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STUDIES ON CELLULOLYTIC BACTERIA AND THEIR ENZYME IN THE RUMEN OF CAMEL (*CAMELLUS DROMEDARIUS*)

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Abstract

This study explained the importance of some microorganisms in camel ruminal fluid as a source of cellulase enyzme. Different strains of cellulolytic bacteria were isolated as *Bacteroides ruminicola, Bacteroides succinogenes, Butyrivibrio fibrisolvens, Ruminococcus flavefaciens* and *Ruminococcus albus*. Pure culture of cellulolytic bacteria *Ruminococcus albus* was experimented for studying factors which affect its celluloytic activity. Increasing in bacterial count resulted in the decrease of remained cellulose concentration in cellulose digestion broth. The activation of cellulase enzyme to digest cellulose occurred until it was saturated and by increasing concentration of substrate, the activation decreased gradually till it became constant. The optimum cellulolytic activity of *Ruminococcus albus* was performed at pH 7.0 and when incubated at 39°C for 72 hours. Addition of ampicillin and gentamicin caused significant inhibition of this bacterium to digest cellulose.

INTRODUCTION

One-humped camel (Camellus Dromedarius) is a domestic animal of an economic importance, and relatively, little is known about the nutrition and digestion of this animal (Maloly, 1972). Hungate (1966) illustrated the importance of the microflora and microfauna in the process of microbial digestion in the ruminants. Bryant and Burkey (1953) observed that the number of bacteria depended upon the ration of the animal.

A wide variation of the microflora inhabitant in the rumen of camels, including approximately 10^{10} to 10^{11} bacteria of about 200 species, has been isolated

(Hungate, 1950). Two types of cellulolytic bacteria, celluloytic cocci (CeC) and cellulolytic rods (CeR) were determined. Cellulase enzyme production is the common dominant for cellulolytic organism which is present in the ruminal fluid. Most cellulolytic organisms are found among bacteria, protozoa and fungi, in more or less anaerobic closed environments, as in guts of herbivorous, digestive juices of invertebrates, and the rumen of cattle and camel. Bacteria are the outstanding cellulose decomposers (Halliwell and Halliwell, 1989).

Since cellulase is one of the most important hydrolytic enzyme, it has many industrial application as in textile manifacture, paper industry, medical drugs and in waste treatment. Several factors affect cellulase production (Ganju *et al.* 1990) such as the composition of culture medium, quantity and quality of cellulose used, the amount of metal salts present, pH, temperature, the adequacy of Co2 supply, and the way by which it was obtained.

Therefore, this study aimed to isolate some cellulolytic bacteria naturally present in the rumen of camel. In addition, it included the influences of bacterial count, cellulose count, cellulose concentration, pH, temperature, time of incubation and antibiotics addition on cellulolytic activity of one of cellulolytic bacteria.

MATERIALS AND METHODS

Ruminal fluid samples were collected immediatly after the evisceration of freshly slaughtered camels (Camellus Dromedarius) in Cairo abattoir. The chosen animals were apparently healthy, 5-7 years old and of body weight ranging between 600-800 kg. Within 1-2 hours after collection, the samples were filtered through a sterile cheese cloth. The filterate was centrifuged at 1000 r.p.m. for one minute to separate most ruminal protozoa. Then, the supernatant was centrifuged again at 5000 r.p.m. for 15 minutes for sedimentation of most ruminal bacteria. Culturing of these bacteria was carried out anaerobically in a specific media, rumen fluid glucose cellulose agar (RGCA) described by Hungate (1950). Anaerobic ruminal bacteria were isolated and identified according to their morphological and biochemical features (Dehority, 1963).

To study the factors affecting the activity of cellulase enzyme produced by these bacteria, pure culture of one strain (*Ruminococcus albus*) was inoculated into a prepared cellulose digestion broth tubes (Hangate, 1950), and incubated at 39^{O}C for 3 days in Co2 gas bag by using gas generating kit (Oxoid, England). The remained cellulose (substrate) concentration in the broth was measured by turbidometer

(Spekol zv), which was inversely correlated with the activity of cellulase enzyme.

The studied factors were: bacterial count (52 $x10^8$, $48x10^7$, $46x10^6$, $42x10^5$, $39x10^4$, $37x10^3$, $35x10^2$, 35x10, 30x10 and 10x10 per ml) with a fixed cellulose concentration (0.2%); different cellulose concentrations (25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 mg/100ml) with a fixed facterial count (58 x 10^8 ml). With a fixed bacterial count ($58x10^8$/ml) and cellulose concentration (0.2%), different factors were studied: various pH of the broth (4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9), incubation temperature (29, 31, 33, 35, 37, 39, 41, 43, 45 and 47°C), incubation period (12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 hours) and addition of antibiotics into the broth in different concentrations (5, 10, 15, 20, 25 mg%), such as ampicillin and gentamicm.

The data were tabulated and statistically analysed by ANOVA test (F-value) according to Snedecor and Cochran (1973).

RESULTS

Five strains of anaerobic ruminal bacteria bacteria were isolated: *Bacteroides ruminicola, Bacteroides succinogenes, Butyrivibrio fibrisolvens, Ruminococcus flavefaciens* and *Ruminococcus albus*. Morphological and biochemical features of these ruminal bacteria were summarized in Table 1.

The influences of bacterial count, cellulose concentration, pH, temperature and incubation time on cellulolytic activity of Ruminococcus albus were illustrated in Table 2. Effect of different doses of ampicillin and gentamicin was shown in Table 3.

DISCUSSION

The five isolated strains of anaerobic ruminal bacteria from rumen of camel (Table 1) were previously recorded as follows: *Bacteroides ruminicola* (Bryant, 1956), *Bacteroides succinogenes* (Hungate *et al*, 1959), *Butyrivibrio fibrisolvens* (Hungate, 1950) and *Ruminococcus albus* (Margherita and hungate, 1963).

Data of bacterial count influencing cellulose digestion (Table 2) pointed out that the cellulolytic effect of *Ruminococcus albus* was increased in activity by increasing of the bacterial count. It is suggested that cellulase enzyme was synthesized in bacterial cells and secreated through its membrane outside the cell during its adherance with cellulose fibers.

Addition of cellulose in different concentrations to ruminal fluid broth

Table 1. Some characteristics of anaerobic ruminal bacteria isolated from the rumen of camel.

Ruminal bacteria	Shape	Gram reaction	Motility	Glucose	Cellulose	Xylan	Starch	Lactate	Glycerol	Substrate
Bacteroids ruminicola	Long rods to coccoid in chains	-	-	+	-	±	±	-	-	glucose
Bacteroides Succinogenes	Rods to coccoid, no chains, pointed ends and curved	-	-	+	+	-	$\overline{+}$	-	-	Cellulose or glucose
Butyrivibrio fibrisolvens	more or less curved rods, often chains and filaments	-	+	+	+	±	±	-	-	glucose cellulose
Ruminococcus flavefaciens	Medium to large cocci usually in short to long chains	±	-	$\overline{+}$	±	±	-	-	-	cellulose cellobiose
Ruminococcus albus	Medium to large cocci usually single and pairs	±	-	$\overline{+}$	±	±	-	-	-	cellulose cellobiose

+ = Positive
- = Negative
± = Most strains utilize
$\overline{+}$ = Few strains utilize.

containing a fixed number of *Ruminococcus albus* ($58x10^8$/ml) (Table 2) showed that, cellulase enzyme digests cellulose till it is saturated. Then, by incrasing concentration of substrate, the activation decreased gradually till became constant. Therefore, addition of higher substrate concentration was accompanied with correspondance increase in cellulose concentration after incubation. These results were consistent with the results obtained by Hassan (1991).

The optimum pH for the cellulase activity of *Ruminococcus albus* was 7.0 (Table 2). The remained cellulose concentration was 17.88 mg%. These results were in agreement with those reported by Hungate (1950) who recorded a sharp increase in cellulase activity when pH value of the culture media was changed from 4.0 to 7.0. Nevertheless, Ali and Hosain (1989) determined that pH 6.0 was the best for celluse production from ruminal bacteria.

The optimum temperature of incubation for cellulolytic activity of *Ruminococcus albus* was 39°C, while, the remained cellulose concentration was 17.88 mg% (Table 2). The previous researches recorded that the effect of temperature on cellulase activity varies according to the species of microorganisms. Aleksidze and Krachaodze (1984) recorded 40°C as an optimum temperature for cellulase activity of *Aspergillus terreus*. On the other hand, Bagga and Sandtiu (1987) detected that the optimum temperature was 37°C for cellulase activity of *Aspergillus nidulans*. The highest degree of cellulose digestion by *Ruminococcus albus* in the present study was obtained after incubation period for 72 hours, where cellulose concentration was 17.88 mg%. It is parallel with the results obtained by Hungate (1950) when he used different types of bacteria isolated from ruminal fluid.

Addition of ampicillin and gentamicin in increasing dose caused significant increase of inhibition of *Ruminococcus albus* to digest cellulose (Table 3). These results were supported by the earlier findings of Kassim and Ghazi (1981) who recorded that penicillin and tetracyclin have an inhibitory effect on the cellulase enzyme of *Aspergillus*.

From the afore-mentioned results, it was concluded that camel ruminal fluid has cellulolytic bacterial strain (*Ruminococcus albus*) which produces cellulase enzyme. The optimum pH, temperature and incubation period for its activity was 7..0, 93°C and 72 hours, respecttively, while, addition of antibiotics (ampicillin and gentamicin) affects its activity.

Table 2. Effect of bacterial count, substrate concentration, pH, temperature and incubation time on cellulose digestion by Ruminococcus albus (remained cellulose concentration).

Bacterial inoculation		Cellulose addition		pH of culture		Incubation temerature		Incubation period	
bacterial count (cells/ml)	* Cellulose conc. (mg%)	substrate conc. (mg%)	** Cellulose conc. (mg%)	pH degree	** Cellulose conc. (mg %)	temp. (°C)	** Cellulose conc. (mg %)	time (hour)	** Cellulose conc. (mg %)
$52x10^8$	5.99 ± 1.87	25	14.88 ± 0.71	4.5	91.42 ± 2.07	29	124.30 ± 1.30	12	116.30 ± 2.88
$48x10^7$	14.88 ± 0.71	50	25.38 ± 0.84	5.0	74.63 ± 1.84	31	99.32 ± 1.30	24	85.83 ± 2.30
$46x10^6$	18.38 ± 1.14	75	39.36 ± 0.84	5.0	61.85 ± 2.77	33	76.95 ± 8.85	36	64.84 ± 1.87
$42x10^5$	23.78 ± 1.14	100	49.36 ± 1.30	6.0	44.36 ± 2.39	35	50.36 ± 1.30	48	46.86 ± 2.28
$39x10^4$	32.57 ± 1.22	125	65.34 ± 0.83	6.5	28.37 ± 1.14	37	30.87 ± 1.51	60	28.87 ± 1.34
$37x10^3$	40.36 ± 0.84	150	75.34 ± 0.83	7.0	17.88 ± 1.30	39	17.88 ± 1.30	72	17.88 ± 1.30
$35x10^2$	59.85 ± 2.12	175	86.83 ± 1.30	7.5	33.56 ± 5.14	41	30.87 ± 1.67	84	17.68 ± 0.84
$35x10^1$	74.34 ± 1.30	200	99.32 ± 1.30	8.0	46.86 ± 2.28	43	44.36 ± 2.39	96	17.86 ± 1.14
$30x10^1$	86.62 ± 2.30	225	103.30 ± 0.89	8.5	66.85 ± 1.64	45	64.84 ± 2.17	108	17.85 ± 2.12
$10x10^1$	95.82 ± 5.56	250	104.30 ± 1.58	9.0	93.82 ± 2.07	47	95.32 ± 1.64	120	17.82 ± 2.64

- Mean ± S.E. (of five samples)
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

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Table 3. Effect of ampicillin and gentamicin on cellulase activity of *Ruminococcus albus* (remained cellulose concentration).

	Antibiotic conc. (mg %)	Cellulose conc. (mg %)
** Ampicillin	5	72.34 ± 2.12
	10	89.82 ± 4.95
	15	119.70 ± 2.12
	20	147.67 ± 2.12
	25	172.26 ± 2.12
** Gentamicin	5	74.84 ± 1.41
	10	87.82 ± 1.30
	15	118.30 ± 2.19
	20	134.29 ± 2.17
	25	156.28 ± 2.07

- Mean ± S.E. (of five samples).
** Significant at $P < 0.01$.

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دراسات عن البكتريا المحللة للسليولوز وانزيماتها فى كرش الجمل

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هذه الدراسة أجريت على بعض الكائنات الدقيقة فى كرش الجمل كمصدر هام لانزيم السليولاز. تم عزل وتصنيف خمسة عترات من البكتريا المختلفة وهى : بكترويدز رومينوكولا، بكترويدز سكسينوجينيس، بيتيريفيبريو فيبريسولفنس، رومينوكوكس فلاف فاسينس، رومينوكوكس البس.تم أختيار ميكروب الرومينوكوكس البس كمصدر لانزيم السليولاز لمعرفة بعض العوامل المختلفة التى تؤثر على نشاط هذا الانزيم على هضم السيلولوز. لوحظ ان نشاط السليولاز يتناقص مع النقصان التدريجى لعدد البكتريا. كما انه بزيادة اضافة السليولوز فى الوسط الغذائى يتزايد نشاط الانزيم حتى درجة التشبع رغم زيادة السليولوز المضاف. وقد وجد ان افضل نشاط لانزيم السليولاز لميكروب الرومينوكوكس البس عند الأس الأيدروجينى ٧ وفى درجة حرارة ٣٩ ٥ م وفترة تحضين ٧٢ ساعة. كما وجد أن اضافة المضادات الحيوية (الأمبسللين والجنتاميسين) لها تأثير مثبط على نشاط انزيم السليولاز.