CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Universiti Putra Malaysia Institutional Repository

UNIVERSITI PUTRA MALAYSIA

IN VITRO STUDIES ON THE ADHESION OF FIBROBACTER SUCCINOGENES STRAIN D3 TO MACROCRYSTALLINE CELLULOSE

SIEO CHIN CHIN

IB 1997 1

IN VITRO STUDIES ON THE ADHESION OF _FIBROBACTER SUCCINOGENES_ STRAIN D3 TO MICROCRYSTALLINE CELLULOSE

By

SIEO CHIN CHIN

Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Institute of Bioscience Universiti Putra Malaysia

June 1997

ACKNOWLEDGEMENTS

I would like to express my appreciation and sincere gratitude to the chairman of the supervisory committee, Associate Professor Dr. Norhani Abdullah for her guidance and advice throughout the course of this study and in the preparation of this thesis. Sincere thanks are also extended to the other members of the supervisory committee, Professor Dr. Ho Yin Wan, and Professor Dato' Dr. Syed Jalaludin Syed Salim, for their invaluable advices and suggestions, and to Dr. Mohd. Ridzwan, Department of Agronomy and Horticulture, Faculty of Agriculture, for his advice on the statistical analysis of the data.

I would also like to extend my sincere thanks to all the staff of the Rumen Microbiology Laboratory, especially Khairul and Jivanathan and staff of the Electron Microscope Unit, especially Mr. Ho Oi Kuan, Puan Aminah Jusoh and Cik Azilah Abdullah Jalil. To my fellow labmates and friends, Geok Yong, Wan Zuhainis, Dr. Jin LiZhi, Thongsuk, Michael and Foong Yee, my grateful thanks for their companionship, humour, support and cooperation.

Finally, I wish to extend my heartfelt thanks and gratitude to my family and my husband, Lai Kok Loong, for their unending support, encouragement and understanding throughout my study.

TABLE OF CONTENTS

		Page
ACKNOWLEDGEMENTS		ii
LIST OF TABLES		vi
LIST OF FIGURES		vii
LIST OF PLATES		viii
LIST OF ABBREVIATIONS		x
ABSTRACT		xi
ABSTRAK		xiv
CHAPTER		
I	INTRODUCTION	1
II	LITERATURE REVIEW	4
	The Rumen Microorganisms and Their Activity	4
	Rumen Protozoa	4
	Rumen Fungi	6
	Rumen Bacteria	8
	Adhesion of Rumen Microbes on Insoluble Nutrients	10
	Electron Microscopic Observation on the Adhesion of Bacteria	12
	Specificity in the Adhesion of Rumen Microbes to Solid Substrates	15
	The Cellulosome	17
	Cellulosome and Rumen Microorganisms	18
	Strategies to Improve Cellulose Digestion	19
III	EFFECT OF PHYSICOCHEMICAL FACTORS ON THE ADHESION OF *F. SUCCINOGENES* STRAIN D3 TO AVICEL	23
	Materials and Methods	24
	Source of Chemicals	24
	Source of Bacteria	24
	Experimental Conditions	24
	Preparation of Modified Scott and Dehority Medium (MOD-SD)	25
	Bryant's Solution	27
	Preparation of Bacteria Culture	28
	Studies on Growth Curve of *F. succinogenes*	28
	Adhesion Test	29

	Adhering Ability of *F. succinogenes* to Avicel at Different Growth Phase	31
	Effect of pH and Temperature on Adhesion	31
	Effect of Enzyme Treatment of Cells on Adhesion	32
	Statistical Analysis	34
	Results	34
	Growth Pattern of *F. succinogenes*	34
	Influence of Growth Phase on Adhesion of *F. succinogenes* to Avicel	37
	Effect of pH and Temperature on the Adhesion of *F. succinogenes*	40
	Effect of Enzyme Treatments on Adhesion of *F. succinogenes*	42
	Discussion	46
IV	ELECTRON MICROSCOPIC STUDY ON THE ADHESION OF *F.SUCCINOGENES* STRAIN D3 TO AVICEL	52
	Materials and Methods	52
	Scanning Electron Microscopy	52
	Transmission Electron Microscopy	54
	Results	55
	Scanning Electron Microscopy	55
	Transmission Electron Microscopy	57
	Discussion	68
V	DETECTION OF CELLULOSE-BINDING PROTEINS (CBPs) OF *F. SUCCINOGENES* STRAIN D3	74
	Materials and Methods	74
	Detection of CBPs	74
	Elution of CBPs	75
	Binding Ability of Eluted Proteins	76
	CBPs of Enzymes Treated Cells	76
	SDS-PAGE Electrophoresis	77
	Detection of Carboxymethylcellulase (CMCase) and Xylanase Activity of CBPs	78

PERPUSTAKAAN
UNIVERSITI PUTRA MALAYSIA

Results.. 79
Detection of CBPs.. 79
Elution of CBPs.. 79
Binding Ability of CBPs.................................... 86
Detection of CBPs on Cells Treated with
Enzymes.. 86
CMCase and Xylanase Activity of CBPs.................. 86

Discussion.. 91

VI GENERAL DISCUSSION.. 99

VII CONCLUSION.. 106

BIBLIOGRAPHY.. 108

APPENDIX.. 120

BIOGRAPHICAL VITA.. 128

LIST OF TABLES

Table		Page
1	Turbidity measurement and total viable cell counts of *F. succinogenes* strain D3 grown in MOD-SD(GC)………….…….	35
2	Percentage of adhesion of *F. succinogenes* to avicel at different periods of growth at pH 6.5 and 39°C..……….….…………..	38
3	Adhesion of *F. succinogenes* strain D3 to avicel at various pHs and temperatures…………..…...............................…………	41
4	Adhesion of *F. succinogenes* strain D3 to avicel after enzyme treatments of cells at recommended pH condition..……..…	43
5	Adhesion of *F. succinogenes* strain D3 to avicel after enzyme treatments of cells at optimum pH condition……..………..	44

LIST OF FIGURES

Figure		Page
1	Growth pattern of *F. succinogenes* strain D3 in MOD-SD(GC) evaluated by turbidity measurement and total viable cell counts..	36
2	Adhesion of *F. succinogenes* to avicel at various stages of growth..	39

LIST OF PLATES

Plate		Page
1	Scanning electron micrographs showing the adhesion of *F. succinogenes* (late exponential phase) to avicel after 10 min of incubation	56
2	Scanning electron micrograph of *F. succinogenes* showing the spike-like structures (S) for adhesion of the bacteria to the substrate and thicker structures (T) that joined the bacteria together. The bacteria were grown for 8 h in MOD-SD medium containing avicel (V)	58
3	Digestion of avicel by *F. succinogenes* after 30 h of incubation	59
4	Pit of digestion at higher magnification	60
5	Transmission electron micrograph of late-exponential-phase *F. succinogenes* strain D3 grown for 8 h in soluble carbohydrate medium	61
6	Transmission electron micrograph of *F. succinogenes* showing initial adhesion to avicel (V) (10 min after incubation with avicel)	63
7	Transmission electron micrograph showing adhesion of *F. succinogenes* to avicel (V) after 18 h of incubation	64
8	Transmission electron micrograph showing adhesion of *F. succinogenes* to avicel (V) after 18 h of incubation in MOD-SD(A)	65
9	Transmission electron micrograph showing the digestion pits or zones in avicel (V) made by *F. succinogenes* after 36 h of incubation in MOD-SD(A)	66
10	Transmission electron micrograph showing the digestion zones in avicel (V) made by *F. succinogenes* after 56 h of incubation in MOD-SD(A)	67
11	Transmission electron micrograph showing the surface material of *F. succinogenes* involved in adhesion of the cells to avicel at different incubation period	69

12	SDS-PAGE protein profiles of degraded *F. succinogenes* strain D3 before and after incubation with avicel, and control samples of medium and buffer used	80
13	SDS-PAGE analysis of washing buffers of avicel incubated with cell lysate of *F. succinogenes*..	81
14	SDS-PAGE of proteins eluted with 1% (w/v) CMC, performed in 7.5% acrylamide separating gel and 4% acrylamide stacking gel..	83
15	SDS-PAGE of proteins eluted with 10% (w/v) cellobiose and 5% (w/v) SDS from avicel incubated with cell lysate of *F. succinogenes*..	84
16	SDS-PAGE analysis of proteins eluted with 5% (w/v) SDS..........	85
17	SDS-PAGE analysis of proteins eluted from avicel treated with CBPs of *F. succinogenes* strain D3..................................	87
18	SDS-PAGE of CBPs from cells treated with or without enzymes..	88
19A	Zymogram analysis of xylanase in cell lysate and CBPs of *F. succinogenes*..	89
19B	Zymograms analysis of CMCase in cell lysate and CBPs of *F. succinogenes*..	90

LIST OF ABBREVATIONS

CMC	-	carboxymethylcellulose
CMCase	-	carboxymethylcellulase
CBPs	-	cellulose-binding proteins
EDTA	-	ethylenediaminetetraacetic acid
kDa	-	kilodalton
O.D.	-	optical density
PAGE	-	polyacrylamide gel electrophoresis
SEM	-	scanning electron microscopy
SDS	-	sodium dodecyl sulphate
TEM	-	transmission electron microscopy

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

IN VITRO STUDIES ON THE ADHESION OF *FIBROBACTER SUCCINOGENES* STRAIN D3 TO MICROCRYSTALLINE CELLULOSE

By

SIEO CHIN CHIN

June 1997

Chairman : Associate Professor Dr. Norhani Abdullah

Faculty : Institute of Bioscience

In the present study, the factors affecting adhesion of *Fibrobacter succinogenes* strain D3 to microcrystalline cellulose avicel were investigated. *Fibrobacter succinogenes* showed the highest percentage of adhesion (85%) during late exponential phase of growth. During lag phase, 50 – 55% of the bacterial cells were adherent and during death phase, 60 – 70% of the cells were adherent. Adhesion of bacterial cells to avicel was significantly ($P<0.05$) affected by pH and temperature and significant ($P<0.05$) interaction between these two factors was also observed. The optimum pH for cell adhesion was 6.5 and the optimum temperature was 39°C. At pH 6.5, the adhering ability of the cells was reduced when the temperature was raised to 50°C and 60°C or lowered to 4°C and 22°C. At this pH, the effect of temperature on adhesion was greater at high temperature than at low temperature. At 50°C and 60°C, only 20 - 27% adhesion was observed but at 4°C and 22°C, 48 - 58% adhesion was obtained. At other combinations of condition (pH 4.0, 5.6, 7.0, 8.0 and temperature

4, 22, 39, 50, 60°C), less than 20% adhesion was observed. The adhering ability of the bacterial cells was also reduced after the cells were treated with proteolytic enzymes such as thermolysin and pronase. Lipase and dextranase did not affect the adhesion of the cells.

The study using scanning electron microscopy and transmission electron microscopy showed that the adhesion of *F. succinogenes* was first mediated by fine structures radiating from the outer layer of the cell and then by the glycocalyx. Initial adhesion by these fine structures was observed after 10 min of incubation with avicel. After 18 h of incubation, the bacteria had digested away the cellulose at the point of contact and penetrated into the substrate. Pits of digestion surrounding the bacteria were particularly evident after 30 h of incubation and larger digestion pits were observed after 56 h of incubation.

Studies were also carried out to detect the cellulose-binding proteins (CBPs) of the cells. In this study, Buffer A supplemented with 1% (w/v) carboxymethylcellulose (CMC), 10% (w/v) cellobiose or 5% (w/v) sodium dodecyl sulphate (SDS) were used to elute CBPs from avicel incubated with cell lysate of *F. succinogenes*. Buffer A supplemented with CMC was found to elute two major proteins (120 kDa and 100 kDa) and a few minor proteins ranging from 35 kDa to 60 kDa. Buffer A supplemented with cellobiose or SDS eluted proteins with approximate weights of 240, 120 and 100 kDa. These three CBPs (240, 120 and 100

kDa) were involved in the adhesion process of the cells as cells with reduced adhering ability after being treated with proteolytic enzymes such as thermolysin and pronase did not show these CBPs. Other than possessing the ability to bind, the 240 kDa CBP showed xylanase activity and the 120 kDa protein showed carboxymethylcellulase (CMCase) activity in the cell lysate. The 100 kDa CBP did not show any of the two enzyme activities shown by 240 kDa and 120 kDa CBPs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

KAJIAN *IN VITRO* KE ATAS PELEKATAN *FIBROBACTER SUCCINOGENES* STRAIN D3 PADA SELULOSA MIKROHABLUR

OLEH

SIEO CHIN CHIN

Jun 1997

Pengerusi : Prof. Madya Dr. Norhani Abdullah

Fakulti : Institut Biosains

Kajian ke atas faktor-faktor yang mempengaruhi pelekatan *Fibrobacter succinogenes* strain D3 pada selulosa mikrokristal avisel telah dijalankan. *Fibrobacter succinogenes* menunjukkan peratusan pelekatan yang tertinggi (85%) pada fasa pertumbuhan eksponen. Pada fasa pertumbuhan 'lag', 50 – 55% daripada sel bakteria bersifat melekat dan pada fasa kematian, 60 – 70% daripada sel bakteria bersifat melekat. Pelekatan sel bakteria pada avisel dipengaruhi oleh pH dan suhu, dan kedua-dua faktor ini berinteraksi secara signifikan ($P<0.05$). Keadaan optimum untuk pelekatan adalah pH 6.5 dan 39°C. Pada pH 6.5, keupayaan sel untuk melekat berkurangan apabila suhu ditingkatkan ke 50°C dan 60°C atau diturunkan ke 4°C dan 22°C. Pada pH ini, suhu tinggi lebih mempengaruhi pelekatan sel berbanding dengan suhu rendah. Pada 50°C and 60°C, hanya 20 – 27% pelekatan diperhatikan tetapi pada 4°C dan 22°C, 48 – 58% pelekatan diperolehi. Pada keadaan kombinasi yang lain (pH 4.0, 5.6, 7.0, 8.0 dan suhu 4, 22, 39, 50, 60°C), kurang daripada 20% pelekatan

diperolehi. Keupayaan untuk sel bakteria melekat juga berkurangan setelah sel ditindak dengan enzim proteolitik seperti termolisin dan pronase. Lipase dan dekstranase tidak mempengaruhi pelekatan sel.

Kajian dengan mikroskop elektron imbasan dan mikroskop elektron transmisi menunjukkan peringkat awal proses pelekatan melibatkan struktur-struktur halus yang berasal dari lapisan luar sel dan seterusnya dilakukan oleh glikokaliks. Pelekatan oleh struktur-struktur halus ini diperhatikan selepas sel dieram selama 10 min dengan avisel. Selepas 18 jam pengeraman, bakteria mendegradasi substrat di tapak pelekatan dan menembusi substrat tersebut. Zon degradasi di sekeliling bakteria diperhati selepas 30 jam pengeraman dan bertambah besar selepas 56 jam pengeraman.

Kajian juga dijalankan untuk mengesan protein pelekat-selulosa pada sel. Dalam kajian ini, larutan penimbal A yang ditambah dengan 1% (w/v) karboksimetilselulosa (CMC), 10% (w/v) selobiosa dan 5% (w/v) sodium dodesil sulfat (SDS) telah diguna untuk menanggalkan protein pelekat-selulosa daripada avisel yang dieram dengan sel lisat *F. succinogenes*. Larutan penimbal A yang ditambah dengan CMC berjaya menanggalkan dua protein utama (120 kDa dan 100 kDa) dan beberapa protein lain yang mempunyai berat molekul antara 35 kDa hingga 60 kDa. Selobiosa dan SDS menanggalkan protein yang mempunyai berat molekul 240 kDa, 120 kDa dan 100 kDa. Ketiga-tiga protein pelekat-selulosa (240 kDa, 120 kDa dan 100 kDa) ini terlibat dalam proses pelekatan kerana sel yang mempunyai

keupayaan untuk melekat yang rendah setelah ditindak dengan enzim proteolitik seperti termolisin dan pronase tidak menunjukkan protein-protein pelekat-selulosa ini. Selain daripada berupaya untuk melekat, protein pelekat-selulosa 240 kDa menunjukkan aktiviti enzim xilanase dan protein pelekat-selulosa 120 kDa menunjukkan aktiviti enzim karboksimetilselulase (CMCase) dalam sel lisat. Protein pelekat-selulosa 100 kDa tidak mempunyai aktiviti enzim seperti yang ditunjukkan oleh protein pelekat-selulosa 240 kDa dan 120 kDa.

CHAPTER I

INTRODUCTION

For thousand of years, ruminants have played a major role in farming production and have provided mankind with meat, milk and clothing. Unlike man, ruminants feed on fibrous plant materials and utilise the carbohydrate components of the plant cell wall as major source of energy. The ability to digest and utilise plant materials is made possible through a unique relationship between the microbes in the rumen and the host animal.

The rumen, which is the largest compartment of the fore-stomach, is inhabited by a complex microbial population and the ability of ruminants to utilise fibrous materials for energy depends very much on the microbial activity and the symbiotic interaction between the microbial population and the host animal.

Among the rumen microorganisms which consist of bacteria, protozoa, fungi and probably other unknown microorganisms (Hungate, 1966), bacteria play a major role in the degradation of cellulosic materials. Early studies using light microscopy have shown that cavities developed in plant particles undergoing digestion in the rumen contain many bacteria (Baker and Harris, 1947). Later, studies using transmission electron microscopy and scanning electron microscopy revealed the

presence of many different morphological types of rumen bacteria adhering to the plant particles while degradation was in process (Akin et al., 1974; Akin and Amos, 1975; Akin, 1976). Digestion of plant cell walls in the rumen is predominantly by cellulolytic bacteria such as *F. succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens* (Amos and Akin, 1978; Windham and Akin, 1984). The close association between the rumen bacteria and the plant tissues often observed during digestion of plant materials (Cheng et al., 1983) suggests that bacterial adhesion is an important aspect of fibre degradation. Loose and irregular pattern of bacterial cell adherence on plant materials often results in less cell wall degradation (Kudo et al., 1987). Adhesion has also been reported to be substrate-specific and it has been postulated that a substrate-binding factor is involved in the recognition of the substrate (Gong and Forsberg, 1989).

Since bacterial adhesion is a prerequisite for the breakdown of cellulosic materials, the mechanisms of adhesion should be studied in order to improve and enhance the degradation of cellulose. Further characterisation of the cellular adhesion mechanisms may enable genetic engineering to play a dominant role in the manipulation of feed digestion through regulation of the genes responsible for the adhesion of microorganisms to the feed materials.

In view of this, an *in vitro* study on the adhesion of *F. succinogenes*, which is one of the major cellulolytic bacteria in the rumen (Bryant and Doetsch, 1954; Malburg and Forsberg., 1993), to crystalline cellulose avicel was carried out to

determine the factors involved in the mechanism of adhesion. The specific objectives of this study are:

a) to study the effects of several physiochemical factors such as the growth phase of bacteria, pH, temperature and enzyme treatments on the adhesion of *F. succinogenes*;

b) to observe the adhesion of *F. succinogenes* on avicel using scanning electron microscopy and transmission electron microscopy; and

c) to detect the substrate-binding factor i.e. cellulose-binding factor of *F. succinogenes*.

UPM

CHAPTER II

LITERATURE REVIEW

The Rumen Microorganisms and Their Activity

The rumen, which is one of the most important compartment of the ruminant's stomach, is inhabited by a complex microbial population. It contains one of the most varied and dense microbial populations known in nature. Of these microorganisms, fungi, protozoa and bacteria are known to be the main inhabitants in the rumen (Czerkawski, 1986). They play a role in the colonisation and degradation of feed material in the rumen and their presence significantly affects the performance of the animal in the utilisation of feed materials. The animal provides a suitable niche for the microorganisms and, in return, the microorganisms degrade the feed ingested by the animal and provide valuable metabolites to the host animal. The population of these microorganisms often fluctuate with the intake of feed and types of diet of the animal (Eadie, 1962).

Rumen Protozoa

The population of rumen protozoa has been estimated to be about 10^5-10^6/ml of rumen contents (Tsuda, 1976), and they have been observed to play a significant role in the primary degradation of plant fragments (Williams, 1989). About 25 - 30% of the total fibre degradation in the rumen is contributed by the protozoa (Williams

PERPUSTAKAAN
UNIVERSITI PUTRA MALAYSIA

and Coleman, 1992). The population density of rumen protozoa is dependent on the frequency of feeding. Higher population of protozoa is observed in animals which are fed regularly and frequently throughout the day (Bonhomme, 1990).

Generally, protozoa can be divided into two classes: ciliates and flagellates. Cilliates are the predominant protozoa in the rumen (Bonhomme, 1990). They are made up of two groups, namely, the holotrich and the entodiniomorphid (Williams, 1986). The entodiniomorphid ingests and digests particulate plant materials, while the holotrich uses mainly soluble carbohydrates (Williams, 1986). Hence, the occurrence of holotrich and entodiniomorphid is very much determined by the type of diet of the host animal. For instance, the numbers of holotrich are higher when the diet contains readily available soluble carbohydrates such as sugar and molasses (Valdez et al., 1977).

Rumen protozoa have been reported to play a number of roles in the rumen. Other than being able to degrade cellulose (Coleman, 1985), protozoa are able to degrade and metabolise the principle protein, carbohydrate and lipid components of the feed materials ingested by the host animal (Bonhomme, 1990). Protozoa also contributes to the microbial turnover in the rumen by predation process where bacteria, which are the nitrogen source for the protozoa, are engulfed by the protozoa. Protozoa play an important role especially in ruminants fed high sugar. The holotrich assimilates soluble sugar and store them as amylopectin (Coleman, 1979). This reduces the rate of fermentation and thus prevents accumulation of high level of

lactate which may cause a rapid lowering of pH in the rumen. In addition to that, rumen protozoa have been implicated to be involved in copper (Cu) metabolism where their presence may alleviate Cu toxicity of the animal (Ivan et al., 1991).

In spite of the roles the protozoa play in the rumen, the protozoa have been considered to be unimportant in ruminant production. Protozoa are found to be unnecessary in improving animal performance since defaunation results in higher growth rate of the animal (Romulo et al., 1988). Furthermore, the unavoidable predatory behaviour of protozoa reduces bacterial growth efficiency and hence reduce the net yield of bacterial amino acids available for intestinal absorption. As 60 - 80% of the protozoa were lysed and degraded in the rumen (Leng, 1988), the protozoa are not considered as an important source of protein to the animal.

Rumen Fungi

Anaerobic rumen fungi are discovered in the rumen only about two decades ago (Orpin, 1975; Bauchop, 1979). Five genera of anaerobic rumen fungi have been identified and they are divided generally into two groups, the monocentric and the polycentric. The monocentric fungi produce a single sporangium and an anucleate rhizoidal system while the polycentric fungi produce an extensive network of rhizomycelium with many sporangiophores on which sporangia develop. Out of the five genera of anaerobic rumen fungi, three genera are in the monocentric group, viz., *Neocallimastix, Piromyces* and *Caecomyces* (Orpin, 1975; 1976; 1977), and two in the

polycentric group, viz., *Orpinomyces* (Barr et al., 1989) and *Anaeromyces* (Breton et al., 1991) [= *Ruminomyces* (Ho et al., 1990)].

Rumen fungi contribute significantly to the prime function of the rumen. The fungi are actively involved in the digestion of plant cell walls to provide fermentation products for the nutrition of the host animal (Orpin and Ho, 1991). All the species of rumen fungi isolated so far are capable of fermenting structural carbohydrates of plant cell walls, especially lignocellulosic tissues (Orpin, 1981; Akin et al., 1983). *In vitro* studies of some species of rumen fungi on the digestion of wheat straw showed that about 40 - 50% of the dry weight of wheat straw fragments was lost in four days. About 50% of the cellulose and hemicellulose was digested and approximately 16% of the lignin in the plant fragments was degraded (Orpin, 1984).

The manner in which rumen fungi colonise the plant materials in the rumen differs markedly from that of the rumen bacteria. The fungus uses its rhizoidal system or hyphae to attach to plant fragments. It was observed that initial colonisation often occurs at the sites of damaged tissues and at the stomata (Orpin, 1977; Akin et al., 1983). Lignified cell walls or recalcitrant tissues such as schlerenchyma and vascular tissues are preferentially colonised (Orpin and Joblin, 1988). Upon attachment of the fungus, the rhizoids or hyphae penetrate into the tissue, colonising the sclerenchyma and vascular tissues, and eventually breaking the tissues into smaller particles (Ho et al., 1988) which in turn will enhance colonisation and digestion by bacteria. The anaerobic fungi penetrate plant cell walls with the help of structures called appresoria

which help the rhizoids in piercing the plant cell wall (Ho et. al., 1988). It has been observed that the penetration of cell walls by the rhizoids not only caused degradation of tissues, which provide surfaces for secondary attack of other rumen microorganisms, but it also prevent the fungus from being washed out by the liquid phase of the rumen contents.

Although the population of fungi (10^3 – 10^5/ml of rumen content) is the lowest among the rumen microorganisms, the presence of fungi in the rumen is essential especially in animal fed low quality forage. Orpin and Joblin (1988) suggested that the presence of fungi is not necessary in animal fed low fibre diet as the rumen bacteria, with a higher population, are capable of digesting this low fibre materials efficiently.

Rumen Bacteria

Rumen bacteria is considered to be one of the most important microbial groups in terms of number, total activity, diversity, consistency in fibre degradation and their ability to survive unfavourable conditions (Akin et al., 1993). In fact, it has been reported that cellulolysis in the rumen is primarily due to the activities of the rumen cellulolytic bacteria (Weimer, 1996).

It is estimated that the population density of rumen bacteria is about 10^{10} - 10^{11} bacteria /ml of rumen content (Trinci et al., 1994). The bacteria range from as small as 1 - 2 μm to as large as 3 - 6 μm in diameter (Czerkawski, 1986), and are made up of all the major morphological forms of bacteria. They may be Gram positive or