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Original Article

Observation of *Ruminococcus* Strains in Captive Asian Elephant (*Elephas maximus*)

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Abstract

Asian elephant is indigenous to many countries including Thailand, but fermenter microorganisms in gastrointestinal tract of the elephant have not fully been investigated. Therefore, this study aimed to observe the cellulolytic bacteria in Genus *Ruminococcus* in large intestines of captive Asian elephants (*Elephas maximus*). Fecal samples were collected from male and female sucklings, young and adult captive Asian elephants. Forty-four elephants were divided into 3 groups as followed: A) > 18 years old (n=24); B) 2-18 years old (n=17) and C) < 2 years old (n=3). The results revealed that there were 214 (42.8%) isolates of *R. flavefaciens*, 105 (21.0%) isolates of *R. bromii*, 90 (18.0%) isolates of *R. obeum*, 54 (10.8%) isolates of *R. callidus* and 37 (7.4%) isolates of *R. albus* from all fecal samples examined. Interestingly, *Ruminococcus* strains could be isolated from the weaned elephants, but were not found in the sucklings ($p<0.05$). In conclusion, cellulolytic bacteria in Genus *Ruminococcus* were isolated from the large intestines of captive Asian elephants. Moreover, the highest prevalence of the bacteria was found in the elephants aged more than 18 years old.

Keywords: captive Asian elephant, cellulolytic bacteria, large intestine, *Ruminococcus spp.*

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บทคัดย่อ

การสำรวจเชื้อแบคทีเรีย *Ruminococcus* ในช้างเลี้ยงเอเชีย (*Elephas maximus*)

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ช้างเอเชียเป็นสัตว์ประจำชาติในหลายประเทศรวมทั้งประเทศไทย แต่การศึกษาเรื่อง จุลชีพหมักย่อยในทางเดินอาหารของช้าง ยังมีน้อยมาก ดังนั้นการศึกษาครั้งนี้ ต้องการสำรวจเชื้อแบคทีเรียย่อยเซลลูโลสในตระกูล *Ruminococcus* ในลำไส้ใหญ่ของช้างเลี้ยงเอเชีย (*Elephas maximus*) ตัวอย่างที่ใช้ในการศึกษาจะได้จากอุจจาระของลูกช้างที่ยังไม่หย่านม ลูกช้างหลังหย่านมและช้างโตเต็มวัย ไม่จำกัดเพศ โดยต้องเป็นช้างเลี้ยง ทั้งสิ้น 44 เชือก ซึ่งแบ่งช้างออกได้เป็น 3 กลุ่ม คือ A) ช้างที่มีอายุตั้งแต่ 18 ปีขึ้นไป (n=24); B) ช้างที่มีอายุระหว่าง 2-18 ปี (n=17) และ C) ช้างที่มีอายุน้อยกว่า 2 ปี (n=3) ผลการศึกษาพบว่าตรวจพบเชื้อ *R. flavefaciens* จำนวน 214 สายพันธุ์ (42.8%), *R. bromii* จำนวน 105 สายพันธุ์ (21.0%), *R. obeum* จำนวน 90 สายพันธุ์ (18.0%), *R. callidus* จำนวน 54 สายพันธุ์ (10.8%) และ *R. albus* จำนวน 37 สายพันธุ์ (7.4%) จากตัวอย่างอุจจาระตามลำดับ เป็นที่น่าสนใจว่าสามารถตรวจพบเชื้อแบคทีเรีย *Ruminococcus* จากตัวอย่างอุจจาระของช้างหลังหย่านมแล้วเท่านั้น ($p<0.05$) ดังนั้นการศึกษาครั้งนี้สรุปได้ว่าสามารถตรวจพบเชื้อแบคทีเรียย่อยเซลลูโลสในตระกูล *Ruminococcus* ในลำไส้ใหญ่ของช้างเลี้ยงเอเชีย ยิ่งไปกว่านั้นพบว่าช้างช่วงอายุมากกว่า 18 ปีมีความชุกของเชื้อแบคทีเรียนี้มากที่สุด

คำสำคัญ: ช้างเลี้ยงเอเชีย แบคทีเรียย่อยเซลลูโลส ลำไส้ใหญ่ *Ruminococcus* spp.

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Introduction

Elephants are herbivorous animals and hindgut-fermenters. The gastrointestinal tract (GI tract) of the elephant is similar to other hindgut-fermenter, including horses and rabbits. Hindgut-fermenters have no gall bladder (Langka, 2002). Biological degradation of dietary fiber; cellulose, hemicellulose, takes place in rumen of ruminant or cecum of horse, rabbit and elephant. Cecal folders assist in the increase in nutrient absorption area through cecal epithelium (McBee, 1971). Moreover, there are many kinds of microorganism inside cecum which mostly are anaerobic bacteria, fungi and protozoa (Forsberg et al., 1997; Koike et al., 2000). These microorganisms play an important role in the cellulose fermentation (Forsberg et al., 1997). Cellulolytic bacteria are most prevalent inside the cecum. They produce enzymes to ferment cellulose and hemicellulose into short-chain fatty acids which are easily absorbed such as primarily acetate, propionate, butyrate, or amino acids (Russell and Wilson, 1996). Microbial ecosystem in rumen of ruminant and cecum of horse are well-studied and used as a good model to study cellulolytic bacteria in elephants. Predominant strains of cellulolytic bacteria in rumen are *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *R. albus* (Julliand et al., 1999; Koike et al., 2000; Chen and Weimer, 2001; Koike and Kobayashi, 2001). *Ruminococcus* spp. has also been isolated from cecum of horse (Julliand et al., 1999). *Ruminococcus* spp. is a non-motile obligatory anaerobic gram-positive coccoid bacterium. It is an important normal flora because it is able to produce xylanase, cellulase and esterase for the biosynthesis of cellulose, hemicellulose and pectin within the gastrointestinal tract (Wang et al., 1997). Thereby, this bacterium is important for herbivorous animals to serve an energy source. Even though identification of this bacterium by a conventional method is recommended, it still depends on the experience of bacteriologist. Therefore, the molecular techniques have been demonstrated and reported in the previous investigations to assist the identification of these bacteria (Wolin, 1981; Russell and Wilson, 1996; Wang et al., 1997; Julliand et al., 1999; Koike et al., 2000;

Chen and Weimer, 2001; Koike and Kobayashi, 2001; Wang et al., 2004; Hastie et al., 2008). Presently microflora, particularly cellulolytic bacterium in GI tract of elephant, has not fully been investigated and needs to be clarified. Therefore, this study aimed to observe the cellulolytic bacteria in Genus *Ruminococcus* isolated from fecal samples of captive Asian elephants (*Elephas maximus*) and to clarify the predominant strain of Genus *Ruminococcus* in elephants of different ages.

Materials and Methods

Elephants: A total of 44 elephants was randomly chosen from three elephant camps in Chiang Mai and Lampang provinces in northern Thailand. The elephants were categorized into three groups based on age (Langka, 2002). Group A consisted of adult elephants aged over 18 years old. Group B consisted of adult elephants aged 2-18 years old. Group C consisted of sucklings aged up to 2 years old. In addition, health status, vaccination and medical history and nutritional management of all the elephants were determined. All procedures performed on animals in this study were approved and supervised by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Chiang Mai University.

Fecal sampling: Fecal collection was taken per rectum in group A and B. Feces were collected beyond the first part of feces in the early morning. Feces were put in sterile tightly closed plastic bag, then kept and transported in an anaerobic jar with Anaerocult (Oxoid, Hampshire, UK) at 4°C as soon as possible. Feces from the sucklings, which were not separated from their mothers and were still nursed, were collected from a newly dropped pile.

Bacterial growth condition and biochemical tests: Feces were subjected to incubate in thioglycolate broth (Sigma Aldrich, St. Louis, MO, USA) at 37°C for 6 hours in an anaerobic chamber. Samples were then cultured on a prereduced rumen fluid-based RGC medium, containing 0.2% each of glucose, cellobiose, maltose and starch as described previously (Ogimoto and Imai, 1981). Culture plates were incubated at 37°C for 48 hours in an anaerobic chamber. Pairs, chains and catalase positive reaction Gram-positive coccoid colonies were collected and subjected to the biochemical tests as follow: urease, starch hydrolysis, cellulose hydrolysis, and fermentation of arabinose, rhamnose, xylose, cellobiose, fructose, lactose, maltose, mannitol, raffinose or sucrose. Positive biochemical test colonies were kept for DNA preparation and PCR.

DNA preparation: Total DNA was taken by the CTAB precipitation method (Ausubel et al., 1999). Briefly, single colony was incubated in thioglycolate broth at 37°C for 6 hours in an anaerobic chamber. Bacterial cells were then lysed with proteinase K solution (bacterial suspension 560 µl, 30 µl 10% SDS solution and 10 µg/ml proteinase K solution; Sigma Aldrich, St. Louis, MO, USA) at 37°C for 1 hour and 5 M NaCl 80 µl together with CTAB-NaCl 100 µl were added. The mixture was mixed thoroughly and incubated at 65°C for 10 min. DNA were purified by phenol-chloroform extraction and precipitated with isopropanol, then resuspended with TE buffer and stored at -20°C until use.

Polymerase chain reaction: PCR detection of 5 major species of *Ruminococcus* spp. was performed with a set of specific primers as described previously (Wang et al., 1997). Total 25 µl of PCR mixture consisted of 50 ng of DNA template with 50 mM Tris-HCl (pH 8.5), 20 mM KCl, 3 mM $MgCl_2$, 0.05% bovine serum albumin (BSA), 0.25 mM of each dNTPs, 0.25 mM of each primer and 1 U of *Taq* polymerase (Takara, Shiga, Japan). PCR reaction was performed with PTC-200 Peltier Thermal Cycler® (AB Applied Biosystems, Foster City, CA, USA). Amplicons were analyzed on 1% agarose gel electrophoresis with 100 bp DNA ladder (Takara, Shiga, Japan) and visualized under UV light. The photo was taken with Geldoc® 2000 (Bio-rad laboratories, CA, USA).

DNA sequencing: PCR products were purified by QIAquick PCR purification kit (QIAGEN, Valencia, CA). Sequence determinations were carried out with the BigDye® Terminator v1.1 Cycle Sequencing Kit (AB Applied Biosystems, Foster City, CA, USA) and generated with the ABI Prism® 310 Genetic Analyzer (AB Applied Biosystems, Foster City, CA, USA). Sequence analysis was conducted with the Applied Biosystems DNA Sequencing Analysis Software Version 5.1 (AB Applied Biosystems, Foster City, CA, USA). The sequences of the isolates were compared to the target gene of *Ruminococcus* spp. database at GenBank website (http://www.ncbi.nlm.nih.gov/genbank/).

Statistical analysis: Comparison of number of strains that were isolated from elephant feces between each group was analysed by ANOVA.

Results

Feces of 44 elephants from three elephant camps in Chiang Mai and Lampang provinces were collected. There were 24 elephants in group A (male = 11, female = 14), 17 elephants in group B (male = 7, female = 10) and 3 elephants in group C (male = 2, female = 1). Each group has an average age of 20.91±4.22, 3.06±3.11 and 1.17±0.29 years old, respectively. They were healthy and had not showed any signs and medical problems at least 2 months prior to sample collections. All elephants were kept as show elephants. All weaned elephants were fed with bananas; sugarcane (*Saccharum spontaneum*) and additional commercial concentrated feed during daytime. About 3 P.M. of everyday, the mahouts took their elephants into deep forest close to the camp and left them there until early morning. The elephants grazed through the area for grass, bamboo leaves or wild sugarcane. However, the lactating females stayed inside the camp with her calves and were fed by their mahouts. Sucklings always stayed with their mothers and received only mothers' milk until weaning at about 2 years old. Some interesting behaviors that were observed at pre-weaning period was coprophagy followed by taking their mothers'

Table 1 Biochemical characteristics of 5 *Ruminococcus* strainsfeed such as banana and wild sugarcane.

Bacteria	Biochemical characteristics												
	sucrose	urease	starch hydrolysis	cellulose hydrolysis	arabinose	rhamnose	xylose	cellobiose	fructose	lactose	maltose	mannitol	raffinose
R. callidus	+	-	-	-	-	-	-	+	-	+	+	-	+
R. albus	+	+	-	+	+	+	+	+	+	+	-	-	-
R. flavefaciens	+	-	-	+	+	+	+	+	-	+	-	-	-
R. bromii	-	+	+	-	-	-	-	-	+	-	+	-	-
R. obeum	+	-	-	-	+	+	+	-	+	+	+	+	+

+: positive test, -: negative test

A total of 500 Gram-positive coccoid were selected. All the isolates showed catalase positive reaction and cell arrangement were diplococci and chains. Biochemical characteristics of each species are explained in Table 1. All the isolates could be classified into 214 (42.8%) isolates of *R. flavefaciens*, 105 (21.0%) isolates of *R. bromii* 90 (18.0%) isolates of *R. obeum*, 54 (10.8%) isolates of *R. callidus* and 37 (7.4%) isolates of *R. albus*. The details of bacteria isolated from each group of elephants are shown in Table 2.

Interestingly, there was no *Ruminococcus* spp. isolated from the sucklings while it was isolated from post-weaning elephants. Statistical analyses by ANOVA indicated that strains of bacteria isolated from elephants in group A and B (weaned) differed from elephants in group C (sucklings) significantly (p<0.05).

PCR method was performed using species-specific primer and the results are shown in Table 1. PCR amplicons of each strain were 286 bp of *R. callidus*, 176 bp of *R. albus*, 213 bp of *R. flavefaciens*, 444 bp of *R. bromii* and 312 bp of *R. obeum*, respectively. The PCR products were sequenced and compared to the database at GenBank. The results indicated that there were 98.3, 95.8, 97.2, 98.5 and 98.7% similarily to target genes of *R. callidus*, *R. albus*, *R. flavefaciens*, *R. bromii* and *R. obeum*, respectively, as described by Wang et al. (1997) (raw data not shown).

Discussion

Anaerobic microorganisms in rumen or cecum of hindgut fermenter like horse have been reported but rarely investigated in elephants (Ogimoto and Imai, 1981; Forsberg et al., 1997; Julliand et al., 1999; Koike et al., 2000). Cellulolytic bacteria are the most numerous microorganisms in rumen of ruminant or cecum of non-ruminant. This bacterium plays a major role in biological degradation of dietary fiber to volatile nutrients. *F. succinogenes*, *R. albus* and *R. flavefaciens* are presently recognized as the three major species of cellulolytic bacteria in rumen (Forsberg et al., 1997). *R. flavefaciens* ferment cellulose and cellobiose with the production of a large amount of succinate, acetate, ethanol and formate, but produce less hydrogen or carbon dioxide (Bryant, 1959). In contrast, *R. albus* is a *Ruminococcus* that do not produce succinate, but give hydrogen and carbon dioxide more than *R. flavefaciens* (Bryant, 1959). *R. albus* differ from other cellulolytic strain in its ability to ferment a large number of carbohydrates more than other cellulolytic strains particularly mannitol which was the only substance fermented by some cellulolytic strain including *R. albus* (Bryant, 1959). However, further investigations on the significance of cellulolytic microorganisms in gastrointestinal tract of elephants and their potential for biological degradation of dietary fiber to volatile nutrients are needed.

Table 2 *Ruminococcus* strains and number of isolates in this study

Bacteria	**No. of bacteria (isolate)**			
	A) ≥ 18 years-old	**B) 2-18 years-old**	**C) ≤ 2 years-old**	**Total (%)**
R. callidus	36	18	0 [a]	54 (10.8)
R. albus	19	18	0 [a]	37 (7.4)
R. flavefaciens	126	88	0 [a]	214 (42.8)
R. bromii	68	37	0 [a]	105 (21)
R. obeum	53	37	0 [a]	90 (18)
Total	302	198	0 [a]	500 (100)

[a]Significantly difference (p<0.05)

Epidemiological investigation of cellulolytic bacteria in cecum of ponies and donkeys in France indicated that the predominant species was *R. flavefaciens*. However, *F. succinogenes* was also isolated, but *R. albus* was rarely found in cecum of ponies and donkeys (Hastie et al., 2008). Moreover, this investigation also suggested that different host species and feeding affected the strains of cellulolytic bacteria in cecum (McBee, 1971). However, the predominant strain of cellulolytic bacteria in cecum of horse at Hokkaido, Japan was *F. succinogenes* and this investigation suggested that season change and feeding affected the amounts of bacteria in cecum (Moore et al., 1993; Julliand et al., 1999). In contrast, *R. bromii*, *R. albus* and *R. obeum* were the most prevalent species, but *R. flavefaciens* and *R. callidus* could not be isolated from mice fecal samples (Simmering et al., 2002). Identification of *Ruminococcus* spp. in primates has also been reported. The most prevalent species was *R. obeum* in human feces but *R. bromii*, *R. callidus* and *R. albus* were rarely isolated. Surprisingly, *R. flavefaciens* could not be isolated from human feces (Simmering et al., 2002). There were *R. lutii* (Russell, and Wilson, 1996) and *R. gnavus* (Wang et al., 1997), which could be isolated only from human feces. Determination of non-human primate *Ruminococcus* spp. has been reported. The study in monkey indicated that *R. bromii*, *R. obeum*, *R. albus* and *R. flavefaciens* were isolated, but *R. callidus* could not be isolated (Russell, and Wilson, 1996). These epidemiological investigations indicated that species or feeding correlated to the species of *Ruminococcus* spp. in large intestine. The results of these previous investigations concluded that *R. flavefaciens* is presently recognized as the major species in ruminant and non-ruminant. Moreover, our present investigations indicated that *Ruminococcus* spp. could not be isolated from the sucklings' feces. Feces of the three sucklings were taken during the experiment at the average age of 1.17 years old. From our observations, the sucklings had no coprophagy habit and the ecology inside the gastrointestinal tract of the sucklings was not suitable for bacterial growth. Therefore, this may be the explanation why the bacteria could not be isolated. This information supported the fact that coprophagy in weaned period assist in the development of cellulolytic microorganisms in gastrointestinal tract of the elephants. Additionally, these results should be useful for the cares and managements of zoo animals.

Conclusion

In conclusion, a cellulolytic bacterium in genus *Ruminococcus* was isolated from captive Asian elephants. The most prevalent species is *R. flavefaciens* which is similar to the major species of cellulolytic bacteria in rumen of ruminant. However, further investigations into the significance of cellulolytic microorganisms in gastrointestinal tract of elephants and their potential for biological degradation of dietary fiber to volatile nutrients are needed.

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References

Ausubel, F.M., Brent, R., Kingstom, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. 1999. Short Protocols in Molecular Biology. 4th ed. New York: John Wiley & Sons, Inc. 1104 pp.

Bryant, M.P. 1959. Bacterial species of the rumen. Microbiol Mol Biol Rev. 23: 125-153.

Chen, J. and Weimer, P. J. 2001. Competition among three predominant ruminal cellulolytic bacteria in the absence or presence of non-cellulolytic bacteria. Microbiology 147: 21-30.

Forsberg, C.W., Cheng, K.J. and White, B.A. 1997. Polysaccharide degradation in rumen and large intestine. In: Gastrointestinal Microbiology, R.I Mackie and B.A. White (eds.), Chapman and Hall, New York, USA: 156-198.

Hastie, P.M., Mitchell, K. and Murray, M.D. 2008. Semi-quantitative analysis of *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and *Streptococcus bovis* in the equine large intestine using real-time polymerase chain reaction. British J Nut. 100: 561-568.

Julliand, V., De Vaux, A., Millet, L. and Fonty, G. 1999. Identification of *Ruminococcus flavefaciens* as the predominant cellulolytic bacterial species of the equine cecum. App Environ Microbiol. 65: 3738-3741.

Koike, S., Shingu, Y., Inaba, H., Kawai, M., Kobayashi, Y., Hata, H., Tanaka, K. and Okubo, M. 2000. Fecal bacteria in Hokkaido native horses as characterized by microscopic enumeration and competitive polymerase chain reaction assays. J Equi Sci. 11: 45-50.

Koike, S. and Kobayashi, Y. 2001. Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens.* FEMS Microbiol Lett. 204: 361-366.

Langka, K. 2002. Manuals of Elephant Health Care. Chiang Mai: Chaing Mai University Press. 160 pp.

McBee, R.H. 1971. Significance of intestinal microflora in herbivory. Ann Rev Eco Sys. 2: 165-176.

Moore, B. E. and Dehority, B. A. 1993. Effects of diet and hindgut defaunation on diet digestibility and microbial concentrations in the cecum and colon of the horse. J Anim Sci. 71: 3350-3358.

Ogimoto, K. and Imai, S. 1981. Atlas of Rumen Microbiology. Tokyo: Japan Scientific Society press. 231 pp.

Russell, J.B. and Wilson, D.B. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH. J Dairy Sci. 79: 1503-1509.

Simmering, R., Taras, D., Schwiertz, A., Le Blay, G., Gruhl, B., Lawson, P.A., Collins, M.D. and Blaut, M. 2002. *Ruminococcus luti* sp. *nov*., isolated from a human faecal sample. Sys App Microbiol. 25: 189-193.

Wang, R.F., Cao, W.W. and Cernigilia, C.E. 1997. PCR detection of *Ruminococcus* spp. in human and animal fecal samples. Mol Cell Probes. 11: 259-265.

Wang, R.F., Beggs, M.L., Erickson, B.D. and Cerniglia, C.E. 2004. DNA microarray analysis of predominant human intestinal bacteria in fecal samples. Mol Cell Probes. 18: 223-234.

Wolin, M.J. 1981. Fermentation in the rumen and human large intestine. Science 213: 1463-1468.