CHARACTERISTICS AND ENZYMATIC PROPERTIES OF SOME RUMINAL CELLULOLYTIC AND HEMI-CELLULOLYTIC BACTERIA ISOLATED FROM BUFFALO AND CATTLE

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ABSTRACT

Isolation and identification of some ruminal active cellulolytic and hemicellulolytic bacteria were carried out from 3 buffaloe and 3 cattle bullocks rumen. Three stains of bacteria were isolated from buffaloe rumen and identified as: *Ruminobacter amylophilus, Prevotella ruminicola* and *Fibrobacter succinogenes*. Meanwhile, four strains of bacteria were isolated from cattle rumen and identified as: *Selenomonas ruminantium* sub. sp. *ruminantium, Selenomonas ruminantium* sub. sp. *bryanti, Lachnospira multiporus* and *Anaerovibrio lipolyctica*.

The activity of crude enzyme extracted from rumen fluid of buffaloe and cattle tended to increase significantly by advancing the time post feeding and the maximum activity was recorded at 9.0 hours post feeding without significant differences among buffaloe and cattle. *Fibrobacter succinogenes* from buffaloe rumen and *Selenomonas ruminatium* sub. sp. *ruminantium* from cattle rumen proved to be the most active bacterial species. The dry matter disappearance of wheat straw, rice straw and date seeds was increased significantly from 2 days up to 8 days of incubation with either *Fibrobacter succinogenes* or *Selenomonas ruminantium*, but it was more pronounced in case of the 1st strain isolated from the buffaloe rumen compared with the 2nd strain isolated from the cattle rumen. The dry matter disappearance of wheat straw, rice straw and date seed were also significantly higher when incubated with the crude enzyme extracted from *Fibrobacter succinogenes* culture compared with the crude enzyme extracted from *Selenomonans ruminantium* sub. sp. *ruminantium* culture. Meanwhile, there was no significant difference between the three roughages on the dry matter disappearances. The crude enzymes extracted from rumen fluid of both animal species were more active than the crude enzymes extracted from the culture filtrates of the most active bacterial strains. The optimal value for the activity of enzyme extracted from *F.* The optimal pH value for the activity of enzyme extracted from *F. succinogenes* and *S. ruminantium* was 6.7, while the optimal temperature for the activity of extracted enzyme from the two strains was 37°C. The effect of some microelements and some vitamins on the enzyme activity was also investigated.

Keyword: *Cellulolytic & hemicellulolytic rumen bacteria, isolation identification, enzymatic activity.*

INTRODUCTION

It is evident that the ruminant animals consume grasses, leaves, twigs and stems rich in cellulose, hemicellulose and legnin. These animals do not produce, the enzymes responsible for degradation of lignocelluloses, but are dependent on associated microbial populations. The rumen provides a relatively uniform and stable environment that is anaerobic, 35 - 40°C and has a pH

of 5.5-7.0. These conditions, which are optimal for the associated microorganisms, and the continuous supply of ingested plant materials permits the development of very dense population of microorganisms. (Hobson and Stewart, 1997; Maklad and Mohamed, 2000). Bendary *et al.* (2002) found that the microflora of buffalo rumen were more efficient in degradation of plant tissue than that of cow and the results obtained indicated that DM, CF fractions and nutrients disappearance percentage of some synthetic and natural cellulosic materials were more pronounced when samples were incubated in buffalo rumen than in cow rumen.

The present investigation was conducted to through some light on the contribution of rumen bacteria in the digestion of lignocellulosic rich feedstuffs. Special emphasis was laid upon the relative presence of the various bacterial species implicated in this regard as influenced by the type of feeds. The factor affecting the production and activity of lignocellulosic enzymes were also attempted.

MATERIALS AND METHODS

This study was carried out at the Department of Botany, Faculty of science, Mansoura University, Sakha Anim. Prod. Res. Laboratories and Nutritional Res. Unit at Ismailia Agric. Res. Station, Anim. Prod. Res. Institute Agric. Res. Center.

During the experimental period all animals were individually fed similar ration and the levels of feeding was at maintenance requirement according to NRC (1989) allowance. Ration contained concentrate feed mixture (CFM), rice straw (RS) and berseem hay (BH). Concentrate feed mixture was offered twice daily at 8.0 a.m and 5.0 p.m. and berseem hay once daily at 9 a.m., while rice straw was given from 10.0 a.m to 4.0 p.m. Fresh water was offered to the animals three times daily. Ruminal contents were collected by ruminal fistula from 6 fistulated bullocks (3 buffalo and 3 cattle) weighing 550 to 650 kg.

For isolation and identification of bacteria, ruminal contents were taken 6 hours after morning concentrate feeding and were squeezed through four layers of cheese cloth into an Erlenmeyer flask with an O_2 free head space. The fluid was anaerobically transferred to centrifuge bottles (CO_2 gas phase) and centrifuged 150 x g at 4°C for 5 min.) to allow sedimentation of feed particles and protozoa. Particle-free fluid from the bottles that contained bacteria was anaerobically transferred (33% vol/vol) using anaerobic glove box (Hoboson and Stewart, 1997) to a medium (pH 6.7) containing 292 mg K_2HPO_4, 240 mg KH_2PO_4, 280 mg $(NH_4)_2$ SO_4, 480 mg NaCl, 100 mg $MgSO_4.7H_2O$, 64 mg $CaCl_2.2H_2O$, 400 mg Na_2CO_3 and 600 mg/l cysteine. HCl (Russel and Martin, 1984 and Callaway and Martin, 1997).

Isolation of bacteria was carried out according the procedure adopted by Hungate (1966) and Russell & Martin (1984) using solid basal medium containing sod-caroxyl methyl cellulose (CMC-Na) as a carbon source, for microbial cultivation and purification microbial strains by subculturing them anaerobicaly using anerojar system (Anero Gen™ 2.5 L).

Identification was carried out according to Krieg & Holt (1984), Sneath *et al.* (1986), Atlas and Bartha (1987), Staley *et al.* (1989), Williams & Holt (1989) and Stewart *et al.* (1997).

Cultures of rumen bacteria were maintained in slants prepared from non-selective media with 0.7-1.2% (w/v) agar.

Cultures were incubated at 39°C until growth is apparent, then stored at 4°C. For long preservation of cultures, glycerol was added to a final concentration of 20% (v/v), to cultures which have been incubated for 12-24 hr at 39°C. Such cultures remain viable for at least one year if stored at –20°C (Stewart *et al.*, 1997).

To collect and prepare ruminal crude enzyme, ruminal fluids were collected from each individual animal (cow and buffaloe) at 0, 1, 3, 6, 9 and 12 post-feeding, squeezed through four layers of cheese cloth, and pH was determined immediately. Aliquots of 20 ml from each rumen fluid sample were centrifuged at 4000 rpm at 4°C for 20 min., then pure supernatant was collected in 25 ml vials, stored at –10°C and used as ruminal crude enzyme (Mankarios and Friend, 1980 and Van Kessel and Russell, 1997).

Crude enzymes for each bacterial strain were obtained from their culture broths after growing for 7 days at 37°C under anaerobic condition by centrifugation at 4000 rpm for 20 min. at 4°C. The supernatant of each strain was collected in volumetric flask, and store at –10°C. Activity of enzymes in both ruminal fluid and culture filtrate were determined by the cup-plate technique as described by (Youssef & Mankariose, 1975).

Dry matter disappearance (DMD) of wheat straw (WS) , rice straw (RS) and the powder of date seed (DS) by cellulolytic and hemicellulolytic enzymes extracted from rumen fluid of buffaloe and cow along with crude enzymes isolated from the most active cellulolytic and hemicellulolytic bacteria (*in vitro*) was carried out according to Odenyo *et al.* (1991).

Eight microelements namely: boron, iron, manganese, molybdenum, nickel, zinc, cobalt and copper, have been tested for their effects on production and activity of cellulolytic enzyme of *F. succinogenes* and *S. ruminatium* sub. sp. *ruminatium*. Each microelement was incorporated at a concentration of 10 mg/L in the basal medium. The basal medium was prepared using tridistilled water supplemented with 1% CMC-Na and adjusted to pH 6.7. 50 ml of the medium were taken in Erelenmeyer flasks (250 ml) supplemented in triplicate with the various microelements (Samaan, 1978 and Mansour and El-Sayed, 1985).

The effect of some vitamins on production and activity of cellulolytic enzymes by *Fibrobacter succinogenes* and *Selenomonas ruminatium* sub. sp. *ruminantium* were studied. The liquid medium contained the most favorable source of carbon (CMC-Na) together with the best microelement (sodium borate) was prepared. Then the media were supplemented in triplicate, after being autoclaved, with the following vitamins. Cyanocobalamine (B_{12}), riboflavin (B_2), Biotin, Pyridoxin (B_6), nicotinic acid (B_5), thiamine (B_1), folic acid, P-amino benzoic acid, vitamin C, pantothenic acid (B_3) and vitamin A. Each vitamin was added at a concentration of 10 µg/L of liquid medium. Moreover, as a natural source of vitamins, yeast extract at a concentration of 0.5 gm/L was also tested. The pH of all media was adjusted to 6.7 before autoclaving (Samaan 1978 and Mansour and El-Sayed (1985). Inoculation, incubation and cup plate technique were used for measuring the production and activity of cellulolytic enzyme as mentioned before.

All collected data were subjected to the statistical analysis as the usual technique of analysis of variance (ANOVA) as mentioned by Steel and Torrie (1980) and was carried out using IRRISTAT software version 3193 (Biometric unit. International rice

Research Institute, Manila, Philippine).

RESULTS AND DISCUSSION

Seven strains of rumen bacteria have been isolated, purified and identified. Bacterial strains isolated from rumen fluid of buffaloe were:

1.*Ruminobacter amylophilus* formerly *Bacteriodes ruminicola* sub. sp. *amylophilus.*

2. *Prevotella ruminicola* formerly *Bacteriodes ruminicola* sub. sp. *ruminicola.*

3. *Fibrobacter succinogenes* formerly *Bacteriodes ruminicola* sub. sp. *succinogenes.*

Whereas bacterial strains isolated from rumen fluid of cattle were:

1. *Selenomonas ruminantium* sub sp. *ruminantium.*
2. *Selenomonas ruminantium* sub sp. *bryanti.*
3. *Lachnospira multiporus.*
4. *Anaerovibrio lipolytica.*

The results given in (Fig. 1) indicated that the activity of crude enzymes extracted from rumen of buffalo and cow tended to increase significantly ($P < 0.05$) by advancing the time post feeding sample and the highest activity was recorded at 9.0 hours post feeding without any significant differences among animal species. The lowest enzymatic activity was recorded one hour post feeding. These results agreed with those of Dehority and Orpin (1997) who investigated that the enzymatic activity was low at one hour after feeding and increased significantly by advancing the time post feeding.

The three different bacterial species isolated from buffalo as well as the four species isolated from cow were tested for their cellulolytic activity using bacterial crude enzymes extracted from Hungate medium supplemented with CMC. Na salt as carbon source were measured by cup plate technique (diameter of clear zone) for total cellulolytic and hemicellulolytic enzymes. Results presented in Table 1 show clearly that the various bacterial isolates, were able to produce cellulytic enzymes, and according to their relative activiteis they conceded as cellulolytic bacteria.

Cellulolytic enzyme production and activity of bacterial isolates, Could be arranged according to the following descending order; in case of buffalo: *Fibrobacter succinogenes* > *Prevotella ruminicola* > *Ruminobacter amylophilus* and in case of cow: *Selenomonas ruminatium* sub sp. *ruminatium* > *Lachinospira multiporus* > *Selenomonas ruminatium* sub sp. *bryanti* > *Anaerovibrio lipolytica.*

Since *Fibrobacter succinogenes* (bacterial isolate from buffaloe) and *Selenomonas ruminantium* sub sp. *ruminantium* (bacterial isolate from cattle) exhibited the highest cellulolytic activity, they were selected for the following experiments.

The effect of interbetween the most active cellulololytic and hemicellulolytic strains and incubation period on DMD of the three tested roughages are presented in Fig. 2. The disappearance of DM of wheat straw, rice straw and date seed were increased significantly ($P < 0.05$) from 2 days up to 8 days of incubation with either *Fibrobacter succinogenes* (isolated from buffalo rumen) or *Selenomonas ruminantium* sub sp. *ruminantium*(isolated from cow rumen). The DMD was pronounced in case of using *Fibrobacter succinogenes* compared with *Selenomonas ruminantium* sub sp. *ruminantium.* DMD was higher in both wheat straw and date seed compared with rice straw using

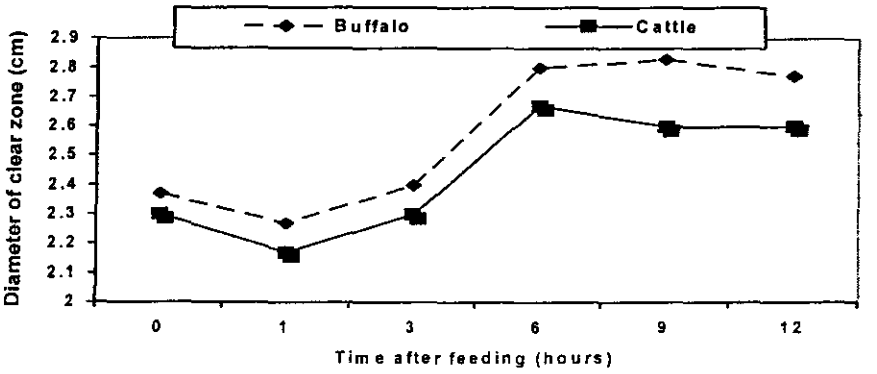

Fig. 1: Cellulolytic activity of crude enzymes extracted from rumen fluid of buffaloe and cattle at different intervals after feeding using cup plate assay on CMC agar.

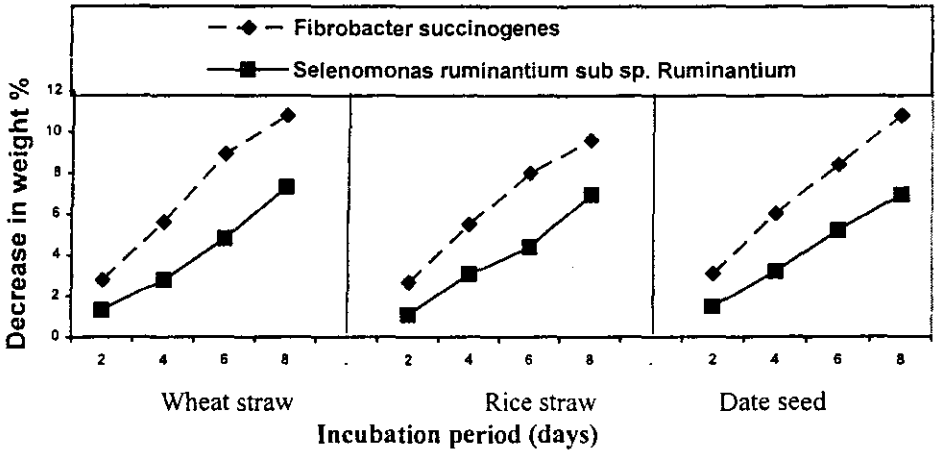

Fig. 2: Dry matter disappearance of wheat straw, rice straw and date seed by the most active cellulolytic and hemicellylolytic bacterial strains (*in vitro*).

Table (1) : Cellulolytic and hemicellulolytic enzymes production and activity of isolated bacterial strains, using cup plate assay.

Animal Species	Bacterial strains	Enzymatic activity Diameter of clear zone (cm)
Buffaloe	*Fibrobacter succinogenes*	2.17 [a]
	Prevotella ruminicola	2.0 7 [a]
	Ruminobacter amylophilus	2.03 [a]
Cattle	*Selenomonas ruminantium* sub sp. *ruminantium*	2.07 [a]
	Lachinospira multiporus	1.70 [b]
	Selenomonas ruminantium sub sp. *bryanti*	1.60 [bc]
	Anaerovibrio lipolytica	1.50 [c]

Means with different superscripts within the same column are significantly different ($P < 0.05$)

Fibrobacter succinogenes or *Selenomonas ruminantium* sub sp. *ruminantium* for incubation. The high content of silica in rice straw can lead to a depressing effect on digestibility and inhibiting the digesting of carbohydrates (Van Soest and Jones, 1968). Results of DMD of the three tested roughage as affected by the crude enzymes extracted from the metabolic liquid culture of the most active bacterial strains or from the rumen fluid of buffalo and cattle are present in Table (2).

Results clearly indicated that the DM disappearance of the three tested roughages were significantly higher ($P < 0.05$) when incubated with the crude enzyme extracted from *Fiberobacter succinogenes* culture (buffalo rumen fluid) compared with the crude enzyme extracted from *Slelenomonas ruminantium* sub. sp. *ruminantium* culture (cattle rumen fluid). There was no significant difference between the three tested roughages on DMD.

The extracted enzymes from rumen fluid of both animals were more active than the crude enzymes extracted from the of the most active bacterial strains.

The results concerning cellulolytic enzyme activity of tested bacteria as influenced by pH variation are presented in Fig. 3. It is obvious that the highest activity, was obtained from buffered reaction mixture maintained at pH 6.7. Thus, pH 6.7 is considered to be the optimum pH value for the enzyme activity of *F. succinogenes* and *S. ruminatium* sub. sp. *ruminantium*. On the other hand, high and weak acidity incomparison with pH 6.7 were unfavourable for enzyme reaction. Such range was suitable for the growth and activity of cellulolytic bacteria (Ozcan *et al.*, 1996 and Abd El-Razik, 1999).

Results presented in Fig. 4 show that different temperature influenced cellulase activity. Maximum cellulolytic activity of the culture filtrates of *Fibrobacter succinogene* and *Selnomonas ruminatium* sub. sp. *ruminantium* was at 37°C. At higher temperature (above 42°C) and lower temperature (below 27°C), activity decreased sharply in all cases. These results agree with habitation of bacterial strains in the rumen of ruminant animals, where the maximum activity of life was at 37°C. A factor of great importance to continue microbial fermentation is the relatively constant temperature of the rumen. The temperature tends to rise following ingestion of food, due to the evaluation of heat in the fermentation process. This evolution of heat has been used as a measure of the fermentation rate (Walker and Forrest, 1964). Oded and Doi (1990) and Peter *et al.* (1996) found that the optimum temperature for β-1, 4-endoglucanase from *Bacillus subtilis*, cellulase enzyme from *Clostridium cellulovorans*, and endoglucanases-xylanases from *Cellulomonas fimi* with both endo-and exo-glucanase activities, respectively, were 37°C.

Concerning the effects of the microelements on the production and activity of the cellulolytic enzyme of *F. succinogenes* and *S. ruminatium* sub. sp. *ruminatium*, the results presented in Table 3 show that enzyme production and activity was highly stimulated by sodium borate manganese chloride and ammonium molybdate as compared with control. Addition of zinc sulphate or nickel sulphate exerted, however, a relatively lower stimulatory effect, whereas ferrous sulphate seemed to be without effect. On the other hand, cobalt chloride and copper sulphate inhibited the enzyme production and activity.

Boron and molybdenum ions have been reported to stimulate cellulolytic activity. This is in conformity with the

Table (2) : Effect of crude cellulolytic and hemicellulolytic enzymes extracted from the metabolic liquid medium of bacteria and from rumen fluid of buffaloe and cattle on dry matter disappearance (DMD) (*in vitro*) .

Roughage	Enzyme source							
	Fibrobacter succingenes		*Selenomonas ruminantium* sub sp. *Ruminantium*		Buffaloe		Cattle	
	DMD gm	DMD %	DMD gm	DMD %	DMD gm	DMD %	DMD gm	DMD %
Wheat straw	0.147 [a]	5.88 [a]	0.093 [a]	3.72 [a]	0.173 [a]	6.92 [a]	0.147 [a]	5.88 [a]
Rice straw	0.133 [a]	5.32 [a]	0.087 [a]	3.48 [a]	0.160 [a]	6.40 [a]	0.143 [a]	5.72 [a]
Date seed	0.143 [a]	5.72 [a]	0.087 [a]	3.48 [a]	0.170 [a]	6.80 [a]	0.150 [a]	6.00 [a]

NS Not significant

Means with different superscripts within the same row are significantly different ($P < 0.05$).

Table (3): Effect of some microelements on crude enzyme production and activity extracted from the metabolism solution of *Fibrobacter succinogenes* and *Selenomonas ruminantium* sub sp. *ruminatium* using cup plate technique.

Microelement	Diameter of clear zonen (cm)	
	Fibrobacter succinogenes	*Selenomonas ruminantium* sub sp. *Ruminatium*
Sodium borate	2.80[a]	2.71 [a]
Manganese chloride	2.25 [c]	2.23 [c]
Ammonium molybdate	2.22 [c]	2.22 [c]
Zinc sulphate	1.96 [d]	1.93 [d]
Nickel sulphate	1.94 [d]	1.92 [d]
Ferrous sulphate	1.91 [d]	1.90 [d]
Cobalt chloride	1.26 [f]	1.23 [f]
Copper sulphate	1.23 [f]	1.21 [f]
Mixture of all	1.64 [e]	1.60 [e]
Control (no microelement)	1.92 [d]	1.91 [d]

Means with different superscripts within the same column are significantly different ($P < 0.05$).

Table (4):Effect of some vitamins on production and activity of cellulolytic enzymes by *Fibrobacter succinogenes* and *Selenomonas ruminatium* sub sp. *ruminantium*.

Vitamins	Clear zone diameter (cm^2)	
	Fibrobacter succinogenes	*Selenomonas ruminantium* sub sp. *ruminantium*
Cyanocoblamine (B_{12})	2.75 [a]	2.70 [a]
Riboflavin (B_2)	2.51 [b]	2.49 [b]
Thiamine (B_1)	2.48 [b]	2.46 [b]
Nicotinic acid (B_5)	2.49 [b]	2.46 [b]
Biotin	2.22 [d]	2.20 [d]
Pyridoxin (B_6)	2.21 [d]	2.20 [d]
Folic acid	1.42 [f]	1.39 [f]
P-aminobenzoic acid	1.41 [f]	1.38 [f]
Pantothenic acid (B_3)	1.21 [g]	1.18 [g]
Vitamin A	1.12 [h]	1.10 [h]
Vitamin C	1.11 [h]	1.09 [h]
Yeast extract	2.31 [c]	2.28 [c]
Control	2.02 [e]	1.89 [e]

There is no significance between the two bacterial isolates used.
Means with different superscripts within the same column are significantly different ($P < 0.05$).

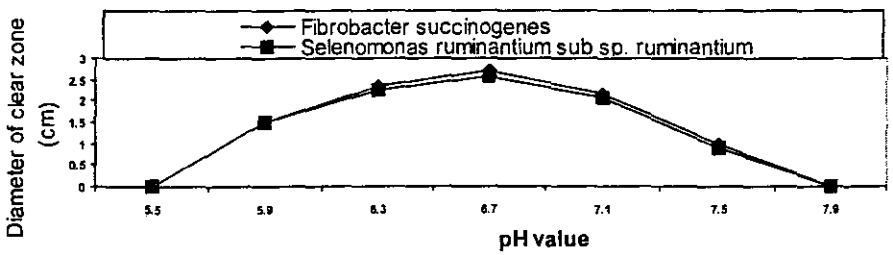

Fig. 3: Cellulolytic enzyme activity of *Fibrobacter succinogenes* and *Seleonomonas ruminantium* sub sp. *Ruminantium* as affected by pH variation

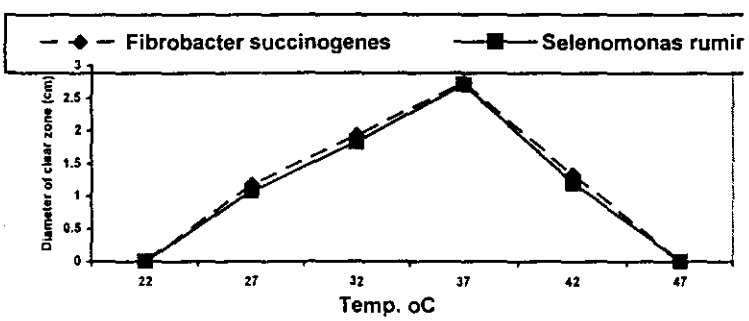

Fig. 4: Cellulolytic enzyme activity of *Fibrobacter succinogenes* and *Seleonomonas ruminantium* sub sp. *ruminantium* as influenced by temperature variation.

studies of Bose and Basu (1965); Naplekova & Anikina (1970) and Mansour and El-Sayed (1985). Manganese, zinc and nickel were also stimulatory for cellulase production and activity. On the other hand, the enzymatic production and activity greatly diminished by the inclusion of copper or cobalt in the medium. In this connection, Jothianandan and Shanmugasundaram (1968) reporetd that copper and cobalt are inhibitory for cellulase formation by *Piricularia oryza* and *Aspergillus niger*. When a mixture of these microelements was supplemented, a slight inhibition of the cellulolyic activity was shown in comparison with control. Thus, presence of cobalt and copper in the mixture might have lowered the stimulatory effect of boron, molybdenum and manganese. Samir *et al.* (1990) suggested that, the inhibition of endoglucanase might be due to the enzyme requirement of metal ions for its optimum activity and/or due to changes in electrostatic banding, which would change the tertiary structure of the enzyme. Heavy metal ions are generally thought to inactivate enzymes by forming covalent salts with cysteine moieties in the enzyme molecule (Abd El-Razik, 1999).

As regards the effect of vitamins on the production and activity of bacterial cellulolitic enzymes the results presented in Table 4 show that vitamin B_{12} and riboflavin (B_2) were of high stimulatory section Thiamine (B_1) and nicotinic acid (B_5) exerted also good stimulation of enzyme production and activity. The least stimulatory effect was exerted by biotin and pyridoxin (B_6).

It is doubtless that viramins in minute quantities are effective in many of the biochemical reactions, which obviously reflects their role as catalysts. Most of the major species of rumen microorganisms also require one or more B vitamins, but their individual requirements differ (Wolin *et al.*, 1997). Scheifinger (1974) demonstrated that biotin is required by *Selenomonas ruminantium* for the decarboxylation of succinate to propionate. Biotin is usually essential and P. aminobenzoic acid (PABA) may be required in *F. succinogenes* growth (Bryant *et al.*, 1959; Stewart *et al.*, 1997). The present investigation clearly indicate that most vitamins of the B-group exerted a pronounced stimulatory effect on cellulase production and activity; the magnitude of response being highest with cyanocobalamine (B_{12}) and riboflavin (B_2) followed by thiamin (B_1), nicotinic acid (B_5), biotin and pyridoxin (B_6). Whereas pantothenic acid (B_3), folic acid, vitamin A, vitamin C and P-aminobenzoic acid appeared to inhibit the enzymatic production and activity. As a natural source of vitamins, yeast extract remarkably stimulated enzymatic production and activity of *F. succingenes & Selenomonas ruminantium* sub sp. *ruminantium*. This confirms the findings of Haenssler (1973) and Mansour & El-Sayed (1985).

Finally, the addition of certain vitamins to the culture medium of the two most active bacterial strain would influence the production and activity of cellulolytic and hemicellulolytic enzymes.

Most of the anaerobic bacteria which degraded cellulose are also able to degrade hemicellulose. It appears that the genes responsible for cellulose and hemicellulose degradation are related to each other and are regulated jointly (Kamra and Pathak, 1996).

Generally, it could be concluded that the activity of crude enzymes extracted from rumen fluid of buffalo and cattle tended to increase significantly by advancing the time post feeding and the maximum activity was recorded at 9.0 hours post feeding, meantime this crude

enzymes were more active than that crude enzymes extracted from the culture filters of the most active bacterial strains. The optimal pH a temper and temperature for the activity of extracted enzyme was 6.7 and 37°C respectively. Where sodium borate, manganeous chloride, ammonium molybdate along with vitamin B_{12}, riboflavin ((B_2), thiamine (B_1) and nictoinic acid (B_5) stimulated the enzyme activity.

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خصائص مميزات إنزيمات بعض البكتريا المحللة للسليلولوز والهيميسوليوز المعزولة من كرش الجاموس والماشية.

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١. معهد بحوث الإنتاج الحيوانى ــ مركز البحوث الزراعية ــ وزارة الزراعة.
٢. قسم النبات ــ كلية العلوم ــ جامعة المنصورة.

تم عزل وتصنيف بعض البكتريا المحللة للسليلوز والهيميسليلوز الأكثر نشاطا من كروش ٣ عجول جاموس و٣ عجول بقرى والتى تضمنت٣ سلالات بكتيرية من كرش العجول الجاموس و ٤ سلالات من كرش العجول البقرى.

إزداد نشاط الإنزيم المستخلص من سائل كرش بتقدم الوقت بعد التغذية وسجلت الإنزيمات أقصى نشاط لها بعد ٩ ساعات من بداية التغذية الصباحية بدون فروق معنوية بين الإنزيمات المستخلصة من الجاموس والأبقار. وكانت بكتريا (*Fibrobacter succinogenes*) المعزولة من الجاموس و *Selenomonas ruminantium* هى اكثر انواع البكتريا نشاطا.

إزداد معدل اختفاء المادة الجافة لتبن القمح وقش الأرز ونوى البلح معنويا بعد تحضينها لمدة ٢-٨ أيام مع كل من *Fibrobacter succinogenes* و *Selenomonas ruminantium* ولكن كان معدل اختفاء المادة الجافة أكبر فى حالة التحضين مع السلالة البكتيرية المستخلصة من كرش الجاموس مقارنة بالبكتيريا المستخلصة من الماشية. ارتفع معدل اختفاء المادة الجافة لتبن القمح وقش الأرز ونوى البلح معنويا عند تحضينها مع الإنزيم الخام المستخلص من *Fibrobacter succinogenes* مقارنة بالإنزيم المستخلص من *Selenomonas ruminantium* ولكن فى نفس الوقت لم يكن هناك فروق معنوية فى معدل اختفاء المادة الجافة للثلاث أعلاف تحت الأختبار.

أظهرت الإنزيمات المستخلصة من كرش الحيوانات نشاطا أكثر مقارنة بالإنزيمات المستخلصة من المزارع البكتيرية. وكانت قيمة pH المثلى لنشاط الإنزيمات المستخلصة من البكتيريا المختبرة كان ٦,٧ بينما كانت درجة الحرارة المثلى لنشاط هذه الإنزيمات هى ٣٧ درجة مئوية.

درس أيضا تأثير بعض العناصر النادرة وبعض الفيتامينات على نشاط الإنزيمات المستخلصة. وكان اكثرها تأثيرا على نشاط الانزيمات بورات الصوديوم وكلوريد المنجنيز وموليبدات الامونيوم من العناصير المعدنية ومن الفيتامينات فيتامين ب١٢ والريبوفلافين (ب٢) والثيامين (ب١) وحمض النيكوتينك (ب٥).