CHARACTERISTICS OF STREPTOCOCCI AND ENTEROCOCCI ISOLATED FROM RUMEN OF MOUFLONS AND EUROPEAN BISONS

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Summary

Streptococci and enterococci, isolates from the rumen content of mouflons and European bisons were isolated. The total counts of these species reached the values (log 10 ± S.E.M.) 7.3 ± 0.21; 6.1 ± 0.06 bacteria per one ml of the rumen content in streptococci and 3.6 ± 0.20; 3.17 ± 0.18 bacteria per one ml of the rumen content in enterococci. Strains isolated were allotted to the species *Streptococcus bovis* (AM1, AM2, AM3, AM4), *Enterococcus faecium* (EH1, EFG2, EC3) and *Enterococcus faecalis* (EFA1, EFD2). Bacteria presented belong to the strains with low urease and α-amylase activities. The majority of isolates were polyresistant. Each strain produced bacteriocin-like substance with effect against at least of one of relatives species as indicators used. The most of inhibition zones were hazy with the width 2-6 mm in diameter.

(**Key Words** : Mouflons, European Bisons, Streptococci, Enterococci, Rumen, Amylase, Urease, Resistance, Bacteriocin)

Introduction

Microbial colonization in the rumen of ruminants comes after their borning immediately. However, the rumen is in the unfunctional state at this time. During this period rumen content contains mainly salivas, desquamatous epithelial cells and phlegma (Fonty et al., 1987). According to Ziolecki and Briggs (1961) the main component in the rumen microflora of young ruminants constitute streptococci, lactobacilli and *E. coli*. Ruminal streptococci and enterococci represent also facultative bacteria which are regularly isolated from the rumen of cattle, sheep or goats (Hungate, 1966, Dehority and Grubb, 1977). Jonecova (1988) described that the total streptococcal counts (per one ml of rumen content) isolated from 1 week old calves represent 5.639 ± 1.190 (log 10 ± S.E.M.) bacteria. The total counts of enterococci at the same time reached the values 4.81 ± 0.33 − 6.02 ± 0.08 bacteria per 1 ml of rumen content (Laukovᵃ et al., 1990). The species *Enterococcus faecium* and *E. faecalis* were found the most frequently in the rumen of ruminants (Latham and Jayne-Williams, 1978, Laukovᵃ, 1992).

This paper describes biochemical and physiological characteristics of streptococci and enterococci isolated from the rumen of mouflons and European bisons because their ecosystem give many possibilities for studies and experiments with the aim to clarify this subject. At the same time, it is chance to choose a suitable strain for genetic studies or for the experiment with gnotobiota.

Materials and Methods

Bacterial strains

Streptococci and enterococci were isolated from the rumen content of four mouflons and European bisons (Zoo, Košice, Slovakia). The animals are moved on the natural pasture (grass, leaves, needle-leaves) in the demarcated wooded area-the fence. In addition, they fed also hay and concentrates. The rumen contents were collected by stomach tube, serially diluted (1:9) and cultured on Azide Blood Agar Base Medium (Imuna, Šarišské Michal'any, Slovakia) with the addition of 60 g sodium chloride per 1 and on Agar Base Medium for faecal streptococci (Imuna) with the addition of 20 g maize starch per 1. These media are selective for growth of enterococci and *Streptococcus bovis*. After obtaining the total counts of isolates, the predominant types of colonies (40) were picked and chosen from each animal for further study. Nine strains of selected bacteria were identified strictly. Others strains were not specified. Strains AM1, AM2, AM3, AM4 were isolated from mouflons.

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Strains EH1, EG2, EC3, EFA1 and EFD2 were isolated from European bisons. Streptococci and enterococci were maintained in Todd-Hewitt Broth and Agar (Imuna).

Biochemical tests

To identify the isolates, the Strepto-Test identification system for streptococci and enterococci (Lachema, Brno, Czech Republic) was used according to the instruction of manufacturer. The isolates were also determined regarding to the morphology of colonies and cells (gram-positive or negative). Hemolysis was detected on VL Agar (Imuna) with the addition of 10% defibrinated sheep blood per 1.

To complete the identification of the isolates, urease [E.C.3.5.1.5.] activity was determined according to Cook (1976). α-amylase [E.C.3.2.1.1.] activity was detected using commercial pelleted S-tests (Chemical Works, Bratislava, Slovakia). The pellets containing specially treated substrate to which the dye was covalently bonded. The enzymatic activities were expressed in nkat. ml^{-1}.

Sensitivity or resistance to antibiotics

Sensitivity or resistance of bacteria to six antibiotics (table 2) was studied using commercial Sensi-La-disks (Lachema). Agarplates with disks were incubated at 37°C according to information of producer. The standard strain *Staphylococcus aureus* ATCC 6538 was incubated simultaneoulsy.

Production of bacteriocin-like substance

This activity was tested according to Skalka et al. (1983). *Streptococcus bovis* BM114 (Horodniceanu et al., 1982), *S. bovis* AO24/85 (obtained from Institute of Experimental Veterinary Medicine, Košice, Slovakia), *Enterococcus faecium* EF1, A26 and *E. faecalis* EFA (own isolates) and *Streptococcus pyogenes* 10535 (The University of Michigan Schools of Dentistry and Medicine, MI, USA) were used as indicator organisms. *E. faecium* AL6 (own isolate) was used as positive control.

Results

The total streptococcal counts isolated from rumen of mouflons and European bisons reached the values (log 10 ± S.E.M.) 7.3 ± 0.21 and 6.1 ± 0.06 bacteria per 1 ml of the rumen content. The total counts of enterococci in the rumen of both animal species reached the values (log 10 ± S.E.M.) 3.6 + 0.20 and 3.17 ± 0.18 bacteria per 1 ml of the rumen content.

Strains presented were facultatively anaerobic, non-motile, gram-positive cocci, occurring in pairs or short chains. All strains fermented lactose and mannitol. Esculin and bile-esculin tests were positive in all bacteria tested. Voges-Proskauer test and hemolysis were shown also as positive in strains presented. Fermentation of other carbohydrates as well as results of other tests are summarized in table 1.

The values of urease activity ranged from 2.47 + 0.06 to 5.59 ± 0.36 nkat. ml^{-1}. In the α-amylase activity were measured the values from 0.77 to 1.80 ± 0.01 nkat. ml^{-1} (table 2).

TABLE 1. PHENOTYPIC CHARACTERISTICS OF STREPTOCOCCI AND ENTEROCOCCI - ISOLATES FROM THE RUMEN CONTENT OF MOUFLONS AND EUROPEAN BISONS

	Strains No.								
	AM1	AM2	AM3	AM4	EH1	EG2	EC3	EFA1	EFD2
Fermentation of									
Sorbitol	−	−	−	V	+	+	+	−	−
Raffinose	V	+	V	+	−	−	−	−	−
Melezitose	V	V	V	V	+	+	V	−	−
Inulin	+	+	+	+	−	V	−	−	−
Glycerol	V	V	V	−	+	+	+	V	V
Other tests									
NaCl-Esculin	−	−	V	V	+	V	V	V	V
Arginine	V	V	V	−	+	+	+	+	+
Hippurate	−	−	−	−	+	+	nd	nd	nd

All strains fermented lactose and mannitol. Esculin, bile-esculin and Voges-Proskauer tests were positive in all strains tested. Hemolysis was found in all bacteria presented.

+ - positive reaction; − - negative reaction; V - variable reaction; nd - not determined; AM1, AM2, AM3, AM4-*S. bovis*; EH1, EG2, EC3 - *E. faecium*; EFA1, EFD2 - *E. faecalis*;

The results of identificational tests showed as well as according to *Bergy's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974), isolates AM1, AM2, AM3, AM4, EH1, EFG2, EC3, EFA1 and EFD2 belong to the species *Streptococcus bovis*, *Enterococcus faecium* and *Enterococcus faecalis* respectively.

The majority of strains presented were polyresistant. *E. faecium* EH1 and *E. faecalis* EFD2 were resistant to all antibiotics used in the test. All bacteria were resistant to penicillin-10 IU (table 2).

The growth of all indicator organisms used in antibacterial activity test was inhibited by bacteriocin-like substance producing by *S. bovis* AM1, AM2 and AM4. Moreover, each strain produced bacteriocin-like substance with effect against at least of one of indicators used. The zones of inhibition were more hazy, reaching 2-6 mm in diameter (table 3).

TABLE 2. ANTIBIOTIC RESISTANCE, UREASE AND α-AMYLASE ACTIVITIES IN STREPTOCOCCI AND ENTEROCOCCI ISOLATED FROM RUMEN CONTENT OF MOUFLONS AND EUROPEAN BISONS

Strain	Urease[a]	Amylase[b]	Antibiotics				
			TCT[c]	AMP[d]	CHC[e]	RIF[g]	STM[h]
AM1	–	1.80 ± 0.01	R	S	S	S	S
AM2	–	0.77 ± 0.05	S	S	S	S	S
AM3	–	1.47 ± 0.10	S	S	S	S	R
AM4	–	1.74 ± 0.12	S	S	S	S	R
EH1	4.22 ± 0.05	–	R	R	R	R	R
EG2	4.98 ± 0.23	–	S	S	S	S	S
EC3	5.59 ± 0.36	–	R	S	S	S	S
EFA1	5.09 ± 0.36	–	R	R	R	S	S
EFD2	2.47 ± 0.06	–	R	R	R	R	R

All strains were resistant to penicillin (10 IU - International Unit per disk); TCT[c] - tetracycline, STM[h] - streptomycin, CHC[e] - chloramphenicol - concentration 30 μg per disk; AMP[d] - ampicillin - concentration 20 μg per disk; rifampincin[g] - concentration 10 μg per disk.

AM1, AM2, AM3, AM4 - *Streptococcus bovis*, EH1, EG2, EC3 - *Enterococcus faecium*, EFA1, EFD2 - *Enterococcus faecalis*; [a] and [b] - nkat. mL^{-1}.

TABEL 3. BACTERIOCIN-LIKE SUBSTANCE PRODUCTION OF ENTEROCOCCI AND STREPTOCOCCI ISOLATED FROM THE RUMEN CONTENT OF MOUFLONS AND EUROPEAN BISONS

Strain No.	Indicator organisms					
	AO24 /85	EF1	BM114	A26	10535	EFA
S. bovis AM1	+	+ +	+	+	+	+
S. bovis AM2	+	+ +	+	+	+	+
S. bovis AM3	–	–	–	–	–	+
S. bovis AM4	+	+ +	+	+	+	+
E. faecium EH1	–	–	–	+	–	nd
E. faecium EG2	+	+	–	+	–	nd
E. faecium EC3	–	–	–	+	–	nd
E. faecalis EFA1	–	–	–	+	–	nd
E. faecalis EFD2	–	–	–	+	–	nd

+ - zone 2-5 mm (diameter zone of inhibition); + + - zone 5 mm (diameter zone of inhibition); nd-not determined.

AO24/85 - *Streptococcus bovis*; Inst. of Exp. Vet. Medicine, Košice, Slovakia EF1, A26 - *Enterococcus faecalis* (own isolates); EFA - *E. faecalis* (own isolate); 10535 - *S. pyogenes* (The University of Michigan Schools of Dentistry and Medicine, M1, USA), BM114- *Streptococcus (Enterococcus) faecium* - Laboratory for streptococci and staphylococci, Inst. Pasteur, Paris, France.

Discussion

The phenotypic characteristics of strains AM1, AM2, AM3, AM4, EH1, EG2, EC3, EFA1 and EFD2 agreed with those of *Streptococcus bovis, Enterococcus faecium* and *Enterococcus faecalis* (Knudtson and Hartman, 1992). In domestic ruminants, *S. bovis* (excepted the first week of animal's life) formed 80-100% of all streptococcal strains isolated from the rumen (Mann et al., 1954). Our findings support these informations as well as those of Kmeť et al. (1988). This author referred the counts of *S. bovis* 6.50 ± 0.61 and 8.58 ± 0.13 (log 10 ± S.E.M.) bacteria per one ml of lamb's rumen content.

It seems that the occurrence of enterococci in rumen of mouflons or European bisons is less counted in comparison with domestic ruminants. Devriese et al. (1992) identified in digestive tract of calves without *E. faecium* and *E. faecalis* also the species *E. avium, E. caecorum, E. durans* and *E. hirae*. Moreover, Laukovâ (1993) reported the other species of enterococci as well as *E. solitarius* and *E. malodoratus* in the rumen content of fallow deers.

On the basis of measured values of urease and α-amylase activity, streptococci and enterococci presented belong to the strains with low urease and α-amylase activities (Jonecovâ, 1988; Laukovâ, 1992). On the other hand, enterococci especially *E. faecjum* or *faecalis* play an important role in hydrolysis of urea (Laukovâ et al., 1990).

The importance have also our findings of α-amylase activity in *S. bovis* isolates mainly regarding to further genetic experiments with them. Because Marekovâ (1992) described α-amylase gene, its cloning and expression in ruminal strain *S. bovis* AO24/85.

Presented enterococci demonstrated the highest resistance to antibiotics than *S. bovis* strains. Penicillin and tetracycline resistance in ruminal enterococci (domestic ruminants in origin) referred Laukovâ et al. (1992). Jonecovâ et al. (1993) presented *S. bovis* isolates (not wild ruminants) to be with the highest resistance to tetracycline (90.5%). In general, *S. bovis* isolates here presented were more antibiotic sensitive.

Regarding to bacteriocin-like substance production, it seems that more active strains were *S. bovis* isolates in comparison with enterococci even if Jonecovâ (1988) reported only 2% of ruminal *S. bovis* strains with bacteriocin-like substance production. However, in these tests were used only relative species as indicators. It has been known that the effect of bacteriocin-like substance production depends not only on indicators used but it depends also on a suitable cultivation medium (Loyola-Rodriguez et al., 1992; Laukova, 1993). In general, enterococcal isolates from domestic ruminants produced bacteriocin substances with a broad spectrum of antimicrobial effect (Laukovâ et al., 1993). It's possible to constate that bacteriocin production can be affected also by source of isolate or isolate's origin.

In conclusion, it is necessary to describe that informations about strains isolated can be used in other specific experiments e. g. isolation, purification of bacteriocins which are commonly used in food industry with protective effect (Mulders et al., 1991; Lewus et al., 1991). Moreover, these informations can be also used by plasmids detection and isolation. Because antibiotic resistance, bacteriocin production, α-amylase or urease activities in streptococci and enterococci can be plasmid encoding as was described by Kuncovâ et al., 1991, Laukovâ et al., 1990, Marekovâ 1992, Jonecovâ et al., 1993, Bondi et al., 1984. On the other hand, it's opportunity to select a suitable strain to follow e. g. a mode of action of bacteriocins in a model of gnotobiotic lambs.

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