Birbal Singh · Gorakh Mal · Sanjeev K. Gautam ·
Manishi Mukesh

Advances in Animal Biotechnology

Springer

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Preface

Biotechnology is a rapidly advancing area with remarkable achievements. This book entitled, *Advances in Animal Biotechnology*, is a compilation of advances in the field of Animal Biotechnology including fishery that are not sheltered in depth in earlier publications. The book is divided broadly into different sections, *viz.*, Gut Microbiome and Nutritional Biotechnology, Assisted Reproduction Biotechnology, Livestock Genomics, Health Biotechnology, and Animal Biotechnology in Global Perspective.

The book covers the syllabi of Animal Biotechnology courses in various universities and competitive examinations at various levels. Researchers, Continuing Graduates, and Academicians, Research Institutions, and Biotech Companies will be benefited.

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He has made significant contribution to delineate stage-specific (heifer, lactating, and involution) transcriptomic signature of buffalo mammary gland using heterologous bovine specific microarray chip and RNASeq technologies. He also worked to establish stage specific transcriptome signature of milk derived mammary epithelial cells during lactation cycle in Sahiwal cows.

Dr. Manishi Mukesh is also engaged in thermoregulation genomics of Indian cattle and buffalo breeds, comparative physiological and transcriptional adaptation of Murrah buffaloes, Sahiwal and Karan Fries cows.

He has contributed immensely towards enriching the genomic information on indigenous cattle and buffaloes by publishing more than 80 research papers in international as well national journals, more than 100 research abstracts, several book chapters and technical bulletins. He has been involved as key resource person during several National Training Programmes.

Part I

Gut Microbiome and Nutritional Biotechnology

1

Metagenomics for Utilizing Herbivore Gut Potential

Abstract

Herbivores possess a mesmerizing gut ecosystem evolved to degrade and convert crude plant biomass and non-protein nitrogen to energy precursors and high biological value proteins. It is high time to unravel valued gut microbiota of herbivores by culture-independent molecular biological and metagenomics tools, and exploit the inferences to actually obtain microorganisms and microbial metabolites of commercial interest, and evolve strategies to improve undesirable processes associated with gut metabolism.

Highlights

- Herbivorous animals has precious gut microbiota and microbial metabolites
- Metagenomics has unravelled a lot about uncultured gut microbiota
- Many bottlenecks has to be resolved to actually utilize the potential of gut microbiota.

Keywords

Herbivores · Rumen · Metagenomics · Hydrolytic enzymes · Therapeutics · Industrial applications

1.1 Introduction

Microorganisms are the primary sources of enzymes and raw materials for industrial applications. Complex microbial ecosystems, such as extremophiles, soil (Yao et al. 2014) and compost, deep sea microbiota, and gut ecosystem of herbivores in particular, are largely underutilized genetic and biological sources of valuable microorganisms, microbial genes and biocatalysts.

The microbes inhabiting the digestive tract of herbivorous animals including vertebrates and invertebrates are of immense interest to nutrition, immunity and overall well-being of the host. As herbivores themselves lack cellulolytic enzymes to degrade plant biomass and phytometabolites therein, and synthesize proteins from non-proteins nitrogen (urea, uric acid etc.), they solely depend on metabolic activities of their gut microorganisms for energy and proteins. The system-wide investigations into microbial gut communities involved in lignocellulose degradation, detoxification of toxic phytometabolites have basic as well as applied prospects (Fig. 1.1). The gut microbial ecosystem is a potent source of valuable microbial metabolites such as enzymes, bacteriocins or antimicrobial peptides (AMPs) (Azevedo et al. 2015; Oyama et al. 2017a, b; Hatziioanou et al. 2017). Therefore, a meticulous understanding of complex gut ecosystem is of interest to microbial ecologists, livestock nutritionists, molecular biologists and industrialists.

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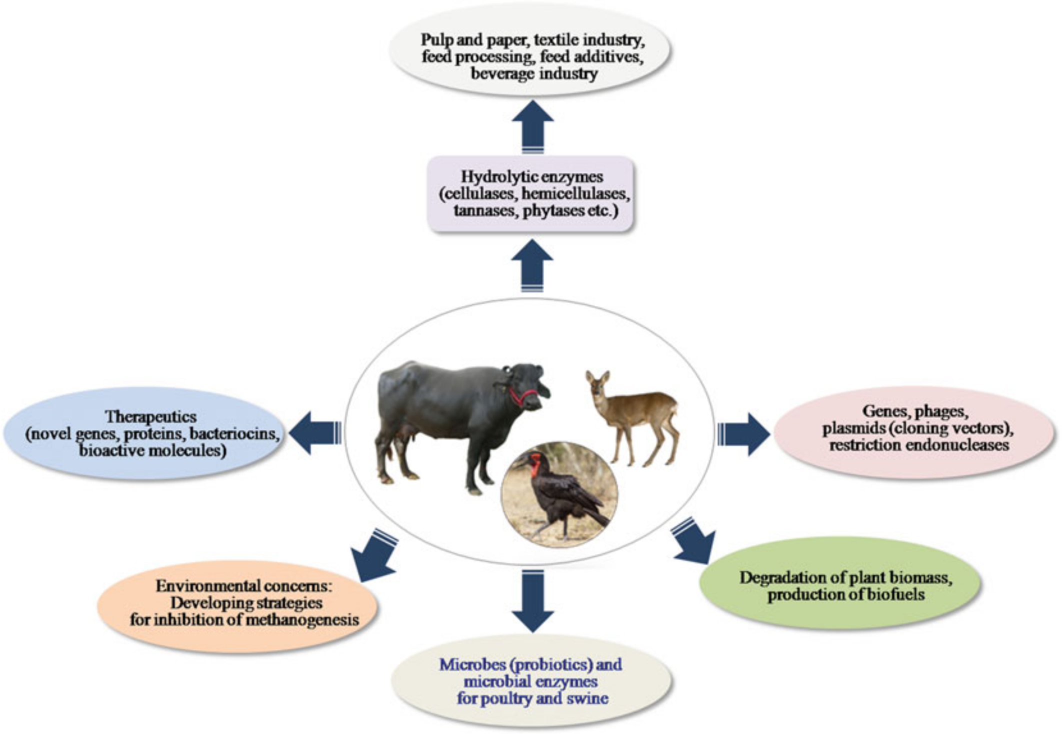

Fig. 1.1 A diagrammatic depiction of herbivore gut ecosystem as a source of well-ordered microbiome for degrading lignocelluloses-rich plant biomass and anti-nutritional plant metabolites such as tannins, oxalates, saponins, fluoroacetate, and non-protein amino acids

Handelsman et al. (1998) have suggested the term metagenomics ('*meta*' Greek, for transcending; more comprehensive), which constitutes a challenging domain to discover enzymes, genes and metabolic pathways from diverse microbial habitats. Soon after development of high throughput genome sequencing methodologies, and bioinformatics methods to analyze the plentiful data, the metagenomics emerged as a potential method to unravel microbial ecosystems, such as gastrointestinal (GI) tract of humans and animals (Singh et al. 2008; Hess et al. 2011; Kohl et al. 2016; Denman et al. 2018; Huws et al. 2018), skin (Chng et al. 2016), genitourinary microbes (Aagaard et al. 2012; Li et al. 2018), and epidemiological studies (Martin et al. 2018). In addition, metagenomics is used to discover extremophiles, marine microbial resources, soil, compost (Matsuzawa et al. 2015), and sewerage (Cai et al. 2018).

1.2 Why Herbivore Gut Ecosystem so Important?

The fact that food and feed processing, textile and surfactants, biofuels, and pharmaceutical industries need a sustained supply of raw materials such as enzymes, has urged the researchers to investigate microbial communities for their beneficial contributions. The herbivores by virtues of their complex and highly efficient gut microbiome very efficiently utilize the hemicelluloses-rich plant biomass, and enable them to adapt to varied agroclimatic situations. The gut microbiota of herbivores is equipped with a wide range of metabolic capabilities including synthesis of energy precursors such as short chain fatty acids (SCFAs) (White et al. 2014; Mayorga et al. 2018; Clemmons et al. 2018), increasing microbial biomass in rumen that is digested in

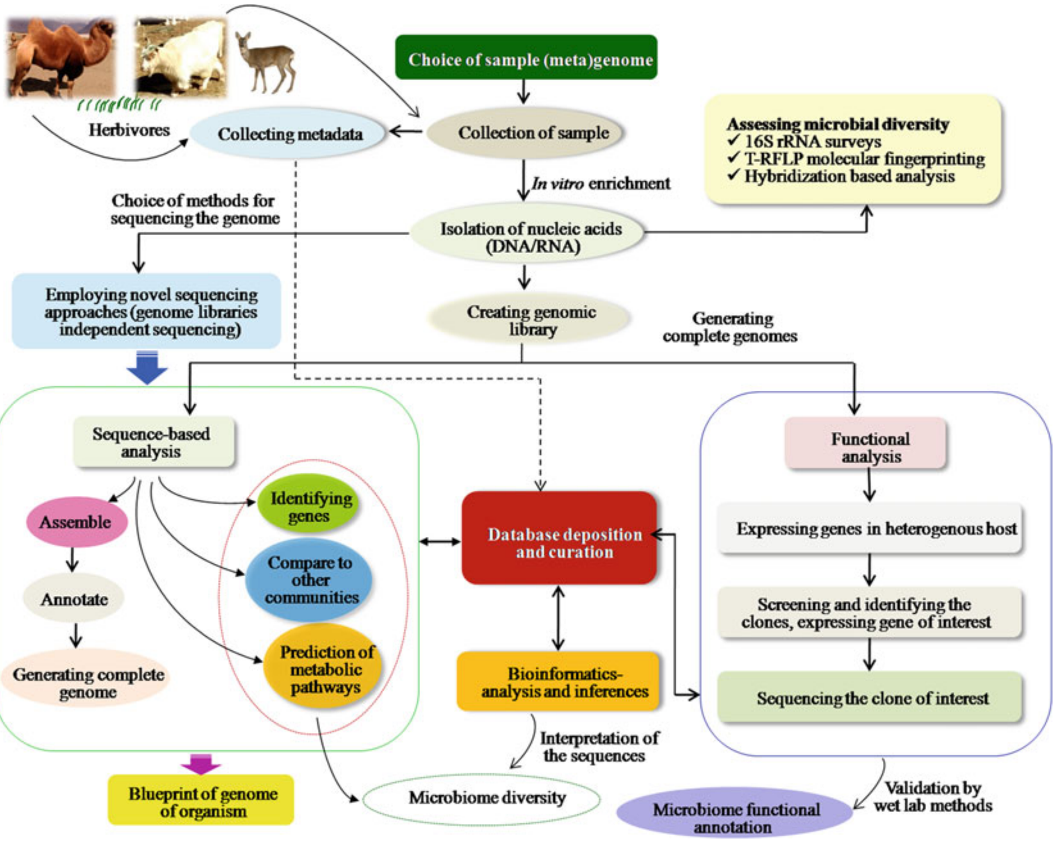

Fig. 1.2 A flowchart presentation of metagenomic profiling of herbivore GI ecosystem. Selection of the species depends on what has to be targeted. For instance wild ruminants harbour more proficient fibrolytic microbiota, while goats, rodents (e.g. Stephen's woodrats, *Neotoma stephensi*) and birds (e.g. Greater Sage-Grouse) may harbour microorganisms with ability to degrade toxic phytometabolites in plants used as forage

abomasum and lower part of GI tract, and used as a source of proteins and amino acids. In addition, some of the AMPs synthesized by bacteria confer protection against invading pathogens.

The culture-dependent microbiological techniques cannot completely describe the microbial complexity of gut ecosystem, molecular biological, and genomics methodologies are developed to expand the blueprint of microbial species inhabiting GI tract. Indeed, the very essential purpose of microbial metagenomics is to decipher non-cultivable microorganisms, their properties and eventually explore them for mercantile applications. The metagenomic analysis (Fig. 1.2), comprises of collection of samples from a niche, enrichment of microorganisms that are low in number in a population, isolation of whole genome of the microorganisms, construction, screening and analysis of metagenomic DNA/RNA libraries.

Technical advances in basic and applied sciences, technologies to construct high efficiency cloning vectors (e.g. bacterial artificial chromosomes, yeast artificial chromosomes to clone eukaryotic genes, and development of bioinformatics methods have progressed the applications of metagenomics. Figure 1.2 summarises the major technical aspects of a representative metagenomic analysis.

1.3 Metagenome Sequence Data Analysis

A typical metagenomic analysis is based on two types of analyses, namely, sequence-based analysis (screening the metagenomic libraries for nucleotide sequences), and function-based analysis (screening the metagenome clones or libraries for a particular metabolite), or a combination of both depending on requirements.

1.4 Sequence-Based Analysis

Identifying potential enzymes or a biomolecules of interest in a metagenome based on sequence-similarity is a rewarding strategy. This approach entails sequencing and analysis of genome or specific phyolegentic clusters, such as 16S rRNA from a taxonomic group. The whole genome sequencing (WGS) produces enormous data. The classic projects may involve multiple samples and billions of sequence reads for further analysis, prediction and interpretations. This requires bioinformatics and *in sillico* methods.

The metagenomic data are analysed based on sequences corresponding to a particular activity or sequences already available in database. Various software packages have been developed for analyzing the metagenome sequence data. For instance, a computer-aided program, named as DOTUR was developed for studying operational taxonomic units and microbial species in a microbial milieu (Schloss and Handelsman 2005). A series of MEGAN (MEtaGenome ANalyzers) programs, originally developed to provide as tool for investigating the taxonomic content of single dataset are available (Huson et al. 2007, 2016). Sargasso Sea data, data obtained from woolly mammoth (*Mammuthus primigennius*) bones was analyzed by MEGAN (Huson et al. 2007).

The web-based mg-RAST (metagenomic Rapid Annotation Using Subsystem Technology), facilitates processing, sharing and analysis of metagenomic data (Meyer et al. 2008).

Currently, a number of software packages are available. The readers should refer to online updates for developments in software packages and bioinformatics tools for microbial metagenomic diversity analysis, sequence similarity, functional annotation, mapping to reference genomes, and quality analysis of metagenomic data.

Several novel cellulases and glycosylases are identified from metagenomic sequences of rumen and GI microorganisms (Lopez-Cortes et al. 2007; Zhao et al. 2010) (Table 1.1).

Table 1.1 Summary of valuable microorganisms and microbial enzymes derived from metagenomics of herbivore GI tract

Microorganisms/niches/enzymes/genes	Host/origin	Inferences and recommendations (Reference)
Bacteria		
Cytophaga-Flexibacter Bacteroides phyla	Buffalo rumen	Cellulose degradation, and abundance of cellulose enzymes (Liu et al. 2009a, b)
Rumen microbes	Camel rumen	Characterization of microorganisms and microbial enzymes responsible for cellulose degradation, and generation of SCFAs. The study highlights the potential of fibrolytic microorganisms and their enzymes in food processing, and producing biofuel and fine chemicals (Gharechahi and Salekdeh 2018)
Gut microorganisms	*Rhinopithecus bieti* (primate)	Description of gut bacterial diversity, and their role in digesting plant biomass (Xu et al. 2015)
Fungi		
Fungal taxa	Murine GI tract	Identification of fungal taxa and their role in GI tract (Toyoda et al. 2009)

(continued)