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Functional Metagenomics from the Rumen Environment—A Review

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Abstract

The rumen microbiome plays an essential role in ruminant physiology, nutrition and pathology as well as host immunity. A better understanding of rumen microbial processes and identification of which populations are responsible for specific functions within the rumen microbiome will lead to better management and sustainable utilization of the available feed base while maintaining a low environmental impact. Recent advance in the culture independent method of microbiology such as metagenomics, unravels potentially the rumen microbial process. There are two basic types of metagenomics studies: Sequence-based and function-based metagenomics. Sequence-based metagenomics involves sequencing and analysis of DNA from environmental samples. Its purpose is to assemble genomes, identify genes, find complete metabolic pathways, and compare organisms of different communities. Whereas functional metagenomics is the study of the collective genome of a microbial community by expressing it in a foreign host usually *Escherichia coli* (*E. coli*). It is a promising approach unearthing novel enzymes even from yet to culture rumen microbiota. Further advances in the screening techniques promise vast opportunities to rumen microbiologists, and animal nutritionist. The identification of novel enzyme through functional metagenomics consists of three parts: rumen sample collection; DNA library construction and screening of individual clone. Functional metagenomics was successfully applied to identify different antibiotics, hydrolytic enzymes, antibiotic resistance genes, and many other functions; moreover, it allowed characterization of genes encoding enzymes with a particular activity, which represents completely novel sequence. There are a number of outputs from functionally screened rumen product such as carbohydrate active enzymes (CAZymes) that can break down plant cell walls. Company involved

commercialization of metagenomics research such as Syngenta, Genencor International, BRAIN etc., has produced many biological molecules of commercial interest. The aim of this paper is to elucidate functional metagenomics, from rumen environment and its potential for commercial purpose.

Keywords

DNA Isolation, DNA Library Construction, Functional Screening

1. Introduction

Unlike monogastric, the forestomach in the ruminants is divided into four compartments, *i.e.* rumen, reticulum, omasum and abomasum or true stomach or glandular stomach [1]. The Ruminants forestomach allows colonization of countless numbers of microbes [2], which are collectively called the rumen microbiome [3]. Out of these groups, bacteria and protozoa predominate the microbial biomass [4]. The rumen microbiome plays an essential role in ruminant physiology, nutrition and pathology as well as host immunity [5]. Rumen microorganisms are able to modulate nutrient absorption and may be among the major determinants of nutrient utilization efficiency and detoxifying plant secondary compounds [6] [7]. However, they are also responsible for methane production [8]. Diet composition and dry matter intake are responsible for altering the rumen microbiome composition [9]. Rumen is one of the most underutilized microbial ecosystems that produce an array of enzymes for digestion and utilization of different plant constituents [4], e.g. Lignocellulolytic enzymes from synergistic relationship of rumen microbiome extract energy from the fiber feed and support digestion of the host. This yields volatile fatty acids (VFAs, acetate, butyrate, propionate), formic acid, H_2, CO_2, and CH_4 [10].

The major obstacles hindering our understanding of the structure and function of the rumen microbiome are that only approximately 15% of rumen bacteria appear to be culturable [11], which highlights the importance of molecular biology approaches to sidestep this limitation and study the rumen system in total [12].

Recent developments in the study of gut microbial communities (microbiomes) through genomics and metagenomics are revolutionizing our understanding of the functions of the ecosystem and the interactions among their members and the host animal [8]. Metagenomics is a fast growing and diverse field within environmental biology directed at obtaining knowledge on genomes of environmental microbes, without prior cultivation, as well as of entire microbial communities. Other terms are also used to describe this: environmental genomics, eco-genomics, community genomics, and mega-genomics [13].

Two approaches have been commonly used for exploring the rich genetic resource provided by rumen microbiome: high throughput screening of cloned

expression libraries made from rumen metagenome DNA for gene products of interest (functional metagenomics) and sequencing based characterization of the aggregate collection of genomes and genes present in rumen microbial communities, at both DNA (metagenomics) and RNA levels (meta-transcript omics) [5].

The term "functional metagenomics", in a broad sense, is meant to reflect a connection between the identity of a microbe, or a community, uncovered via metagenomics and their respective function(s) in the environment [13]. By combining different approaches, investigation at a functional level (e.g. cellulose degradation, hydrogen metabolism) rather than a phylogenetic one is more pragmatic approach to uncover new protein [8] [14]. That helps to better assign function, role and significance to differences in microbial community structure.

Functional screening technology was first applied to rumen materials to mine novel enzymes in 2005 [15]. Despite the accumulated body of information, there is still an incomplete understanding of the functioning and ecology of the rumen microbiome and its behavior from yet to be culture microbes. For instance, the complete mechanism of plant polysaccharide degradation, the quintessential rumen function, is not yet elucidated [16]. So, this manuscript is intended to highlight functional metagenomics approach from the rumen environment.

2. Microbes Involved in Various Rumen Functions

The rumen harbor very complex consortium of bacteria, protozoa, archaea, fungi and bacteriophages, where the interaction among them results in better feed degradation [17]. According to Woese's classification all microbes in the rumen ecosystem can be distinguished into three domains: Bacteria (bacteria), Archaea (methanogens), and Eucarya (protozoa and fungi) [18]. **Table 1** illustrates the physical, chemical, and microbiological characteristics of rumen ecosystem.

3. Methodology

3.1. Rumen Functional Metagenomics Methodology

The Rumen microbiome functional metagenomics protocol consists of three parts: Environment (rumen fluid) sample collection; DNA library construction and isolate or screening of individual clones. A DNA library consists of random fragments of DNA (genes from rumen metagenome) insert into a circular DNA vectors called e.g. Plasmid/fosmids. These circular vectors are then put into a microbe usually *E. coli*, so they can be replicated during the microbe's life cycle. The next step is to isolate or screen individual clones following particular function (e.g. antibiotic resistance).

3.2. Rumen Samples Collection Method

The standardization of collection and processing methods for rumen samples is

Table 1. Physical, chemical, and microbiological characteristics of rumen ecosystem.

Physical properties	
Dry matter (%)	10 - 18
Osmolality	250 - 350 mOsmol/Kg^{-1}
pH	5.5 - 6.9 (Mean 6.4)
Redox potential	−350 to −400 mV
Temperature	38˚C - 41˚C
Chemical properties	
Amino acids and oligopeptides	<1 mmol·L^{-1} present 2 - 3 h post feeding
Ammonia	2 - 12 mmol·L^{-1}
Dietary (cellulose, hemicelluloses, pectin) component	Always present
Endogenous (mucopolysaccharides)	Always present
Gas phase (%)	CO_2 65; CH_4 27, N_2 7; O_2 0.6, H_2 0.2
Growth factors	Good supply; branched chain fatty acids, long chain fatty acids, purines, pyrimidines, other unknown
Lignin	Always present
Minerals	High Na; generally good supply
Nonvolatile acids (mmol·L^{-1})	Lactate < 10
Soluble carbohydrates	<1 mmol·L^{-1} present 2 - 3 h post feeding
Trace elements/vitamins	Always present; good supply of B vitamins
Volatile fatty acids (mmol·L^{-1})	Acetate 60 - 90, propionate 15 - 30, butyrate 10 - 25, branched chain and higher 2 - 5
Microbiological properties	
Anaerobic fungi	10^{3-5} g^{-1} (6 genera)
Bacteria	10^{10-11} g^{-1} (>200 species)
Bacteriophage	10^{7-9} g^{-1} particles ml^{-1}
Ciliate protozoa	10^{4-6} g^{-1} (25 genera)

Source: [19].

crucial to reduce the level of errors that may affect the analysis and interpretation of the data [20]. **Table 2** illustrates different rumen sampling and processing techniques.

3.3. Rumen Microbiome DNA Extraction Methods

Sampling and DNA extraction methods result in adequate yields of microbial DNA that also accurately represents the microbial community are crucial [22]. Environmental samples DNA fragments size varied in a range between less than 10 kb and more than 400 kb, depending on the sample and the mechanical, chemical, or enzymatic protocols used for the DNA extraction [23]. Different Author evaluated the phylogeny of rumen microbes using different rumen microbiome DNA extraction methods. **Table 3**, illustrate the different DNA extraction evaluation method and its output.

Table 2. Rumen sampling and processing techniques.

Ref.	Evaluation method	Host animal	Out put
[21]	**Sampling technique** (cannulation vs. stomach tube) and **Site** (dorsal sac vs. ventral sac) on the rumen microbiome and fermentation parameters	Han woo steers.	Rumen microbiome and fermentation parameters are not affected by different sampling techniques and sampling sites. A stomach tube can be a feasible alternative method to collect representative rumen samples.
[20]	**Processing method** rumen liquor that was either immediately frozen or samples that were stored as cell pellets on the key microbial group	Fistulated Brahman steers	Regardless of the processing method used, both identified the key microbial groups. However, immediately freezing samples might alter the abundance of species

Table 3. DNA extraction evaluation method and its output.

Ref.	Evaluation method	Host animal	Output
[24]	Comparison of the bacterial profile of intracellular (iDNA) and extracellular DNA (eDNA) Rumen fluid treatment (cheesecloth squeezed, centrifuged filtered), Storage temperature (RT, −80˚C) and Cryo protectants (PBS-glycerol, ethanol)	cow rumen	Intracellular DNA extraction using bead-beating method from cheesecloth sieved rumen content mixed with PBS-glycerol and stored at −80˚C was found as the optimal method to study ruminal bacterial profile.
[22]	Fifteen different DNA extraction methods	cow and sheep rumen	There is significant differences in microbial community between extraction methods, e.g. Relative abundances some bacteria e.g. *phyla Bacteroidetes* and *Firmicutes* DNA extraction methods that involved phenol-chloroform extraction and mechanical lysis steps tended to be more comparable.
[25]	DNA extraction such as: Repeated bead beating (RBB), Phenol dependent bead beating (PBB), Fast spin DNA kit for soil (FDSS), and PQIAmini. On observed microbial communities from fibrous and liquid rumen fractions	Dairy cows.	All four extraction procedures yielded DNA suitable for further analysis of bacterial, archaeal and anaerobic fungal communities using quantitative PCR and pyrosequencing of relevant taxonomic markers.
[26]	Ten improved DNA extraction methods	Yak	hexadecyltrimethylammomium bromide-lysozyme using physical lysis by bead beating is recommended for the DNA isolation of the rumen microbial community. It also showed that the bead-beating step is necessary to effectively break down the cell walls of all of the microbes, especially Gram-positive bacteria.

4. Constructing Functional Metagenomics Libraries

The metagenomics for different enzyme discovery involves creating of a metagenomics library from rumen sample and screening the library clones for specific enzymes [27]. Early studies on rumen microorganisms depended on retrieval

of genes from libraries of genomic DNA via functional screening or, lately, via PCR amplification of genes and their homologs [28]. Procedurally, clone library construction involves obtaining a DNA or RNA extract from a mixed microbial community of interest, such as rumen sample. Ribosomal RNA Gene is then amplified using PCR or RT-PCR. Amplicons are purified and inserted into a vector such as plasmid containing antibiotic resistance genes [29]. Expression library then screened for a target reaction with a specific substrate [30] [31]. The clone in the expression vectors followed by activity-based screening has endless possibilities of unlocking concealed potential in uncultured microbial world [32].

There are two distinct strategies taken in metagenomics, according to the primary goal. First, large insert libraries (cosmid, fosmid, or bacterial artificial chromosomes (pBACs)) are constructed for archiving and sequence homology screening purposes: to capture the largest amount of the available genetic resources available in the sample and archive it for further studies/interrogation. Second, small insert expression libraries, especially those made in lambda phage vectors, are constructed for activity screening [14].

The DNA extraction procedure and size sorting using denaturing gradient gel electrophoresis is a critical step when constructing large-insert libraries (e.g., fosmid, cosmid) and small insert expression libraries (e.g., those in lambda phage and plasmid vectors) [14]. The choice of a vector depends largely on the length of the inserts. Plasmids are suitable for cloning smaller than 10-kb DNA fragments, and cosmids (25 - 35 kb), fosmids (25 - 40 kb), or BACs (100 - 200 kb) can be used to clone larger fragments [33]. Among these vectors, plasmids have high copy numbers and strong vector-borne promoters. Nevertheless, these apparent merits do not improve the hit rate significantly [33]. Cosmid or fosmid based libraries are often preferred due to their large and consistent insert size and high cloning efficiency [27].

For the construction of a library, most researchers use *E .coli* as a surrogate host [33]. In most such cases, the host for the cloned DNA has been the workhorse of the molecular geneticist [34]. Various types of *E. coli* strains are available as highly efficient competent cells from commercial sources.

However, most function-based approaches for metagenomic screening are hindered by the biased and insufficient expression in *E. coli* due to transcription-translation machinery of *E. coli* is not compatible with the expression of genes harvested from environmental microbes. This can result in a very low proportion of positive clones being obtained from one round of screening of metagenomic libraries (in some cases less than 0.01%) [35]. There is an urgent need to develop a greater range of alternative hosts with good expression of foreign genes of metagenomic origins [36]. Furthermore, technical challenge in library construction such as insufficient amount and length of the extracted DNA Parks and Graham [37], inefficient transcription of target genes as well as improper assembly of the corresponding enzymes [38] and DNA shearing [39].

Development of new host systems using microbes, namely, *Streptomyces spp.*,

thermus ther-mophilus, *Sulfolobus solfataricus and Proteobacteria* [40] [41] [42] [43], have widened the choice of host and compatible enzyme assay systems. *E. coli*, owing to its ease of transformation and being the best genetically characterized bacterium, has been the choice host for heterologous gene expression in metagenomic studies [32].

5. Screening Strategies to Obtain Metagenome Derived Biocatalysts

Functional screening has become an increasingly important field for discovering novel biomolecules for applications in biotechnology and medicine [5]. Most activity screenings of metagenomics libraries are based on the cultivation of metagenomics clones on indicator plates allowing analysis of defined enzyme activities via bio catalytic conversion of an indicator substrate that leads to the formation of a clear or colored halo surrounding the "positive" colony [44]. Several parameters are important for successful screening of metagenomics libraries, such as the abundance of the gene in the library, the average insert size, the host-vector system, the use of an adequate host organism that is able to express the target gene, the assay method, the efficiency of heterologous gene expression in a surrogate host and the throughput of screening methods is relatively low [5] [33] [45]. **Table 4**, illustrate recent examples of functional screening strategies employed to obtain metagenome-derived biocatalysts.

With synchronized advances in the HTS (high throughput screening) methods and the choice of transformation systems with wide available range of hosts for heterologous gene expression, it is now possible to screen up to 50,000 clones per second or over one billion clones per day [46]. Functional screening technology was first applied to rumen materials to mine novel enzymes in 2005 [15].

Table 4. Recent examples of functional screening strategies employed to obtain metagenome derived biocatalysts.

Screening approach	Target gene	Detection method	Inducer	Source	Host, vector
Agar plate screening	b-Glycosidases	Phenotypical detection	AZCL-xylan, xyloglucan	Cow dung	*E. coli*, *phage*
Agar plate screening	Genes resistant to toxic elements	Phenotypical detection	Several antibiotics	Dairy cow manure	*E. coli*, *fosmid*
Agar plate screening	Genes resistant to toxic elements	Phenotypical detection	Several antibiotics	Cheese food matrix	*E. coli*, *fosmid*
Microtiter plate screening	Cellulase	Absorbance measurement	Dinitrophenol-cellobioside	Soil, Buffalo rumen, etc.	*E. coli*, *fosmid*
GMD, FACS	Screening for antibiotics	Fluorescence	*S. aureus*	3 strains of Staphylococcus obtained from an ARSculture collection	*E. coli* *S. cerevisiae*, *plasmid*
Microfluidics (water in oil droplets), FACS	Hydrolases	Fluorescence	Sulfate monoester Phosphate triester	Variety of sources (soil, degraded plant material, cow rumen)	*E. coli*, *plasmid*

Note: AZCL, azurine-cross-linked; GMD, gel micro-droplet; FACS, fluorescence-activated cell sorting; ARS, agriculture research service. GMD: the microfluidic gel microdroplets. Source: [36].

5.1. Agar Plate Screening Method

It is the oldest method of screening, in which many have used it as a state of the art hydrolytic enzyme screening methodology. This method for functional metagenomics screening gives a simple and straightforward approach to identify novel enzymes that function under diverse conditions. Novel hydrolytic enzyme such as lipases, esterases, cellulases, proteases, laccases, glycosylases, nitrilases, and dehalogenases, have been identified using this method [47]. The agar plate screening method helps to pin point genes responsible for the resistance to the toxic elements such as antibiotic, extreme salt concentration, extreme pH, and heavy metals [37]. And the assay are based on the production of a chromophore or fluorophore in colonies incubated with a chromogenic or fluorogenic substrate, with the throughput of 10^3 - 10^6 clones per day [48] [49]. The method has successfully isolate large number of unique enzymes from various environments [37].

5.2. Microarray Based Screening

The idea behind DNA microarray-based approach is, screening metagenomics libraries for the presence of selected genes. It efficiently spot a genomic target region [50]. Unlike agar plate, DNA micro array is sequence-based screening method of metagenomics library [51]. The protocol includes identification biological photoreceptors based on a homology search in already sequenced, annotated genomes. The similarity of novel DNA sequences to already identified genes encoding for functional proteins is the basis for microarrays approach [52]. The use of microarrays to profile libraries offers an effective approach for characterizing many clones rapidly [51]. This format is referred to as a metagenome microarray (MGA) [14]. However, the difficulty and limitation of this approach is related to achieving high hybridization efficiency and that the target genes derived from conserved regions of already known protein families reduce our chances for obtaining fundamentally new proteins [35].

5.3. Microtiter Plate Screening

Microtiter plates approach involves incubation of bacterial culture with enzyme substrate in the microwells [32]. The use of microtiter plate assays is a conventional and straightforward high-throughput approach to protein library screening [53]. The method provides high-throughput at minimal expenses in time, money and work effort [54]. The protein property of interest can be directly or indirectly measured in the microtiter plate, most commonly via spectrophotometry or fluorometry [53]. With the occurrence of substrate conversion, in microtiter plates a visual signal emerges, such as color or fluorescence, which is used to identify colonies expressing an enzyme with desirable properties [36].

5.4. Fluorescence Activated Cell Sorting (FACS) Base Screening

Fluorescence-activated cell sorting (FACS) is an emerging technology having a

powerful tool for screening enzyme libraries due to its high sensitivity and its ability to analyze as many as 10^8 mutants per day [55]. FACS enables the identification of biological activity within a single cell based on cell size, shape, and fluorescence [56]. FACS have many advantages: 1) it deposits single events into a variety of vessels quickly and accurately; 2) the laminar flow fluidics of FACS prevents disruption of cells during sorting and 3) the contamination is limited because of the small volume of each droplet [57]. FACS can easily couple to a number of different high-throughput screening methods due to its powerful cell sorting capacity such as droplet sorting and reporter-based screening [36]. Recently, this system incorporates a laser with multiple wavelength capabilities screen up to 50,000 clones per second, or over one billion clones per day [58].

5.5. Microfluidics Based Screening

Microfluidic base screening platform has equipped with high-throughput screening technology which give an advantage over the other method due to its suitability for cell based assay, low analysis cost, and easy handling pico liter volumes of liquids [59]. The method allows high-throughput screening with rapid analysis of thousands of chemical, biochemical, genetic or pharmacological tests in parallel [60]. Micro droplets are produced in large numbers at speeds of thousands of droplets per second and a single droplet functions as a reaction chamber. Cells, enzyme variants, substrates and products are confined in the picoliter volume of the droplets, where reactions take place [61]. Subsequently, the droplets are sorted according to fluorescence or color of the product. The coupling of microfluidics with FACS results in the ultrahigh-throughput screening of metagenomic libraries. However, the major bottleneck of such technique is the detection method, which is mostly limited to fluorescent signal. In the future, other detection methods, such as mass spectrometry, nuclear magnetic resonance (NMR) and colorimetric assay, may be combined with microfluidic devices to accelerate the discovery of novel biocatalysts or other genes with important functions in the microbiota [36].

6. Functionally Screened Rumen Product

Rumen associated functionally screened microbiome product and application of highly active enzymes for commercial applications will provide a new dimension in agroindustry's and also decreasing the methane release into atmosphere [28] [62]. Rumen microorganisms produce a series of enzymes known as carbohydrate active enzymes (CAZymes) that can break down plant cell walls. There are four types of CAZymes that are distinguished based on protein sequence, gene sequence, and structural similarities: glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), and carbohydrate esterases (CEs); these CAZymes cooperatively contribute to dietary cellulose, hemicellulose, and pectin deconstruction [63] [64]. Some of the CAZy family such as GH3 (b-glucosidase), CE6 (esterase) from cow and GH (Cellodextrinase) from Buffalo were obtained using functional metagenomics approach [65] [66] [67]. Some of

the Author that characterize CAZymes includes Hess *et al.*, [68], identified 27,755 putative cow rumen carbohydrate active genes and expressed 90 candidate proteins, of which (51) 57% were enzymatically active against cellulosic substrates. Cheng *et al.*, [69] functionally screened high temperature resistant and pH tolerance industrial relevance novel esterase and xylanases from cow rumen metagenomics. Zhao *et al.*, [70], screened substrate specific and good thermal stable three lipases derived from dairy rumen microflora. Pope *et al.*, [71] also identified laccase from reindeer rumen by metagenomic approaches which able to degrade lignin. Wichmann *et al.*, [72], identified 80 unique antibiotic enzymes together with a novel clade of chloramphenicol acetyl-transferases from cow manure. Thirabunyanon *et al.*, [73], found a novel probiotics strain of *Bacillu* subtilis having inhibitory activity against Salmonella enteritidis infection. Some of identified enzymes together with screening method from various reports are given in **Table 5**.

Advances in functional metagenomics have paved industry with an unprecedented chance to bring biomolecules of metagenomic origin into a commercial success. There are a number of companies involved commercialization of metagenomic research such as Diversa Corp, BASF, DSM, Syngenta, Genencor International, and BRAIN AG have commercialized many biological molecules of commercial interest **Table 6** [74].

Table 5. Metagenome studies on rumen enzymes.

Source	Enzyme/enzyme family	Sequencing method	Screening method
Cow	Cyclodextrinases	-	Function based
		Shotgun sequencing	sequencing and functional screening
	Endoglucanase	Pyrosequencing 454 GS FLX	Function based
	α-Glucuronidase	-	Function based
	Glycoside hydrolases	Sanger sequencing	Function based
	Carbohydrate active enzymes	Pyrosequencing	Function based
	Mannanase-xylanase glucanase	Sanger sequencing	Function based
	Lipases		Function and sequence based
Bovine	Endoglucanase	Sanger sequencing	Function based (BAC vector)
	Endoglucanase	-	Function based (fosmid vector)
	Glycoside hydrolases	-	Function based
	Glycoside hydrolases	Pyrosequencing 454 GS FLX	Sequence based
Swamp Buffalo	Endoglucanase	-	Function based
	Carbohydrate active enzymes	Ion torrent PGM next-generation sequencing	Sequence based
Yak	Glycoside hydrolases	-	Function based
Sheep	Xylanase	-	Function based (fosmid vector)
Goat	Endoglucanase	Shot gun sequencing	Sequence based
-	Feruloyl esterase	-	Function based

Source: [4].

Table 6. Commercialization of metagenomics technologies.

Company	Target products	Classes	Products and market	Commercial interest
BASF http://www.corporate.basf.com/	Enzymes	Amylase Hydratase	Acidophilic gluco amylase	Food industry, aiding with the digestion of starch
Bioresearch Italia, SpA (Italy)	Anti-infectives	Vancomycin	Dalbavancin	Development of human gene targeted therapeutics and novel anti-infective
B.R.A.I.N http://www.brain-biotech.de/	Bioactive peptides and enzymes for pharmaceuticals and agrochemicals	N.D.	Nitrile hydratases Cellulases	Degussa AG Partnership for the industrial processe
Cubist pharmaceuticals https://www.merck.com/	Anti-infectives	N.D.	N.D.	Various commercial relationships. Variety of products in Stage I, II and III trials
Diversa http://www.diversa.com/	Enzymes	Nitrilase Glycosidase Phytase	Discovery of 100 novel nitrilases Production of Lipitor Pyrolasee 160 and Pyrolasee 200; Phyzymee XP	Drug, lowering cholesterol levels Broad spectrum b-mannanase and b-glucanase added to animal feed to break down indigestible phytate in grains and oil s eeds to release digestible
	Biometabolites	Fluorescent protein	Discovery Pointe Green-F P* and Cyan-FP*	Novel green and cyan flu orescent proteins for potential u se in drug discovery, ommercial screening and academic research
Diversa and Invitrogen https://www.thermofisher.cn/cn/zh/home.html	Enzymes	DNA polymerase	hermal Acee and Replicasee DNA for research and diagnostics	Research and diagnostics
EMetagen	Enzymes; antibiotics; small active molecules	Polyketides	eMetagen Gene and Pathway Banks eLarge clone DNA libraries encoding biosynthetic pathways for 5000 to 20,000 secondary metabolites	Food, agriculture, research and other commercial applications Pharmaceuticals: antimicrobial, anticancer and other bioactive properties
Kosan Technology http://www.kosan.com/	Antibioti cs	Polyketides	Adriamycin, Erythromycin, Meva-cor, Rapamycin, Tacrolimus (FK506), Tetracycl ine, Rapamyc in,	Therapeutic drugs
Genencor http://www.genencor.com/	Enzymes	Lipase, Protease	Washing powder and alkaline tolerant protease.	Cleaning industry
Libragen http://www.libragen.com/	Antibiotics and biocatalysis for pharmace uticals	N.D.	Anti-infective and antibiotic discovery Biocatalysis discovery for pharmaceuticals (partnership with Synkem)	Medicine; synthesis of pharmaceuticals
Prokaria http://www.prokaria.is/	Enzymes	Rhamnosidase b-1,6 Gluconase; Single stranded DNA ligase	Food and agricultural industry	Food industry

Continued

Proteus http://www.proteus.fr/	Enzymes; anti-biotics; antigens	Not specified	Research and diagnostics Products for the agricultural, environmental, food, medical and chemical industries	Anti-phytopathogenic fungal agent Development of novel biomolecules
Xanagen http://www.xanagen.com/	Libraries	Gene products	Unspecified	Services in library construction, screening and annotation

N.D. no details available or products still under development. Source: [74].

Currently, the major laboratories working in the area of rumen metagenomics include DOE Joint Genome Institute-Genome Technology, USA; USDA, USA; INRA, France; CSIRO, Australia; and AgResearch, New Zealand, and Agricultural University India [4].

7. Conclusion

Functional metagenomics screening technology is the powerful tool of future research arena with a potential of mining environmentally as well as commercially important biocatalyst. Rumen sample collection, DNA library construction and screening of the clone are the standard procedure for rumen functional metagenomics work. Screening technology ranges from the old technique such as Agar plate to high throughput advanced technology such as mass spectrometry, nuclear magnetic resonance (NMR) and colorimetric assay. The four rumen environment origin enzymes (CAZymes) have a significant contribution to agro industry and pharmaceutical Company.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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