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Review Article

RUMINANT AND MICROBIAL PECTINASE

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ABSTRACT
Local feed resources, particularly low-quality roughages and agricultural crop-residues, are of prime importance for ruminants raised in the tropics. These feeds exhibit close relationships with rumen ecology, microbes and rumen fermentation patterns. A number of dietary factors could influence rumen fermentation especially the basal roughage source, its physical form and fermentation end-products. Therefore there is a need to develop in vitro methods that do not require the need to surgically modify ruminants to obtain rumen fluid to study rumen degradation. Lactose in combination with peptone supported maximum pectinase production. This study deals with types and classification of pectinolytic enzymes, their mode of action, production techniques and the methods of activity assay. Furthermore, it provides the possible applications of these enzymes in ruminant nutrition.

Key Words: *Ruminant Nutrition, Fermentation, Microbial Pectinase, Silage.*

INTRODUCTION
The importance of rumen microbial ecology and diversity of microorganisms in the ruminant forestomach is highlight by McSweeney and Makkar (2005) in response to recent trends in global livestock production. The microorganisms in the digestive tracts of ruminant livestock have a profound influence on the conversion of feed into end-products which can impact on the animal and the Environment. The rumen microbial ecosystem is an anaerobic environment, which defines the microorganisms that have adapted to this lifestyle yet. It was Pasteur (1860) who described those microorganisms could survive and prosper in the absence of oxygen using the process of fermentation. Feed when ingested by ruminant animals are subjected to microbial degradation in the rumen. The end products of the degradation process, i.e. ammonia, amino acids, peptides and volatile fatty acids, are utilized for the synthesis of microbial biomass. The ultimate rumen pH value appeared to exert an effect on the type of rumen microorganisms.four chamber in ruminant stomach show in figure 1 (Russell, 2002)

Stomach Compartments
1. Reticulum
2. Rumen
3. Omasum
4. Abomasum

A higher proportion of a ruminant's digestive system is stomach.

Reticulum Characteristics
1. Located next to heart
2. Honeycomb appearance
3. Catches metal and hardware
4. Pathways
5. Esophagu
6. Rumen
7. Omasum
8. No enzymes secreted

Rumen Characteristics

1. Left side of abdomen
2. Papillae lining
3. Muscular pillars
4. Fermentation vat
5. Primarily anaerobic

Some aerobic microbes Not functional at birth

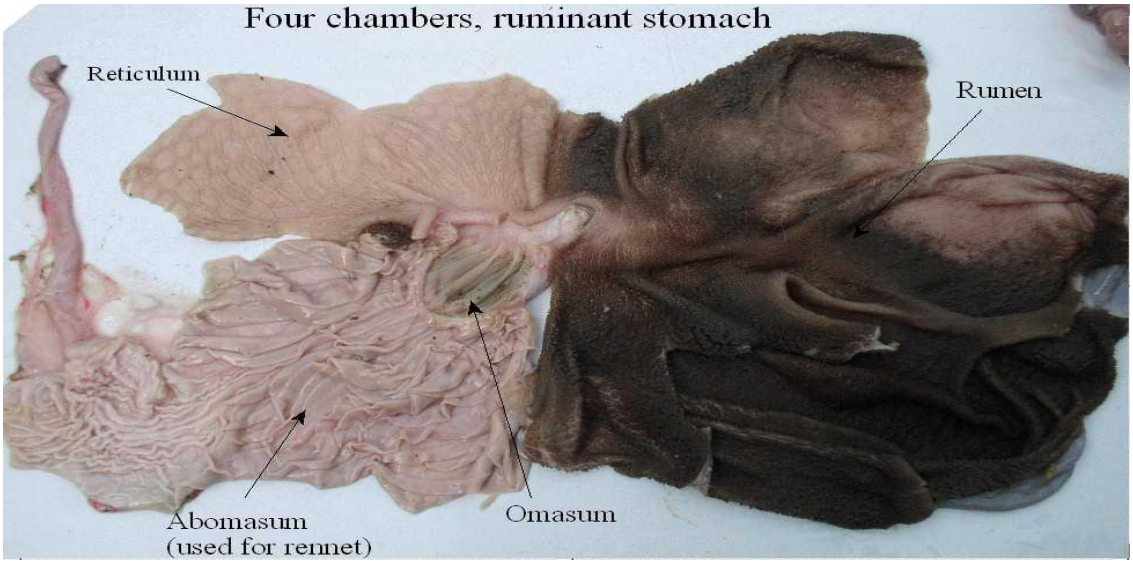


Figure 1: Four chamber of ruminant stomach

Rumen Function

1. Storage
2. Soaking
3. Physical mixing and breakdown
4. Fermentation
5. Synthesizes some vitamins
6. Synthesizes AA and protein
7. Breaks down fibrous feeds into VFAs

Digestive Fluids

1. Saliva
2. Gastric juices from stomach
3. Pepsinogen
4. Rennin
5. HCL

Volatile fatty acids (VFA) are produced in large amounts through ruminal fermentation and are of paramount in that they provide greater than 70% of the ruminant's energy supply (Matsuda *et al.*, 2011).

- **Acetic acid** is utilized minimally in the liver, and is oxidized throughout most of the body to generate ATP. Another important use of acetate is as the major source of acetyl CoA for synthesis of lipids.
- **Proprionic acid** is almost completely removed from portal blood by the liver. Within the liver, proprionate serves as a major substrate for gluconeogenesis, which is absolutely critical to the ruminant because almost no glucose reaches the small intestine for absorption.

- **Butyric acid**, most of which comes out of the rumen as the ketone beta-hydroxybutyric acid, is oxidized in many tissues for energy production.

Microbial fermentation can supply 70 to 100% of amino acid requirement in the form of microbial protein, and 70 to 85% of energy requirement in the form of short chain fatty acids (SCFA) (Dewhurst *et al*., 1986). Since protein requirement for milk or growth is obtained from the microbial protein synthesized in the rumen and the undegraded dietary protein (UDP), high microbial protein production in the rumen can decrease the need for undegraded dietary protein. Further, a proportionally high carbon fixation into microbial cells can reduce fermentable carbon losses in the form of carbon dioxide and methane (Beever *et al*., 1999). Rumen microbial biomass contributes hydrolytic enzymes for fiber digestion, in addition to serving as a source of amino acids for tissue protein synthesis.

Pectinase Enzyme

Pectins are polysaccharides ubiquitous in the plant kingdom and constitute the major component of plant cell walls. The pectinases are a group of related enzymes capable of degrading pectin. Therefore this group of enzymes have been used for decades in the food and winemaking industry for the processing of fruit juices (Mohnen, 2008) (*Prade et al.,* 1999) (Ribeiro *et al.,* 2010).

Different types of micro-organisms have been exploited for the production of enzymes. Pectinolytic enzymes have been reported to be produced by a large number of bacteria and fungi list in following table-1,

Table 1: Some bacteria and fungi produce pectinase

Bacteria	Fungi
Bacillus spp., *Clostridium* spp., *Verticillium spp.*, *Pseudomonas spp., Penicillium spp*	*Aspergillus spp.*,

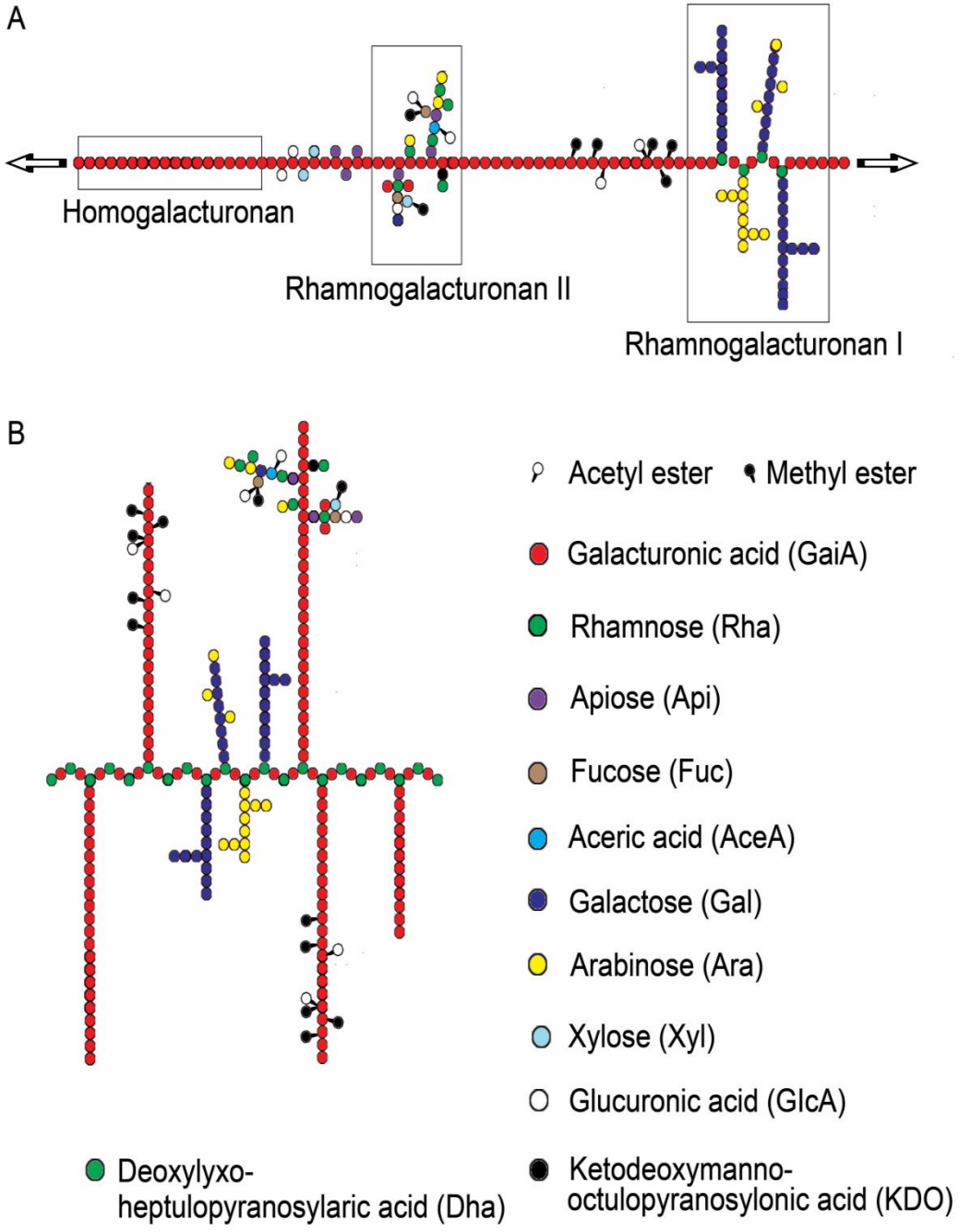
Pectic enzymes are widely distributed in nature. Pectin is first isolated in 1820s and shown to be the key substances in making jams and jellies. Jam and jellies have been produced for many years, at least since the 18th century. Pectinases are a heterogeneous group of enzymes that degrade Pectin. Microbially derived Pectinases find more use due to their advantage over plant and animal derived pectinases. The reasons being cheap production, easier gene manupilations, faster product recovery, further microbial enzymes are usually free of harmful substances These include Endopolygalacturonases(EC 3.2.1.1.5), Exopolygalacturonases(EC 3.2.1.67) , Pectate lyases (EC 4.2.2.2), Pectin lyases(EC 4.2.2.10) and Pectin methyl esterases(EC 3.1.11)(Marounek *et al.,* 1999).

The Substrate

Pectic substance is the generic name used for the compounds that are acted upon by the pectinolytic enzyme. They are high molecular weight, negatively charged, acidic, complex glycosidic macromolecules (polysaccharides) that are present in the plant kingdom. They are present as the major components of middle lamella between the cells in the form of calcium pectate and magnesium pectate (Jayani *et al.,* 2005).

The polymers are intended only to illustrate the some of the major domains found in most pectins rather than definitive structure (Willats *et al.,* 2006).

Various substrates that are being used are sugarcane bagasse, wheat bran, rice bran, wheat straw, rice straw, saw dust, corn cobs, coconut coir pith, banana waste, tea waste, sugar beet pulp, apple pomade, orange peel etc. (Pilar *et al.,* 1999).



Schematic representations of the conventional (A) And recently proposed alternative (B) Structures of pectin

Figure 2: The basic structure of pectin

Pectic substances are a group of galacturonan polymers with neutral sugars (largely arabinose and galactose) substitutions (Jung, 1997). Pectic substances are found in the middle lamella and other cell wall layers (Van Soest, 1994. The most important pectinolytic activity represents pectin lyase (EC 4.2.2.10) (Wojciechowicz, 1982). Grasses contain from 3 to 4% of pectin in the dry matter, leguminous

plants from 5 to 12%, and sugar beet pulp 25% (Aspinall, 1970; Van Soest, 1983; Cassida *et al.*, 2007). The American chemical society classiffy pectic substances into four main types as reported by Alkorta et al. (1998) as follows:

- Protopectin is the insolubal pectic substance in intact tissue. Protopectin on restricted hydrolysis yields pectin or pectic acid
- Pectic acid is the soluble polymer of galacturonans that contains negligible amount of methoxyl groups Normal or acid salt of pectic acid are called pectates
- Pectinic acids is the polygalacturonan chain that contains >0 and <75% methylated galacturonate units. Normal or acid salts of pectinic acid are referred to as pectinates
- Pectin (Polymethyl galacturonate) is the polymeric material in which, at least, 75% of the carboxyl groups of the galacturonate units are esterifid with

Methanol . It confers rigidity on cell wall when it is bound to cellulose in the cell wall

The esterification level and the distribution of esterified residues along the pectin molecule change according to the plant life cycle and between different species. Thus, the ability of some phytopathogenic microorganisms to poduce a variety of pectinolytic enzymes that differ in their characteristics, mainly in their substrate specifity, can provide them with more efficacies in cell wall pectin degradation and consequently more success in the plant infection (Herron *et al*, 2000). Silage is cut green plant material that is sealed in a concrete pit without air and water. Silage can be stored for approximately two years and still have up to 85% of the energy and protein value of the original fodder crop. Silage is a form of conserved grass (or other crop) (show in figure 3) that is made by farmers during the summer months when the grass supply is plentiful and not required for grazing. Enzymes promote the breakdown of complex feed molecules into smaller chemical fractions such as glucose or amino acids that are digestible by the ruminant animal. Common enzyme-based silage additives contain cellulases, hemicellulases, xylanases, amylases, and pectinases. Cellulases, hemicellulases, and pectinases are enzymes that degrade the fiber portion of forages.These multiple hydrolytic enzyme activities required to degrade the cell wall in the rumen are attained by a niche of diversified ruminal microbes that produce enzymes capable of cleaving certain linkages within the cell wall.Research data also suggests that hemicellulases and pectinases are more effective than cellulases at reducing fiber content. Unfortunately, hemicellulases and pectinases break down fiber fractions (hemicellulose, pectin) that are more easily digested by ruminants.

Ruminal microorganisms that interact with feed sparticles can be functionally described as three distinct subpopulations: 1) those associated with the ruminal fluid, 2) those loosely attached to feed particles and 3) those firmly attached to feed particles (Cheng and McAllister, 1997). Microorganisms associated with the ruminal fluid include those newly detached from feed particles, as well as those that survive on soluble feed components withinruminal fluid and have little direct involvement in the digestion of insoluble feed particles (Latham, 1980).

Figure3: Silage made by farmers

Microbial Pectinase

Source

Samples can be collected from different sites such as Fruit market waste,Lemon farm, Garden sites,Guava farm , Dumped fruit juice waste ,Sugarcane bagasse, Wheat bran,Rice bran, Wheat straw, Rice straw, Corn cobs, Banana waste, Sugar beet pulp, Apple pomade,Citrus peel, sunflower, Orange peel etc. (Pilar *et al.,* 1999).

Pectinase production technique

In developing nation, there is noticeable of food and processing industries, leading to an increase in the demand for pectic enzymes. Unfortuately, many of these nations depend largely on imported pectinase.

Microbial pectinases production techniques: There are two fermentation techniques we can use for pectinases production, as many other enzymes (Murad and Foda,1992) these techniques are,

1) Solid Sate Fermentation (SSF)
2) Submerged fermentation (SmF)

Solid state Fermentaion :- This process occurs in the absence or near absence of free water in the space between substrate particles. In this system, water is present in the solid substrate whose capacity for liquid retention varies with the type of material (Lonsane *et al.,* 1985).

Submerged fermentation :- in submerged fermentation (SmF) the nutrients and microorganisms are both submerged in water (Grigelmo-migeul and Martin-Belloso, 1998). Approximately 90% of all industrial enzymes are produced in SmF, frequently using specifically optimized, genetically manipulated microorganisms. In this respect SmF processing offers an insurmountable advantage over SSF (Kashyap, *et al.,* 2003).

The factors affecting microbial pectinases production: Environmental and nutritional factors are known to have market on enzyme production by microorganisms. There are, therefore, variation in optimum conditions for pectic enzyme production. Some of the cultural factors that production of pectic enzymes are presented in the study.

Initial pH of growth medium: According to Shoichi *et al.* (1985) the initial pH of the medium has a great effect on the growth of the organism, on the membrane permeability, also on the biosynthesis and stability of the enzymes (Murad,1998; Murad and Salem,2001). Optimum production of pectic enzymes from many moulds has been reported to be within the acidic pH (pH 5.0) in addition, piccoli-Valle *et al.*(2001) observed that a high polygalacturonase and pectin esterase activity was showed by P.griseoroseum in more acid pH of 4.5 and 5.

Incubation period: Mximum production of pectic enzyme from different moulds varies from 1 to 6 days (Ghildyal *et al.,* 1981). Castilho *et al.* (2000) reported that the higherst polygalacturonase activities are obtain by *A.niger* after 70 h 0f fermentation period. Moreover, Sarvamangala and Dayanand (2006) observe a gradual increase in the production of pectinase from dessede sunflower head by *A. niger* after 72 h of fermentation period in submerged and up to 96 h in solid-state conditions.

Nitrogen source: The effect of organic and inorganic source**s** on the production of pectinase are extensively studies. The obsevvation of Hours *et al.* (1988) suggested that lower levels of $(NH_4)_2 SO_4$ (0.16%) added to the growth medium as inorganic nitrogen sources is not influence pectinase yield. In addition Galiotou-Panayotou and Kapantai (1993) observed that ammonium phosphat and ammonium sulphat is influence production of pectinase positively but also recorded the inhibitory effects of ammonium nitrate and potssium nitrate on pectinase production.

Extraction and Purification

The crude enzyme source can beextraction and purification by different chromatography like ion exchang chromatography, gel dhromatography, etc.

Application in Ruminant Nutrition

Ruminants have an extensive array of microbial enzymes produced in the rumen and these enzymes play an important role in the ruminant digestive process. Pectinolytic activity in the rumen arises from protozoal, fungal and bacterial sources, one of the major pectinolytic bacterial species inhabiting the

rumen, *Lachnospira multiparus*, produces a pectin lyase and a pectin methylesterase (Silley, 1985). Use supplemental enzymes in the rumen may be important since the digestibility of organic matter in the rumen is not reach 100% and even small changes in digestibility can influence the efficiency of ruminal fermentations. As a result, many strategies have been developed to stimulate the digestion of the fibrous components in ruminant feeds. One particularly promising technology is supplementation of fibrolytic enzymes (Cellulases, xylanases and pectinases) to ruminant's diets (Selinger *et al*., 1996). Although extensive studies are Carrie out to investigate the effects of cellulases and xylanases supplementation to ruminant diets on their productivity, study effects of pectinases supplementation to ruminant diets is lacking.This ten fold increase in activity of polygalacturonase activity attributed to citrus pulp suggests that the formation of extracellular polygalacturonase was induced by the increase in total pectic substances in the diet.

Finally, we recommended in this study giving more attention to production of microbial pectinases and more attention to their application especially in animal feed production.

Other applications are shown in following table

Table 2: Other application of pectinase (Emma & Melina-2010) (Sonia Ahlawat -2009) (Amanjot kaur-2010)

AREA	APPLICATION
Juice industry	Juice clarification
Textile industry	They are capable of depolymerising the pectin breaking it into low molecular water soluble oligomers improving absorbency and whiteness of textile material andavoiding fiber damage
Pulp and paper industry	Effective in biobleaching of mixed hard wood and bamboo kraft pulp,
Wine industry	Improve wine characeristics of colour and turbidity, improvement of chromaticity and Stability of red wines
Biological applications	In protoplast fusion technology and plant pathology
Coffee and tea fermentation	Fermentation by breaking pectins present in tea Leaves
Oil extraction	By avoiding emulsification formation

CONCLUSION

Even though the ruminal ecosystem represents a sophisticated microbial community for attacking fibrous substrates, digestion of these feeds in the rumen is still less than desirable. This usage of pectinase for ruminants feed production can reduces the feed viscosity, which increases absorption of nutrients, liberates nutrients,either by hydrolysis of non-biodegradable fibers or by liberating nutrients blocked by these fibers and reduces the amount of feces.

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