Rumen: An Underutilised Niche for Industrially Important Enzymes

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Abstract

Rumen is one of the most underutilised microbial ecosystems, harbouring a diverse population of microbial species. These species thrive in this ecosystem by producing an array of enzymes for digestion and utilisation of different plant constituents. The search for novel and efficient fibrolytic cultures/enzymes will foster the development of different applications such as biofuel production from lignocellulosic biomass. Exploring and exploiting these efficient cultures/enzymes using biotechnological interventions for enhanced production are necessary before their efficient application in industries. Recent advances in molecular biology such as metagenomic studies with high-throughput screening methods are enabling the development of novel strategies for effective delivery and enhancement of these enzymes. This chapter takes a holistic review of most extensively studied enzymes produced in the rumen and their role in digestion of fibre and other associated plant cell wall polymers.

Keywords

Rumen bacteria • Rumen fungi • Enzymes • Metagenomics

17.1 Introduction

Enzymes are known to be the sustainable biocatalysts of high demand with continuously increasing industrial sector. The application of potential enzymes over the conventional chemical and thermal methods offers additional advantages such as reduction in the use of hazardous chemicals, creation of safer working environment, lowering the cost of process and product formulation, reduction in consumption of water and energy

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and enhancement of efficient use of non-renewable resources.

The rumen is an ideal habitat for the growth of anaerobic microorganisms encompassing bacteria, fungi, protozoa, archaea and bacteriophages. Out of these groups, bacteria and protozoa predominate the microbial biomass. The fungi make up only 8–20 % of microbes and occupy an important niche in the rumen because of their specific affinity towards lignin and ability to produce esterases for hydrolysing ester linkages between lignin and hemicelluloses or cellulose and thus help break down digesta particles. Further, their rhizoidal system causes physical disruption by penetrating inside recalcitrant feed stuff, which also allows bacteria to gain access to otherwise non-available sites. The bacteria being most abundant and diverse are further classified into different groups based on their enzymatic activities such as fibrolytic, amylolytic, proteolytic, etc. The diversity arises due to different microbial communities as well as multiplicity of fibrolytic enzymes produced by individual microorganisms when they encounter different plant polymers. Due to its diverse microbial population, the rumen works as a specialised fermentation vessel facilitating the microbial degradation of ingested plant materials. Therefore, the rumen has been described as microbial cell factory for biorefineries as it harbours a rich source of microbial cell and could be a potential model to study the higher organisational levels of microbial communities, finally leading to a new concept for metabolic engineering (Sauer et al. 2012). The digestion is mainly affected by the plant materials, its nature, components and structure as well as the microbial factors such as microbial load, communities, competition and others. The different characteristics of microbial population in rumen such as survival under anaerobiosis, predatory activities of rumen protozoa, recalcitrant plant components and toxic effects of plant secondary metabolites make it an ideal source for bioprospecting (Selinger et al. 1996; Wang and McAllister 2002). The majority of the enzymes discovered or studied belong to different classes that help to degrade different plant cell wall polymers (Table 17.1).

Table 17.1 Major enzyme activities required for hydrolysis of plant cell wall components

Substrate	Linkage	Enzyme required
Cellulose	β-1,4-Glucose linkage	Endo-β-1,4-glucanase
Cellulose (non-reducing ends)	β-1,4-Glucose linkage	Exo-β-1,4-glucanase
Cellobiose	β-1,4-Glucose linkage	β-1,4-Glucosidase
Soluble cello-oligomers	β-1,4-Glucose linkage	Cellulodextrinase
Cellulose/xylan	β-1,4-Glucose linkage	Xylocellulase
Xylan	β-1,4-Xylose linkage	Endo-β-1,4-xylanase
Xylobiose	β-1,4-Xylose linkage	β-1,4-Xylosidase
Arabinoxylan	α-1,3-Linkage	α-L-arabinofuranosidase
Glucuronoxylan	α-1,3 or α-1,2 linkage	α-Glucuronidase
Acetylxylan	Acetylester bond	*O*-Acetyl xylan esterase
Ferulic acid cross bridges	Feruloylester bond	Ferulic acid esterase
p-Coumaric acid cross bridge	*p*-Coumaryl ester bond or linkage	*p*-Coumaric acid esterase
Laminarin	β-1,3-Glucanase	β-1,3-hexose linkage
Lichenin	β-1,3- and β-1,4-hexose linkages	Mixed linkage β-1,3-β-1,4-glucanase
Polygalacturan	α-1,4-Galacturonide linkages	Pectate lyase
Pectin	α-1,4-Galacturonide linkages methyl ester bond	Pectin lyase
		Pectin methylesterase
Tannins	Depside linkage	Tannin acyl hydrolase

Modified from Wang and McAllister (2002)

17.2 Plant Cell Wall Components

Ruminal digestion of plant materials consists of numerous complex processes involving a number of microbial species and their enzymatic machinery. At the microbial level, digestion of plant materials is poorly understood. The major bacterial species associated are known; however, it is in the last decade that researchers began to isolate the pure anaerobic cultures and study the various enzymatic activities and physiological factors that influence the expression of these enzymes.

17.2.1 Cellulose

Cellulose is the most important and abundant structural part of the plants which is a homopolymer of glucose. The plant cell wall is composed of fibrils of cellulose which accounts for 20–30 % dry weight of the primary cell wall. Cellulose molecules associate with each other to form microfibrils and have crystalline formulations.

17.2.2 Hemicellulose

Hemicellulose is composed mainly of xylans with a backbone structure of β-1,4 linkage in xylose residues and attachment of various side chains (e.g. acetic acid, arabinose, coumaric acid, ferulic acid, glucuronic acid, 4-O-methylglucuronic acid). Xylan polymers may be cross-linked to other hemicellulose backbones or to lignins through ferulic acid or 4-O-methylglucuronic acid. It may also be linked to cellulose fibrils forming an extensive network of cross-links. The varying branching patterns of the surrounding structures result in different types of hemicelluloses structures.

17.2.3 Pectin

Pectic substances are prominent structural constituents of primary cell walls and middle lamella. It is one of the most complex biomolecules and can be composed of as many as 17 different monosaccharides with at least seven different polysaccharides. The predominant structure of pectin consists of homogalacturonan (HG) which is an unbranched molecule composed of polyβ -1,4- D-galacturonic acid (PGA) with α-1,4-linked residues of D-galacturonate. The galacturonic acid (GA) residues can be methyl esterified at C-6 and some of the hydroxyl groups on C2 or C3 can be acetylated. Blocks of more than 10 unesterified GA residues generally yield pectin molecules, which are sensitive to calcium cross-linking (Daas et al. 2001). The rhamnogalacturonan backbone may be interspersed with either rhamnose or galacturonic acid residues substituted with methyl ester groups or sugar side chains (Jarvis 1984; McNeil et al. 1984; Rombouts and Pilnik 1986).

17.2.4 Phytic Acid

Phytic acid (phytate) is a complex of calcium or magnesium with myo-inositol and is regarded as the primary storage form of phosphorus and inositol in almost all seeds. It is considered as an anti-nutritional constituent of plant-derived feeds. As a reactive anion, it forms a wide variety of insoluble salts with minerals including phosphorus, calcium, zinc, magnesium and copper. Phytic acid is also known to form complexes with protein and proteolytic enzymes.

17.2.5 Lignins, Polyphenols and Toxic Components

Lignin is a complex polymer of aromatic compounds which account for the most abundant polymer on earth. Instead of sugar monomers like cellulosic compounds, it is composed of up to three different phenyl propane monomers, namely, *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol which are methoxylated to various degrees. The lignin degradation has been reported in rumen; however, so far the lignin degradation has not been reported in pure rumen bacterial isolate.

Besides lignin, the rumen microorganisms have been reported to detoxify various plant toxic components. The exposure to these components usually results in loss of animal productivity. The most widely studied toxins include mimosine T-2 toxins, nitrotoxins, pyrrolizidine alkaloids, *trans-aconitate* and tannins. They reduce animal productivity by reducing intake, feed palatability, enzyme activity, ruminal fermentation rates, nutrient availability and wool growth or by inducing toxicosis. Tannins based on their molecular structure are classified as hydrolysable (HTs) and condensed tannins (CTs, proanthocyanidins). Hydrolysable tannins contain a carbohydrate (generally D-glucose) as a central core with hydroxyl groups esterified with phenolic groups (Haslam 1989). These HTs are metabolised to gallic acid, pyrogallol and other products by rumen microbes that are potentially toxic to the ruminants. On the other hand, CTs do not have a central carbohydrate core and are complexes of oligomers and polymers of flavonoid units linked by carbon-carbon bonds with a molecular weight of 2,000–4,000 kDa (Hagerman and Butler 1981; Foo et al. 1986). Their multiple phenolic hydroxyl groups lead to the formation of complexes primarily with proteins limiting their availability to the animal (Makkar 2003).

17.3 Microbial Enzymes

Rumen is a rich source of fibrolytic enzymes such as cellulase, xylanase and β-glucanases. A large number of anaerobic bacteria, protozoa and fungi possess very efficient cellulolytic machinery which helps in increasing the efficiency of feed conversion (Table 17.2). The fibrolytic enzymes find their application in saccharification of lignocellulosic wastes for production of biofuels, removing certain forms of polysaccharides (arabinoxylan and β-glucan) in cereals that may interfere with nutrient absorption and promote intestinal disturbances.

17.3.1 Cellulases

The researchers have gained interest in rumen bacteria and fungi for a number of biotechnological applications, mainly for the production of cellulases which are capable of hydrolysis of 1,4 β-D-glycosidic linkages in cellulose to its monomers. Based on structure and functionality, these cellulases have been categorised as:

- Endocellulase which cleaves internal bonds at amorphous sites that create new chain ends.
- Exocellulase cleaves two to four units from the non-reducing ends of the cellulose molecule produced by endocellulase.
- Cellobiase or β-glucosidase hydrolyses the exocellulase product into individual monosaccharides (Bhat and Bhat 1997; Lynd and Zhang 2002).

In some microbes, the produced cellulases may exist as free enzymes, where different enzymes act on different parts of cellulose (Fig. 17.1). In some microbes, degradation of cellulose is accomplished by large multi-enzyme complex known as cellulosome. The attachment of this complex to cellulose fibres is achieved by non-cellulolytic bacteria via cellulose-binding proteins such as scaffolding cellulosome-integrating proteins and large glycosylated proteins (Fig. 17.2). Alongside the cellulosome structure, *Ruminococcus* also uses a cellulose-binding protein type C that involves fimbrial structures that interact with cellulose. With *R. albus*, the cellulases appear to be organised into highly structured, high molecular weight and extracellular complexes whereas *B. fibrololvens* produces extracellular polysaccharides having complex sugar composition. *F. succinogenes* possesses the membrane-associated cellulases and xylanase activities. Apart from bacterial species, the cellulolytic activities have also been reported in rumen protozoal population.

17.3.2 Hemicellulases

The hemicellulases include a variety of different types of enzymes. The initial attack on hemicelluloses has been reported by celloxylanase which displays good activity either on cellulose or xylan. This enzyme is produced by cellulolytic bacteria but do not grow on xylan as substrate; therefore, it helps in the initial disruption of plant cell fibre.

Table 17.2 Rumen microbial groups possessing fibre degrading activities

	Cellulolytic	Hemicellulolytic	Pectinase
Rumen bacteria			
Fibrobacter succinogenes	+		+
Ruminococcus albus	+		+
R. flavefaciens	+		+
Butyrivibrio fibrisolvens	+		+
Eubacterium cellulosolvens	+		+
Clostridium longisporum	+	+	
Cl.locheadii		+	+
Prevotella ruminantium		+	+
Eubacterium xylanophilum		+	
Ruminobacter amylophilus		+	
Succinimonas dextrinosolvens		+	
Selenomonas ruminantium		+	
Selenomonas lactilytica		+	
Lachnospira multiparus		+	+
Streptococcus bovis		+	+
Megasphaera elsdenii			
Rumen protozoa			
Eudiplodinium maggii	+	+	+
Ostracodinium dilobum	+	+	+
Epidinium caudatum	+	+	
Metadinium affine	+	+	+
Eudiplodinium bovis	+	+	+
Ophryoscolex caudatus	+	+	+
Polyplastron multivesiculatum	+	+	+
Diplodinium pentacanthum	+		
Endoploplastron triloricatum	+		
Ophryoscolex tricoronatus	+		
Ostracodinium gracile	+		
Entodinium caudatum	+	+	
Isotricha intestinalis	+	+	+
Isotricha prostoma	+	+	+
Rumen fungi			
Neocallimastix frontalis	+	+	+
N. patriciarum	+	+	+
N. joyonii	+	+	
Caecomyces communis	+	+	
Piromyces communis	+	+	+
Orpinomyces bovis	+	+	+
Anaeromyces sp.	+	+	

Later on the activities of xylan degraders help in degradation such as mannanase, arabinofuranosidase, ferulic acid esterase and xylanase. The hemicellulase activities have been reported in cell free extracts for different protozoal species. Higher cellulolytic activity was observed in entodiniomorphid ciliates, whereas holotrich ciliates were reported to possess weak hemicellulolytic activity. The site of action of hemicellulases is shown in Fig. 17.3. These enzymes act on the various structures surrounding cellulose thereby exposing the cellulose for microbial attack.

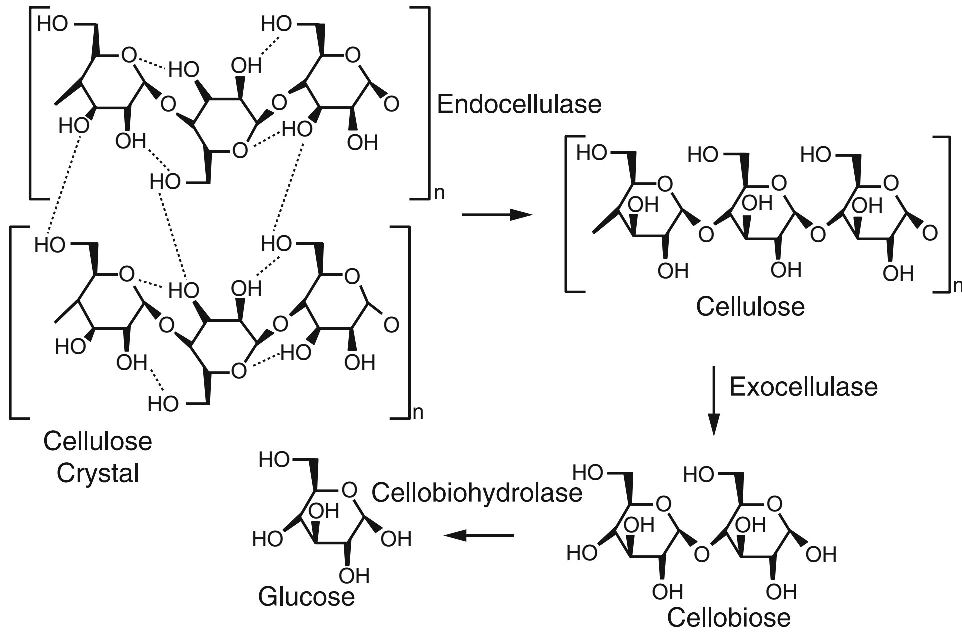

Fig. 17.1 Basic cellulose structure broken down by the three types of cellulases (Anonymous 2009)

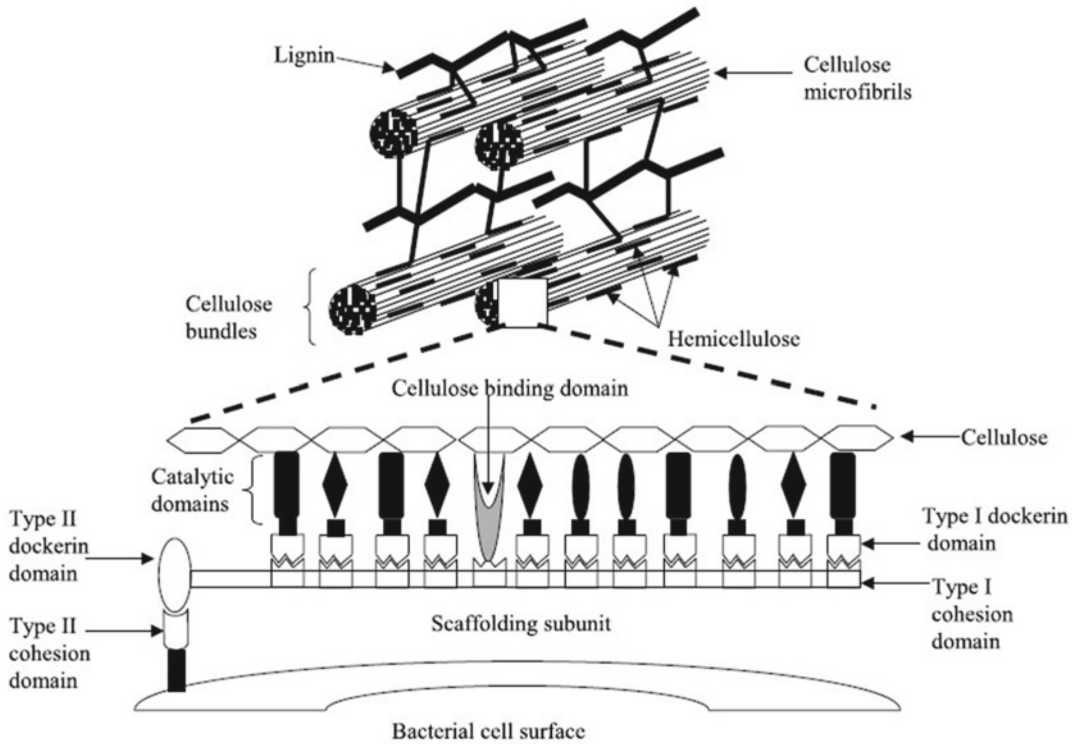

Fig. 17.2 Cellulosomal complex attached to lignocellulose (Krause et al. 2003)

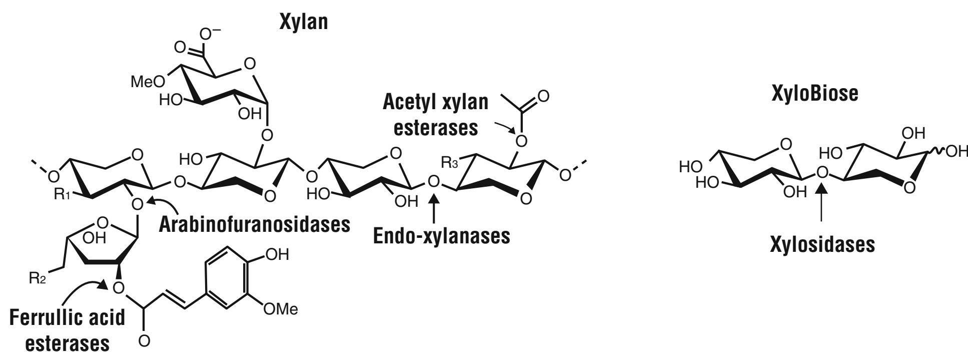

Fig. 17.3 Site of action of different hemicellulases (Shallom and Shoham 2003)

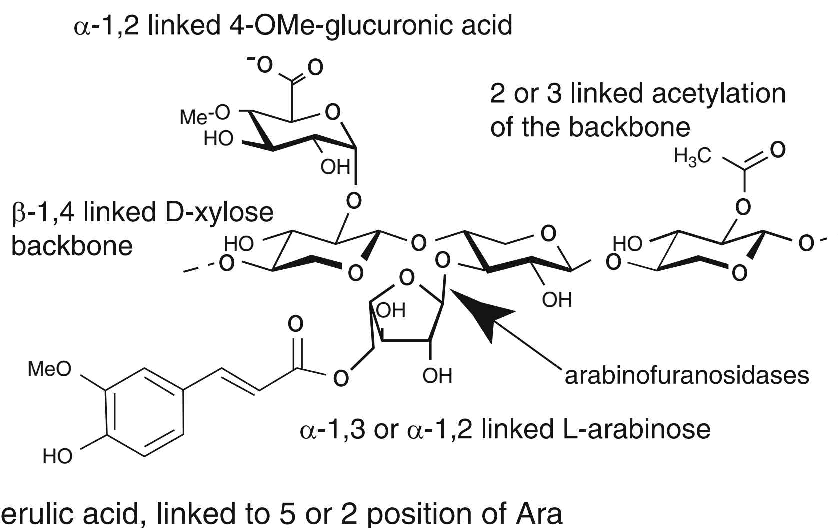

Fig. 17.4 The site of attack by FAFases on a xylan backbone (Taylor et al. 2006)

17.3.2.1 Mannanase

Mannan is a fundamental component of hemicelluloses. It consists of β-1,4 linkage between mannose monomers forming the hemicelluloses cross-linkages (Hogg et al. 2003). The mannan component is degraded to β-1,4-manno-oligomers by β-mannanases followed by action of β-mannosidases which reduce them to the monosaccharide mannose (Shallom and Shoham 2003).

17.3.2.2 Arabinofuranosidase

Arabinose is found in conjunction with xylan as hemicellulose component of plant cell wall. The arabinose units are attached to xylan via α-1,2, 1,3, 1,5 or linked to C2 or C3 position on arabinoxylan. The AFase hydrolyse the terminal, non-reducing arabinofuranosyl. The arabinose units can be cleaved off the xylose backbone by arabinofuranosidase activity of *B. fibrisolvens* and *B. ruminicola*.

17.3.2.3 Ferulic Acid Esterase

Ferulic acid esterase (FAEases) is a group of enzymes that form a subclass of carboxylic ester hydrolases. These enzymes hydrolyse the bond between hydroxycinnamates and sugars (Rashamuse et al. 2007) releasing ferulic acid (Fig. 17.4).

17.3.2.4 p-Coumaric Acid Esterase

p-Coumaric acid esterase or p-coumaroyl esterase is a very crucial enzyme for efficient degradation of lignocellulosic biomass. This enzyme helps in breakage of ester linkages that connect lignin to hemicelluloses, releasing p-coumaric acid. Very interestingly, none of the rumen bacterial group produces this and is exclusively produced by anaerobic fungi (Borneman et al. 1990), therefore further strengthening their ecological role and significance in rumen microbial ecosystem.

17.3.2.5 Xylanases

The xylan consists of β-1,4 linked xylopyranosyl residues and contains side chains with acetyl group and L-arabinofuranosyl residues. The xylanases are responsible for the hydrolysis of xylan by breaking the glycosidic linkages in xylan backbone (Shallom and Shoham 2003). Similar to cellulases, the xylanases are composed of three enzymes: endoxylanase, β-xylosidase and acetyl xylan esterase (Fig. 17.5). All the three enzymes hydrolyse the xylan molecule, rendering the D-xylan sugar usable (Kosugi et al. 2001).

17.3.3 Pectinase

There are a few rumen microorganisms possessing pectinolytic activities containing enzymes pectin lyase, polygalacturonase and pectin methylesterase (Fig. 17.6). One of the major pectinolytic bacterial species inhabiting the rumen, *Lachnospira multiparus*, produces a pectin lyase and a pectin methylesterase (Silley 1985). Apart from bacterial species, the rumen protozoa and fungi also possess the pectinolytic system (Orpin 1984; Bonhome 1990; Gordon and Philips 1992; Chesson and Forsberg 1997).

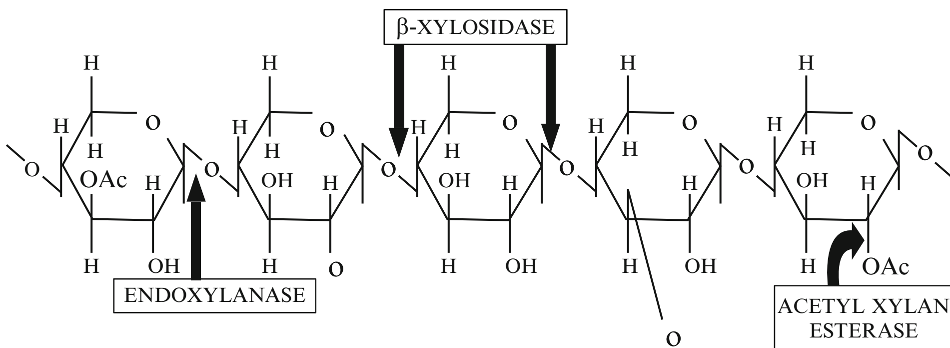

Fig. 17.5 A hypothetical xylan structure showing different sites of microbial attack (Beg et al. 2001)

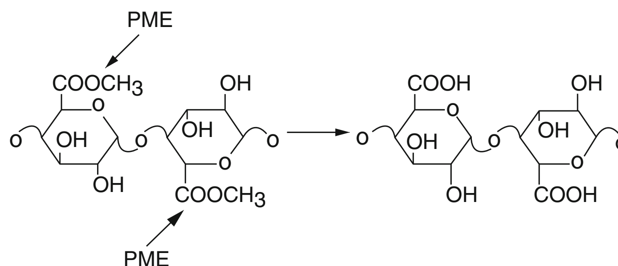

Fig. 17.6 Site of action of pectin methyl esterase (Jayani et al. 2005)

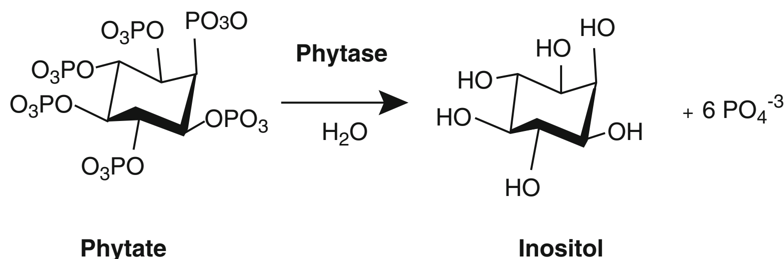

Fig. 17.7 Action of phytase on phytic acid (Mittal et al. 2011)

17.3.4 Phytase

The presence of phytase in the rumen microbes makes them able to utilise the phosphorus in phytic acid (Fig. 17.7). However, the metabolic activity of phytate degradation has been thoroughly characterised, and the study of the genetics of this process is relatively new. A recombinant phytase has been produced from *E. coli* by cloning the gene from *Selenomonas ruminantium* JY35 which possessed four to eight times higher specific activity (400 to 800 μmol phosphate released from phytate/min/mg protein) than the commercial preparation from *Aspergillus niger*. The phytase production does not depend on the coordinated activities of the enzymes such as cellulolytic enzymes; therefore, it can be genetically manipulated easily (Piddington et al. 1993; Van Hartingsveldt et al. 1993).

17.3.5 Polyphenol Degrading Enzymes

Tannin acyl hydrolase: Tannase catalyses the breakdown of hydrolysable tannins such as tannic acid, methyl gallate, ethyl gallate, n-propylgallate and isoamyl gallate. Tannase hydrolyses tannic acid completely to gallic acid and glucose through 2,3,4,6,-tetragalloyl glucose and two kinds of monogalloyl glucose. Tannase hydrolyses only those substrates that contain at least two phenolic hydroxyl groups in the acid component. The esterified carboxylic group must be on the oxidised benzene ring and must not be ortho to one of the hydroxyl groups (Fig. 17.8). Tannase activity has been reported mainly in *Streptococcus gallolyticus*, *Selenomonas ruminantium* and other inhabitants of gastrointestinal tract of ruminants such as *Enterococcus faecalis* and some other unidentified Gram-negative bacteria as reviewed by Goel et al. (2005).

17.3.6 Enzymes Involved in Biohydrogenation

The role of fatty acids in human health is very well documented. Conjugated linoleic acid (CLA) is one such polyunsaturated fatty acid that has attracted a substantial attention from the scientists from all around the world, because of its possible health effects. CLA comprises a group of positional and geometric isomers of linoleic acid and is produced as an intermediate during biohydrogenation of polyunsaturated fatty acids in the rumen of animals. Since these dietary unsaturated fatty acids are toxic to rumen microorganisms, as a defence mechanism, they secrete various enzymes to hydrolyse and hydrogenate these unsaturated fatty acids (Harfoot and Hazlewood 1988). In rumen, bacteria play the primary role in biohydrogenation (Jenkins et al. 2008). Mainly two types of bacteria, i.e. group A and group B, are involved in the biohydrogenation process (Fig. 17.9). Group A bacteria can hydrogenate linoleic acid (LA) or linolenic acid (LNA) to trans-Vaccenic acid (TVA) and are not able to hydrogenate the last step, i.e. conversion of TVA to stearic acid (SA), for example, *B. fibrisolvens* MDT5 (Fukuda et al. 2006), *Micrococcus* spp., *Ruminococcus* spp. and *Lactobacillus* spp.

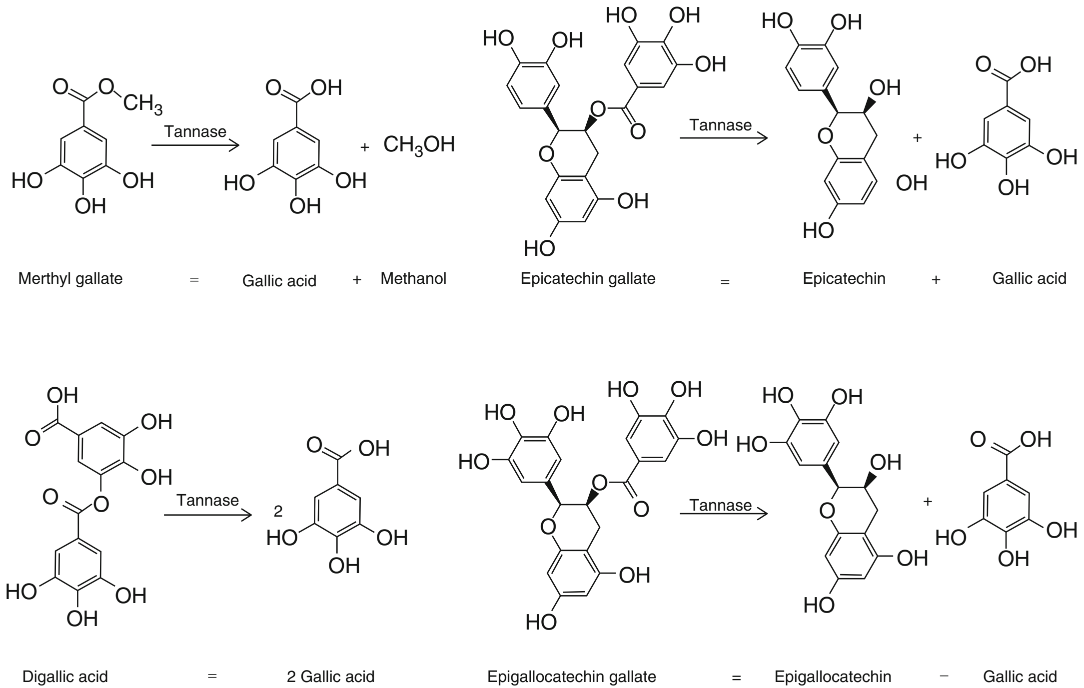

Fig. 17.8 Mode of action of tannase on different polyphenols present in plant cell wall (Rodríguez et al. 2011)

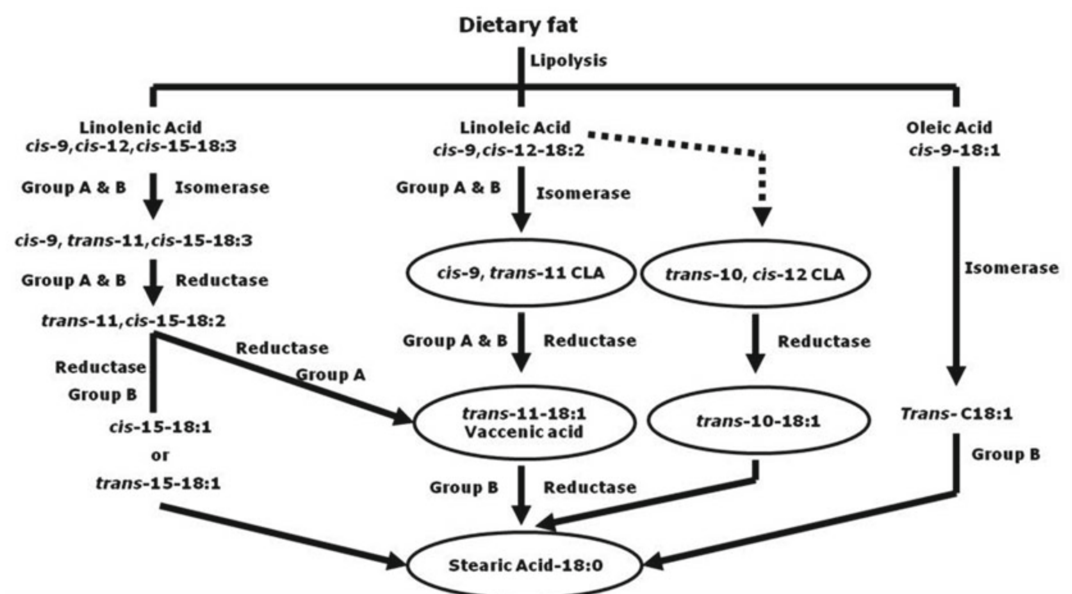

Fig. 17.9 Biohydrogenation of dietary lipids into saturated fatty acids (Griinari and Bauman 1999)

On the other hand, group B bacteria can complete all steps of biohydrogenation, i.e. conversion of LA/LNA and even oleic acid (OA) to SA, for example, *B. proteoclasticum* (Paillard et al. 2007). Many strains of *Megasphaera elsdenii* can also produce significant amounts of *trans*-10, *cis-12* CLA (Kim et al. 2002). Rumen protozoa are not involved in biohydrogenation (Boeckaert et al. 2010). The biohydrogenation activity of anaerobic fungi is reported to be very slow than rumen bacteria. Among different genera, *Orpinomyces* sp. is described as chief CLA producer (Nam and Garnsworthy 2007).

17.4 Application of Molecular Techniques in the Screening of Rumen Enzymes

From the last decade, the research has been focussed on identifying the genes encoding unique feed degradative enzymes which can be used to fortify the existing livestock production systems or to deliver novel enzymes for other industrial applications. Few ruminal microorganisms have been exploited for identification of genes and studying the expression of those genes in different microbial expression systems such as *E. coli* or *Pichia pastoris* (Table 17.3).

With the advancement in application of molecular techniques, the research has been focussed on metagenomics of the rumen microbial community. It is a method to study the DNA of entire population of microorganisms (Handelsman 2004). The metagenomics for different enzyme discovery involves creating of a metagenomic library from rumen sample and screening the library clones for specific enzymes. The advantage of metagenomics over conventional way is that it allows screening of thousands of clones in a relatively short time and enable the potential discovery of a large number of different enzymes from a sample.

Worldwide research groups are working towards the development of metagenomic approaches to characterise the structure and function of rumen microbiota in order to identify the factors that may improve the functioning of the rumen and limit undesirable environmental effects (Table 17.4). Metagenomics is the application of modern genomic techniques to the study of communities of microbes directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species. Metagenomics represents a strategy for discovering diverse enzymes encoded in nature. In Table 17.4, a few studies done on metagenomics of rumen towards the identification of new enzymes which have potential biotechnological applications are listed. The metagenomic research has generated genetic information on the entire microbial community, which is important because 90 % of microbes cannot be isolated or cultured. The metagenomic method provides a global microbial gene pool without the need to culture of the microorganisms.

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. From India, Anand Agricultural University has recently used Ion Torrent PGM next-generation sequencing technology to characterise general microbial diversity and the repertoire of microbial genes present, including genes associated with dormancy and sporulation in Mehsani buffalo rumen metagenome (Singh et al. 2014). An European project on rumenomics is underway between European partners from UK, Sweden, France, Italy, Finland and Switzerland.

17.5 Conclusions

Although rumen contains various kinds of microbes that can be used for various purposes, microbes/enzymes for lignocellulose-based biorefinery can play a crucial role in the present environment. Plant biomass being most abundant and mostly unused provides us a valuable renewable natural resource that can be exploited for various purposes ranging from production of fuels, chemicals, food or feed. However, due to

Table 17.3 Genetic characterisation of rumen microorganisms for different enzymes

Enzyme	Organism	Gene	Reference
Endoglucanase	*B. fibrisolvens* H17c	*endI*	Berger et al. (1989)
	F. succinogenes AR1	*endAFS*	Cavicchioli et al. (1991)
	F. succinogenes BL2	*endC*	B'era et al. (1996)
	F. succinogenes SD35	*end-1*	Ozcan et al. (1996)
	F. succinogenes S85	*endB*	Broussolle et al. (1994)
	P. ruminicola AR20	*celA*	Vercoe and Gregg (1992)
	P. ruminicola 23		Matsushita et al. (1991)
	R. albus F-40	*Egl*	Ohmiya et al. (1989), Duguchi et al. (1991)
	R. albus F-40	*egIV*	Karita et al. (1993)
	R. albus SY3	*celA, celB*	Poole et al. (1990)
	R. albus 8	*celA*	Attwood et al. (1996)
	R. albus AR67	*celA*	Vercoe and Gregg (1993)
	R. flavefaciens FD-1	*celE*	Wang et al. (1993)
		celB	Vercoe and Gregg. (1993)
	N. frontalis MCH3	*celA*	Fujino et al. (1995)
	N. patriciarum	*celB*	Zhou et al. (1994)
	Orpinomyces joyonii	*celA*,	Liu et al. (1996)
		celB2	Ye et al. (2001)
	F. succinogenes S85	*Cel9B, Cel5H and Cel8B*	Qi et al. (2007)
Xylanase	*B. fibrisolvens* 49	*xynA*	Mannarelli et al. (1990)
	B. fibrisolvens H17c	*xynB*	Lin and Thomson (1991)
	F. succinogenes S85	*xynC*	Paradis et al. (1993), Zhu et al. (1994)
	P. ruminicola 23	*xynA*	Whitehead (1993)
	P. ruminicola B_14	*xynB*	Gasparic et al. (1995)
	R. flavefaciens 17	*xynA, xynB, xyn D*	Zhang and Flint (1992), Zhang et al. (1994), Flint et al. (1993)
	N. patriciarum	*xynA*	Gilbert et al. (1992)
		xynB	Zhou et al. (1994)
	N. patriciarum 27	*xynC*	Tamblyn et al. (1993), Selinger et al. (1995)
	N. patriciarum	*xynCDBFV*	Liu et al. (2005)
	Orpinomyces sp. PC-2	*xynA*	Chen et al. (1995)
β-Glucosidase	*R. albus* F-40	*pRA201*	Takano et al. (1992), Ohmiya et al. (1985)
	B. fibrisolvens H17c	*Bg1A*	Lin et al. (1990)
β-Glucanase	*F. succinogenes*	–	Liu et al. (2005)
	P. rhizinflata	*eglA*	Liu et al. (2005)
Peptidase	*Prevotella albensis M384*	*DPP-IV*	Walker et al. (2003)
Cellodextrinase	*B. fibrisolvens* H17c	*cedI*	Berger et al. (1990)

Modified from Selinger et al. (1996)

Table 17.4 Metagenome studies on rumen enzymes

Enzyme/enzyme family	Source	Screening method	Sequencing method	Reference
Cyclodextrinases	Cow	Function based	–	Ferrer et al. (2005)
	Cow	Sequential and functional screening	Shotgun sequencing	Hess et al. (2011)
Feruloyl esterase		Function based	–	Wong et al. (2013)
Endoglucanase	Cow	Function based	Pyrosequencing 454 GS FLX	Pozo et al. (2012)
	Bovine	Function based (BAC vector)	Sanger sequencing	Gong et al. (2012)
	Swamp Buffalo	Function based	–	Cheema et al. (2012)
	Buffalo	Function based	–	Rungrattanakasin et al. (2011)
	Bovine	Function based (fosmid vector)	–	Rashamuse et al. (2013)
	Buffalo	Function based (cosmid vector)	–	Liu et al. (2009)
	Cow	Function based	–	Shedova et al. (2009)
	Goat	Sequence based	Shot gun sequencing	Lim et al. (2013)
α-Glucuronidase	Cow	Function based	–	Lee et al. (2012)
Glycoside hydrolases	Bovine Ruminal Protozoan	Function based	–	Findley et al. (2011)
	Yak	Function based	–	Zhou et al. (2012)
	Yak	Function based (BAC vector)	Pyrosequencing	Dai et al. (2012)
	Bovine	Sequence based	Pyrosequencing 454 GS FLX	Brulc et al. (2009)
	Yak	Function based (cosmid vector)	–	Bao et al. (2011)
	Cow	Function based	Sanger sequencing	Zhao et al. (2010)
Carbohydrate active enzymes	Buffalo	Sequential screening	Ion torrent PGM next-generation sequencing	Patel et al. (2014)
	Cow	Function based	Pyrosequencing	Wang et al. (2013)
Mannanase-xylanase-glucanase	Cow	Function based	Sanger sequencing	Palackal et al. (2007)
Xylanase	Sheep	Function based (fosmid vector)	–	Wang et al. (2012)
Lipases	Cow	Function and sequence based	–	Liu et al. (2009)

the recalcitrant nature of biomass, its hydrolysis and further degradation are very difficult, hence also limiting industrial exploitation. Since the use of different pretreatment methods is time consuming and costly, the use of lignocellulolytic microbes of rumen can be an excellent way forward for applications in various industries including agriculture, chemicals, ethanol, animal feed, biofuel, food, paper and textiles. Furthermore, being anaerobic in nature, these microbes unlike their aerobic counterparts do not present any problems for bioprocess development.

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