diversity that is astonishing in its magnitude and is open to tap the vast genetic potential of microorganisms to obtain products and processes of biotechnological value. The use of metagenomics for exploiting the whole microbiome of a given environmental sample has met a lot of success through breakthrough discoveries as far as the industrial sector is concerned. The quest for novel biocatalysts through metagenomics has yielded positive results with novel enzymes being unlocked from the genetically untapped resources that find applications in varied fields. The production of biosurfactants and antibiotics using metagenomics has given a ray of hope for addressing the issues of oil leakage and drug resistance, respectively. Furthermore, the degradation of synthetic compounds, essential to protect the environment from pollution, has been addressed by metagenomics. Metagenomics thus provides a means to view the structural and functional genomics of microbial diversity and paves a way to discover novel genes for obtaining newer and useful industrial enzymes and bioactive molecules with better properties. This recent trend to access novel and natural sequences through the direct cloning of metagenomic DNA is significantly contributing to the screening and identification of hitherto unexplored microbial consortia for valuable bioactive compounds, biocatalysts, and other useful products and processes. However, the success of these techniques demands the development of faster high-throughput screening systems.

Though metagenomic approaches have identified a number of novel genes encoding cellulolytic, pectinolytic, proteolytic, lipolytic enzymes, and so forth and many interesting novel enzymes have been discovered, the fact remains that the majority of biocatalysts are still uncharacterized [65]. Further, since the functional heterologous expression of genes from unculturable microbes is not always successful, approaches like metatranscriptomics and stable isotope probing (SIP) could be adapted for the high resolution identification in the hunt for novel biocatalysts.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Yasir Bashir is grateful to Tezpur University, India, for providing the Institutional fellowship. Necessary facilities provided by the Department of Molecular Biology & Biotechnology, Tezpur University, are thankfully acknowledged.

References

[1] J. Handelsman, M. R. Rondon, S. F. Brady, J. Clardy, and R. M. Goodman, "Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products," *Chemistry and Biology*, vol. 5, no. 10, pp. R245–R249, 1998.

[2] V. Torsvik, J. Goksoyr, and F. L. Daae, "High diversity in DNA of soil bacteria," *Applied and Environmental Microbiology*, vol. 56, no. 3, pp. 782–787, 1990.

[3] J. A. Gilbert and C. L. Dupont, "Microbial metagenomics: beyond the genome," *Annual Review of Marine Science*, vol. 3, pp. 347–371, 2011.

[4] T. Nguyen, D. You, E. Kanaya, Y. Koga, and S. Kanaya, "Crystal structure of metagenome-derived LC9-RNase H1 with atypical

DEDN active site motif," *The FEBS Letters*, vol. 587, no. 9, pp. 1418–1423, 2013.

[5] O. López-López, M. E. Cerdán, and M. I. Gonzalez-Siso, "New extremophilic lipases and esterases from metagenomics," *Current Protein and Peptide Science*, vol. 15, pp. 445–455, 2014.

[6] M. Schallmey, A. Ly, C. Wang et al., "Harvesting of novel polyhydroxyalkanaote (PHA) synthase encoding genes from a soil metagenome library using phenotypic screening," *FEMS Microbiology Letters*, vol. 321, no. 2, pp. 150–156, 2011.

[7] S. Cheema, M. Bassas-Galia, P. M. Sarma, B. Lal, and S. Arias, "Exploiting metagenomic diversity for novel polyhydroxyalkanoate synthases: production of a terpolymer poly(3-hydroxybutyrate-co-3-hydroxyhexanoate-co-3-hydroxyoctanoate) with a recombinant Pseudomonas putida strain," *Bioresource Technology*, vol. 103, no. 1, pp. 322–328, 2012.

[8] P. Lorenz, K. Liebeton, F. Niehaus, and J. Eck, "Screening for novel enzymes for biocatalytic processes: accessing the metagenome as a resource of novel functional sequence space," *Current Opinion in Biotechnology*, vol. 13, no. 6, pp. 572–577, 2002.

[9] P. D. Schloss and J. Handelsman, "Biotechnological prospects from metagenomics," *Current Opinion in Biotechnology*, vol. 14, no. 3, pp. 303–310, 2003.

[10] D. B. Wilson, "Cellulases and biofuels," *Current Opinion in Biotechnology*, vol. 20, no. 3, pp. 295–299, 2009.

[11] M. Hess, A. Sczyrba, R. Egan et al., "Metagenomic discovery of biomass-degrading genes and genomes from cow rumen," *Science*, vol. 331, no. 6016, pp. 463–467, 2011.

[12] Y. Yeh, S. C. Chang, H. Kuo, C. Tong, S. Yu, and T. D. Ho, "A metagenomic approach for the identification and cloning of an endoglucanase from rice straw compost," *Gene*, vol. 519, no. 2, pp. 360–366, 2013.

[13] A. Bhat, S. Riyaz-Ul-Hassan, N. Ahmad, N. Srivastava, and S. Johri, "Isolation of cold-active, acidic endocellulase from Ladakh soil by functional metagenomics," *Extremophiles*, vol. 17, no. 2, pp. 229–239, 2013.

[14] H. Nacke, M. Engelhaupt, S. Brady, C. Fischer, J. Tautzt, and R. Daniel, "Identification and characterization of novel cellulolytic and hemicellulolytic genes and enzymes derived from German grassland soil metagenomes," *Biotechnology Letters*, vol. 34, no. 4, pp. 663–675, 2012.

[15] T. M. Alvarez, J. H. Paiva, D. M. Ruiz et al., "Structure and function of a novel cellulase 5 from sugarcane soil metagenome," *PLoS ONE*, vol. 8, no. 12, Article ID e83635, 2013.

[16] C.-J. Duan, L. Xian, G.-C. Zhao et al., "Isolation and partial characterization of novel genes encoding acidic cellulases from metagenomes of buffalo rumens," *Journal of Applied Microbiology*, vol. 107, no. 1, pp. 245–256, 2009.

[17] S. Voget, H. L. Steele, and W. R. Streit, "Characterization of a metagenome-derived halotolerant cellulase," *Journal of Biotechnology*, vol. 126, no. 1, pp. 26–36, 2006.

[18] F. Ferrato, F. Carriere, L. Sarda, and R. Verger, "A critical reevaluation of the phenomenon of interfacial activation," *Methods in Enzymology*, vol. 286, pp. 327–347, 1997.

[19] K. Jaeger and T. Eggert, "Lipases for biotechnology," *Current Opinion in Biotechnology*, vol. 13, no. 4, pp. 390–397, 2002.

[20] P. K. Ghosh, R. K. Saxena, R. Gupta, R. P. Yadav, and S. Davidson, "Microbial lipases: production and applications," *Science progress*, vol. 79, p. 2, 1996.

[21] S. Nagarajan, "New tools for exploring old friends-microbial lipases," *Applied Biochemistry and Biotechnology*, vol. 168, no. 5, pp. 1163–1196, 2012.

[22] Q. Peng, X. Wang, M. Shang et al., "Isolation of a novel alkaline-stable lipase from a metagenomic library and its specific application for milkfatflavor production," *Microbial Cell Factories*, vol. 13, no. 1, 2014.

[23] M. Lee, C. Lee, T. Oh, J. K. Song, and J. Yoon, "Isolation and characterization of a novel lipase from a metagenomic library of tidal flat sediments: evidence for a new family of bacterial lipases," *Applied and Environmental Microbiology*, vol. 72, no. 11, pp. 7406–7409, 2006.

[24] F. Hårdeman and S. Sjöling, "Metagenomic approach for the isolation of a novel low-temperature-active lipase from uncultured bacteria of marine sediment," *FEMS Microbiology Ecology*, vol. 59, no. 2, pp. 524–534, 2007.

[25] J. Selvin, J. Kennedy, D. P. H. Lejon, G. S. Kiran, and A. D. W. Dobson, "Isolation identification and biochemical characterization of a novel halo-tolerant lipase from the metagenome of the marine sponge Haliclona simulans," *Microbial Cell Factories*, vol. 11, article 72, 2012.

[26] T. D. Ngo, B. H. Ryu, H. Ju et al., "Structural and functional analyses of a bacterial homologue of hormone-sensitive lipase from a metagenomic library," *Acta Crystallographica D: Biological Crystallography*, vol. 69, part 9, pp. 1726–1737, 2013.

[27] J. Fu, H.-K. S. Leiros, D. de Pascale, K. A. Johnson, H.-M. Blencke, and B. Landfald, "Functional and structural studies of a novel cold-adapted esterase from an Arctic intertidal metagenomic library," *Applied Microbiology and Biotechnology*, vol. 97, no. 9, pp. 3965–3978, 2013.

[28] J. Chow, F. Kovacic, Y. Dall Antonia et al., "The metagenome-derived enzymes LipS and LipT increase the diversity of known lipases," *PLoS ONE*, vol. 7, no. 10, Article ID e47665, 2012.

[29] A. Glogauer, V. P. Martini, H. Faoro et al., "Identification and characterization of a new true lipase isolated through metagenomic approach," *Microbial Cell Factories*, vol. 10, article 54, 2011.

[30] L. Chang, M. Ding, L. Bao, Y. Chen, J. Zhou, and H. Lu, "Characterization of a bifunctional xylanase/endoglucanase from yak rumen microorganisms," *Applied Microbiology and Biotechnology*, vol. 90, no. 6, pp. 1933–1942, 2011.

[31] F. Cheng, J. Sheng, R. Dong, Y. Men, L. Gan, and L. Shen, "Novel xylanase from a holstein cattle rumen metagenomic library and its application in xylooligosaccharide and ferulic acid production from wheat straw," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 51, pp. 12516–12524, 2012.

[32] Y. S. Jeong, H. B. Na, S. K. Kim et al., "Characterization of Xyn10J, a novel family 10 xylanase from a compost metagenomic library," *Applied Biochemistry and Biotechnology*, vol. 166, no. 5, pp. 1328–1339, 2012.

[33] D. Verma, Y. Kawarabayasi, K. Miyazaki, and T. Satyanarayana, "Cloning, expression and characteristics of a novel alkalistable and thermostablexylanase encoding gene (Mxyl) retrieved from compost-soil metagenome," *PLoS ONE*, vol. 8, no. 1, Article ID e52459, 2013.

[34] X. Gong, R. J. Gruniniger, R. J. Forster, R. M. Teather, and T. A. McAllister, "Biochemical analysis of a highly specific, pH stable xylanase gene identified from a bovine rumen-derived metagenomic library," *Applied Microbiology and Biotechnology*, vol. 97, no. 6, pp. 2423–2431, 2013.

[35] E. Orhan, D. Omay, and Y. Güvenilir, "Partial purification and characterization of protease enzyme from *Bacillus subtilis* and *Bacillus cereus*," *Applied Biochemistry and Biotechnology A Enzyme Engineering and Biotechnology*, vol. 121, no. 1–3, pp. 183–194, 2005.

[36] S. Biver, D. Portetelle, and M. Vandenbol, "Characterization of a new oxidant-stable serine protease isolated by functional metagenomics," *SpringerPlus*, vol. 2, article 410, 2013.

[37] F. Niehaus, E. Gabor, S. Wieland, P. Siegert, K. H. Maurer, and J. Eck, "Enzymes for the laundry industries: tapping the vast metagenomic pool of alkaline proteases," *Microbial Biotechnology*, vol. 4, no. 6, pp. 767–776, 2011.

[38] T. J. Neveu, C. Regeard, and M. S. Dubow, "Isolation and characterization of two serine proteases from metagenomic libraries of the Gobi and Death Valley deserts," *Applied Microbiology and Biotechnology*, vol. 91, no. 3, pp. 635–644, 2011.

[39] P. L. Pushpam, T. Rajesh, and P. Gunasekaran, "Identification and characterization of alkaline serine protease from goat skin surface metagenome," *AMB Express*, vol. 1, article 3, 2011.

[40] R. Singh, S. Dhawan, K. Singh, and J. Kaur, "Cloning, expression and characterization of a metagenome derived thermoactive/thermostable pectinase," *Molecular Biology Reports*, vol. 39, no. 8, pp. 8353–8361, 2012.

[41] H. Wang, X. Li, Y. Ma, and J. Song, "Characterization and high-level expression of a metagenome-derived alkaline pectatelyase in recombinant *Escherichia coli*," *Process Biochemistry*, vol. 49, pp. 69–76, 2013.

[42] A. Bordbar, K. Omidiyan, and R. Hosseinzadeh, "Study on interaction of α-amylase from Bacillus subtilis with cetyl trimethylammonium bromide," *Colloids and Surfaces B: Biointerfaces*, vol. 40, no. 1, pp. 67–71, 2005.

[43] S. Sharma, F. G. Khan, and G. N. Qazi, "Molecular cloning and characterization of amylase from soil metagenomic library derived from Northwestern Himalayas," *Applied Microbiology and Biotechnology*, vol. 86, no. 6, pp. 1821–1828, 2010.

[44] J. Vidya, S. Swaroop, S. K. Singh, D. Alex, R. K. Sukumaran, and A. Pandey, "Isolation and characterization of a novel α-amylase from a metagenomic library of Western Ghats of Kerala, India," *Biologia*, vol. 66, no. 6, pp. 939–944, 2011.

[45] D. E. Gillespie, S. F. Brady, A. D. Bettermann et al., "Isolation of antibiotics turbomycin A and B from a metagenomic library of soil microbial DNA," *Applied and Environmental Microbiology*, vol. 68, no. 9, pp. 4301–4306, 2002.

[46] F. Chang and S. F. Brady, "Discovery of indolotryptoline antiproliferative agents by homology-guided metagenomic screening," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 7, pp. 2478–2483, 2013.

[47] H. K. Allen, L. A. Moe, J. Rodbumrer, A. Gaarder, and J. Handelsman, "Functional metagenomics reveals diverse β-lactamases in a remote Alaskan soil," *The ISME Journal*, vol. 3, no. 2, pp. 243–251, 2009.

[48] S. F. Brady, C. J. Chao, J. Handelsman, and J. Clardy, "Cloning and heterologous expression of a natural product biosynthetic gene cluster from eDNA," *Organic Letters*, vol. 3, no. 13, pp. 1981–1984, 2001.

[49] Z. Feng, D. Chakraborty, S. B. Dewell, B. V. B. Reddy, and S. F. Brady, "Environmental DNA-encoded antibiotics fasamycins A and B inhibit FabF in type II fatty acid biosynthesis," *Journal of the American Chemical Society*, vol. 134, no. 6, pp. 2981–2987, 2012.

[50] G. Wang, E. Graziani, B. Waters et al., "Novel natural products from soil DNA libraries in a streptomycete host," *Organic Letters*, vol. 2, no. 16, pp. 2401–2404, 2000.

[51] H. K. Lim, E. J. Chung, J. Kim et al., "Characterization of a forest soil metagenome clone that confers indirubin and indigo production on Escherichia coli," *Applied and Environmental Microbiology*, vol. 71, no. 12, pp. 7768–7777, 2005.

[52] J. J. Banik and S. F. Brady, "Recent application of metagenomic approaches toward the discovery of antimicrobials and other bioactive small molecules," *Current Opinion in Microbiology*, vol. 13, no. 5, pp. 603–609, 2010.

[53] L. L. Williamson, B. R. Borlee, P. D. Schloss, C. Guan, H. K. Allen, and J. Handelsman, "Intracellular screen to identify metagenomic clones that induce or inhibit a quorum-sensing biosensor," *Applied and Environmental Microbiology*, vol. 71, no. 10, pp. 6335–6344, 2005.

[54] Human Microbiome Project Consortium, "Structure, function and diversity of the healthy human microbiome," *Nature*, vol. 486, pp. 207–214, 2012.

[55] C. F. Maurice, H. J. Haiser, and P. J. Turnbaugh, "Xenobiotics shape the physiology and gene expression of the active human gut microbiome," *Cell*, vol. 152, no. 1-2, pp. 39–50, 2013.

[56] J. Kennedy, N. D. O'Leary, G. S. Kiran et al., "Functional metagenomic strategies for the discovery of novel enzymes and biosurfactants with biotechnological applications from marine ecosystems," *Journal of Applied Microbiology*, vol. 111, no. 4, pp. 787–799, 2011.

[57] M. Pacwa-Płociniczak, G. A. Płaza, Z. Piotrowska-Seget, and S. S. Cameotra, "Environmental applications of biosurfactants: recent advances," *International Journal of Molecular Sciences*, vol. 12, no. 1, pp. 633–654, 2011.

[58] N. G. K. Karanth, P. G. Deo, and N. K. Veenanadig, "Microbial production of biosurfactants and their importance," *Current Science*, vol. 77, no. 1, pp. 116–126, 1999.

[59] A. Y. Burch, B. K. Shimada, P. J. Browne, and S. E. Lindow, "Novel high-throughput detection method to assess bacterial surfactant production," *Applied and Environmental Microbiology*, vol. 76, no. 16, pp. 5363–5372, 2010.

[60] M. Morikawa, M. Ito, and T. Imanaka, "Isolation of a new surfactin producer Bacillus pumilus A-1, and cloning and nucleotide sequence of the regulator gene, psf-1," *Journal of Fermentation and Bioengineering*, vol. 74, no. 5, pp. 255–261, 1992.

[61] I. M. Banat, "The isolation of a thermophilic biosurfactant producing *Bacillus sp.*," *Biotechnology Letters*, vol. 15, no. 6, pp. 591–594, 1993.

[62] I. Siegmund and F. Wagner, "New method for detecting rhamnolipids excreted by *Pseudomonas* species during growth on mineral agar," *Biotechnology Techniques*, vol. 5, no. 4, pp. 265–268, 1991.

[63] T. Uchiyama and K. Watanabe, "Substrate-induced gene expression (SIGEX) screening of metagenome libraries," *Nature Protocols*, vol. 3, no. 7, pp. 1202–1212, 2008.

[64] D. Paul, G. Pandey, J. Pandey, and R. K. Jain, "Accessing microbial diversity for bioremediation and environmental restoration," *Trends in Biotechnology*, vol. 23, no. 3, pp. 135–142, 2005.

[65] M.-N. Xing, X.-Z. Zhang, and H. Huang, "Application of metagenomic techniques in mining enzymes from microbial communities for biofuel synthesis," *Biotechnology Advances*, vol. 30, no. 4, pp. 920–929, 2012.

[66] J. Neveu, C. Regeard, and M. S. Dubow, "Isolation and characterization of two serine proteases from metagenomic libraries of the Gobi and Death Valley deserts," *Applied Microbiology and Biotechnology*, vol. 91, no. 3, pp. 635–644, 2011.