SUPPORTING PAPERS
ISBN 979-97243-9-2

ISOLATION OF CHITINOLYTIC BACTERIA FROM BUFFALO RUMEN FLUIDS AND MEASUREMENT OF ITS ACTIVITY

Widyantoro, Z. Bachruddin, S. Prawirokoesoemo, B.P. Widyobroto, Wibowo, and L.M. Yusiati

Laboratory of Nutritional Biochemistry Department of Animal Nutrition
Faculty of Animal Science Gadjah Mada University

ABSTRACT

The study was conducted to study the activity of selected chitinolytic microbe isolates from buffalo rumen fluids. One hundred ml buffalo rumen fluids was put into 250 ml Erlenmeyer flask, meanwhile seven test tubes in duplo containing nine ml sterile aquadest were prepared and to each tube was added with buffalo rumen fluids of 10^{-1} dilution which was then gradually diluted again up to 10^{-7}. The final diluted fluids were inoculated on agar media and was incubated at 39°C for 48 hours. The microbes colonies grew on the agar media were then placed in chitin media of liquid form and again was incubated at 39°C for 48 hours and then subjected to microbes protein analysis. The selected isolates having highest microbes protein content were then incubated again on liquid chitin media. The respective selected isolates were tested to study its ability to degrade crab shells chitin (C-7170 Sigma) at different pH values. The ability of the selected chitinolytic microbes to degrade chitin of crab shells chitin was evaluated based on the results of analysis of the reducing sugars, N-NH_3 and microbe protein. The results of the study indicated that differences on pH did not cause significant differences on the variables being tested, but not so in the case of length of incubation time.

Keywords: Buffalo Rumen Fluids, Chitinolytic Microbe Isolates, Ph Values, Reducing Sugars, N-Ammonia, Microbes Protein.

INTRODUCTION

Chitinolytic bacteria in rumen fluids is favourable for animal host. Enzyme chitinase is excreted by bacteria chitinolytic and it hydrolysis of chitin on β-(1,4) glycosidic bond with N-acetylglucosamin (GlcNAc) as the product (Wang and Chang, 1997). GlcNAc is a part of cell wall bacteria. Hydrolysis of GlcNAc which releases group by acetylase will produce glucosamin and acetic acid (Kopěcný et al., 2006; Kopěcný and Hodrová, 2006). Acetic acid will be used as energy source while glucosamin will be used for synthesis of N-acetylmuramic (NAM). GlcNAc and NAM are compounds of cell wall bacteria (=peptidoglycan)(Lehninger, 2000).

The study was conducted to study the activity of selected chitinolytic bacteria isolates from buffalo rumen fluids. Chitin crab shell (C-7170 Sigma) being used as substrate were incubated by chitinolytic bacteria isolates in McDougall reagent. All samples were incubated in waterbath (39°C/48 h). Chemical analysis of the incubated

SUPPORTING PAPERS
ISBN 979-97243-9-2

product consisted of reducing sugars by Anthron method (Plummer, 1978), N-NH_3 (Weatherburn, 1967) and bacteria protein (Plummer, 1978).

MATERIALS AND METHODS

MATERIALS

Male buffalo fistulated ± 3,5 years old used as rumen fluids donor, sets of glasses equipment, electrical weighing (Sartorius), autoclave, revrigerator, spectrophotometer (Genesys 20), ultracentrifugation (Centrifuge 5810 R), waterbath (Memmert) and chemicals reagent.

METHODS

One hundred mL buffalo rumen fluids was put into 250 mL Erlenmeyer flask, mean while seven test tubes in duplo containing nine mL sterile aquadest were prepared and each tube was added with buffalo rumen fluids of 10^{-1} dilution which then gradually diluted again up to 10^{-7}. The final diluted fluids were incubated on agar-chitin media and incubated in oven (39°C/48 h).

The microbes colonies grew on the agar-chitin media were then placed in chitin media of liquid form and again was incubated in oven (39°C/48 h) and then subjected to microbes protein analysis. The selected isolates leaving highest microbes protein content were then incubated again on liquid chitin media.

The respective selected isolates were tested to study ability to degrade crab shell chitin (C-7170 Sigma) at differences values (6.2, 6.6, and 7.0). The ability of the selected chitinolytic bacteria to degrade chitin of crab shell was evaluated by analysis of the reducing sugars by Anthron method (Plummer 1978), N-NH_3 by Weatherburn method (1967) and concentration of bacteria protein by Lowry method (Plummer, 1978).

RESULTS AND DISCUSION

In vitro treatment on materials waste shrimp in different pH treatment

Reducing sugar concentration. The result of statistical analysis on reducing sugar concentration in in vitro treatment showed in Table 1.

Table 1. Concentration of reducing sugar (mg/ml) in in vitro treatment on differenct pH values

Incub.	pH			Average
(hours)	6,2	6,6	7,0	
0	0.1209	0.1246	0.1260	0.1238[b]
48	2.3748	2.6280	2.3740	2.4598[a]
Average[ns]	1.2479	1.3763	1.2500	

ns =non significant different
[a,b] Superscript significantly different

SUPPORTING PAPERS
ISBN 979-97243-9-2

Table 2. Concentration of N-NH_3 (mg/100 ml) in in vitro treatment on differenct pH values

Incub. (hours)	pH			Average
	6,2	6,6	7,0	
0	0.1079	0.0802	0.0803	0.0895[b]
48	0.3724	0.6040	0.3979	0.7342[a]
Average[ns]	0.2402	0.3421	0.2391	

ns =non significant different
[a,b] Superscript significantly different

Table 3. Concentration of bacteria protein (mg/ ml) in in vitro treatment on differenct pH values

Incub. (hours)	pH			Average
	6,2	6,6	7,0	
0	0.3455	1.0690	0.0859	0.5001[b]
48	13.4125	14.4959	0.3208	14.0704[a]
Average[ns]	6.8790	7.7825	7.2034	

ns =non significant different
[a,b] Superscript significantly different

Statistical analysis concentration of reducing sugar on different pH values in in vitro treatment was not significant different. Chitinolytic bacteria was able to degrade chitin although in different pH values. Chitin is analog of crude fiber. Crude fiber was degraded by rumen microbe directly on pH 6.0 and 7.0 and optimaly on pH of 6.2 (Van Soest, 1994).

Statistical analysis concentration of reducing sugar on 0 hour and 48 hours incubation showed significantly different values. Feed particles which stayed in rumen for a long period will degraded more (Van Soest, 1994) so that concentration of reducing sugar will be higher on 48 hours incubation.

N-NH_3 concentration. The result of statistical analysis concentration on N-NH_3 in in vitro treatment showed in Table 2. Statistical analysis concentration of N-NH_3 being produced from different pH values in in vitro treatment was not different significantly different (Table 2) but on pH 6.6 value N-NH_3 concentration is higher than pH 6.2 and pH 7.0.

Concentration of N-NH_3 on 0 hour and 48 hours incubation were significantly different. Feed particle which stayed in rumen for along period will be degraded more (Van Soest, 1994) so that concentration of N-NH_3 value higher than on 48 hours incubation.

Concentration of bacteria protein. The result of statistical analysis concentration on bacteria protein in in vitro treatment showed in Table 3. Statistical analysis concentration of bacteria protein being produced from differenct pH values in in vitro was not significant different (Table 3) but on pH 6.6 value bacteria protein concentration is higher than pH 6.2 and pH 7.0.

Concentration of bacteria protein average on 0 hour and 48 hours incubation were significantly different. Particle of Feed particle which stayed in rumen for along period will be degraded more (Van Soest, 1994) so that concentration of bacteria protein will be higher than on 48 hours incubation.

SUPPORTING PAPERS
ISBN 979-97243-9-2

CONCLUSION

Concentration of reducing sugars, concentration of N-NH_3 and concentration of bacterial protein in in vitro trial on treatment of pH different values showed not significant different. But on pH 6.6 value concentration of reducing sugars, concentration of N-NH_3 and concentration of bacterial protein higher than pH 6.2 and 7.0.

On 0 hour and 48 hours incubation concentration of reducing sugars, concentration of N-NH_3 and concentration of bacterial protein are significantly different.

REFERENCES

Kopêcný, J., B. Hodrová, and C.S. Stewart.2006. The isolation and charac-terization of a rumen chitinolytic bacterium. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dobt=Abstra... 7/18/2006

Kopêcný, J., and B. Hodrová.2006. Chitinolytic enzymes in the rumen microbial ecosystem. www.brfo.uni-ij.si/zoo/publikacije/zbornik/PDF/72-1998-85-93.pdf-273k-View as html-More from this site-Save... 8/4/2006

Nelson, D.L. and M.M. Cox.2000. Lehninger Principles of Biochemistry. 3rd Edition. Worth Publishers, 41 Madison Avenue. New York, NY 10010.

Plummer, D.T.1978. An Introduction to Practical Biochemistry. Tata McGraw-Hill Publishing Company Ltd. New Delhi.

Van Soest, P.J.1994. Nutrition Ecology of the Ruminant. O & B Books Inc. Corvalis. Ithaca. London.

Weatherburn, M.W.1967. Phenol hypochlorite reaction for determination af ammonia. Anal. Of Chem. 39:971-974.