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Study of the animal to animal variability in bacterial community structure in the goats' rumen

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Abstract. The use of classical culture and, more recently, modern molecular techniques to study microbial diversity in the rumen show a high animal to animal variability and, sometimes, contradictory information on diurnal variation pattern in their numbers. This work aimed to study the animal variation in diversity of rumen bacterial community within an established flock of Granadina goats. In a first period (I), three adult goats cannulated in the rumen were fed alfalfa hay for a month and samples of rumen contents collected at 0, 2 and 4 hours after the morning feeding in two non consecutive days. In two consecutive additional periods of one month duration, rumen content was transferred between animals and samples collected 2 h after the morning feeding in two non consecutive days. Total DNA was extracted from rumen samples and t-RFLP used to monitor bacterial dynamics. In Period I the bacterial profiles of each animal clustered separately, and were very similar along the day with no apparent differences between sampling day. In Periods II and III the animal was still the main clustering factor. Our results show that within an established flock of goats each animal harbours its own bacterial community and that there seems to be a day-to-day stability.

Keywords. Animal variability – Alfalfa hay – Molecular techniques – Rumen – t-RFLP.

Etude de la variabilité animale dans la structure de la communauté bactérienne ruminale de caprins

Résumé. *La culture classique et les techniques moléculaires montrent une haute variabilité parmi des animaux dans la diversité microbienne du rumen et, parfois, une contradiction concernant la variation diurne de la quantité de bactéries. Ce travail étude la variabilité animale dans la diversité de la communauté des bactéries du rumen chez chèvres de la rase Granadina cannulées dans le rumen. Dans une première période (I) les animaux ont été nourris au foin de luzerne pour l'entretien pendant un mois et des échantillons du contenu du rumen ont été collectées à 0, 2 et 4 heures après la nourriture de la matinée et dans deux jours consécutifs. Pour évaluer la cohérence des différences observées dans la période I, le contenu du rumen a été transféré entre les animaux dans les périodes II et III au cours des lesquelles les animaux ont également été nourris avec de foin de luzerne. Des échantillons du contenu du rumen ont été prélevés après 2 h de la nourriture du matin et pendant deux jours consécutifs. L'ADN total a été extrait des échantillons le "Terminal restriction fragment polymorphism (t-RFLP)" utilisée pour étudier la dynamique bactérienne. Dans la période I les profils bacteriennes de chaque animal se sont regroupées séparément et ils étaient très semblables le long de la journée avec aucune différence apparente entre l'échantillonnage provenant de différent jours. Dans les périodes II et III les différences entre les animaux dans la structure de la communauté des bactéries ont été réduites après l'échange de contenu du rumen bien que l'animal semble être le facteur clé de clustering. Dans le cadre d'un troupeau de chèvres établis chaque animal port sa propre communauté des bactéries et il semble y avoir une stabilité jour à jour au cours d'une couple de semaines, ce qui a des implications importantes pour la conception des protocoles expérimentaux.*

Mots-clés. *Variabilité des animaux – Foin de luzerne – Techniques moléculaires – Rumen – t-RFLP.*

I – Introduction

The microbial population in the rumen is composed of a complex mixture of bacteria, protozoa, fungi, archaea and phage (Dehority, 2004). Each of these groups comprises a collection of species that are genetically and biochemically diverse. This versatility complements the intricate physical structures of the microbial community within the ruminal ecosystem and enables ruminant animals to consume a wide variety of feedstuffs without causing large fluctuations in the amounts or types of microbial products produced (Wolstrup *et al.*, 1974). However, the species composition of the rumen microbial community has been shown to vary greatly between individuals, different feeding regimes, intake levels, frequency of feeding and the postprandial time at which the rumen is sampled (Swain *et al.*, 1996).

These early works studying the composition of rumen microbial communities were based on traditional cultivation-based approaches, which can only detect a low proportion of the whole microbial population in the rumen (Edwards *et al.*, 2004). Advances in the development of molecular techniques for analysis of complex microbial communities have bypassed the necessity for cultivation and made considerable progress in characterization of the rumen microbial ecosystem (Wright *et al.*, 2004).

This work aimed to use modern molecular techniques to study the animal to animal variation and the day to day stability in the rumen bacterial community structure within an established flock of Granadina goats fed alfalfa hay at maintenance level.

II – Material and methods

The trial was divided in 3 periods. In period I, three adult goats cannulated in the rumen were fed alfalfa hay at maintenance level (Prieto *et al.*, 1990) for a month and then samples of rumen contents collected at 0, 2 and 4 hours after the morning feeding on days 31 (d1) and 45 (d2). To evaluate the consistency of differences amongst animals observed in Period I, 1.5 kg of rumen content were swapped between animals in two additional periods (II and III), during which animals were also fed alfalfa hay for one month and samples collected on days 31 (d1) and 45 (d2) 2 hours after the morning feeding. In Period II the content was transferred from animal 1 to 2, from 2 to 3 and from 3 to 1. In Period III the rumen content was transferred from animal 2 to 1, from 3 to 2 and from 1 to 3.

Approximately 50 g of samples of rumen contents were freeze-dried and thoroughly mixed by physical disruption using a bead beater (Mini-bead Beater; BioSpec Products, Bartlesville, OK, USA) before DNA extraction, which was performed from approximately 50 mg sub-sample using the QIAamp® DNA Stool Mini Kit (Qiagen Ltd, West Sussex, UK) following the manufacturer's instructions. DNA samples were used as templates for terminal restriction fragment length polymorphism (t-RFLP) analysis (Hongogh *et al.*, 2005) to study total bacterial community structure: PCR was performed using a 16S rRNA bacterial-specific primer pair, cyanine labelled 27F (5'-AGA GTT TGA TCC TGG CTG AG-3') and unlabelled 1389R (5'-AGG GGG GGT GTG TAG AAG-3'). The PCR product was purified (Millipore MultiScreen® PCR-96 plate with 20 inches Hg vacuum). The DNA concentration within each sample was determined by spectrophotometry (Nanodrop® ND-1000 spectrophotometer) and then diluted to 20ng/□l. Restriction enzyme digestion was performed using HhaI at 0.25 U/µl. Analysis of terminal restriction fragments in samples was performed by using the Bray-Curtis distance between binary profiles (presence/absence of peaks) to construct dendrograms. Analysis of the T-RFLP peaks profiles was carried out by CAP 4 software (Pisces Conservation Ltd., Lymintong, Hampshire, UK).

III – Results and discussion

In Period I, as shown in Fig. 1, the bacterial profiles of each animal clustered separately, and were very similar along the day with no apparent differences between d1 and d2. Animals 2 and 3 seemed to harbour a more similar bacterial community compared to animal 3, whose samples are in a separated cluster.

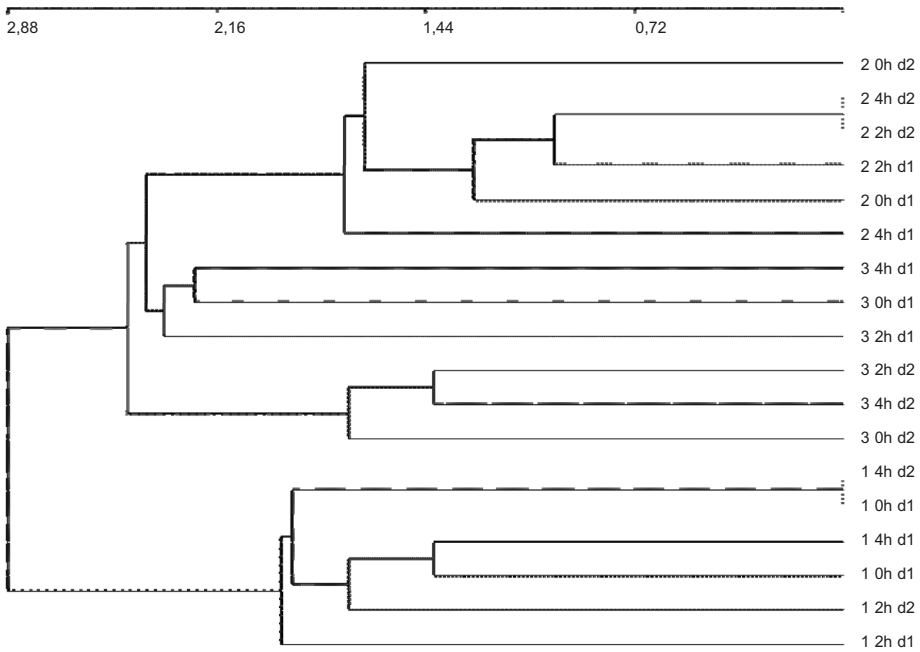

Fig. 1. Dendrogram illustrating the effect of animal (1, 2 and 3), sampling time (0, 2 and 4 hours) and day (d1 and d2) on total bacteria (16s rRNA gene) t-RFLP patterns in Period I. The scale bars shows Bray-Curtis distances.

In Periods II and III (Fig. 2) the similarity within each individual in t-RFLP profiles was reduced after exchanging rumen contents, which is shown by samples from Period I clustered together and those from periods II and III being less similar. However, the animal seemed to be the main clustering factor, as the dendrogram displays 3 main clusters.

Recent studies have shown that bacterial communities co-diversified with their hosts, being mainly influenced by animal species and diet preference, but with high bacterial-host specificity (Ley *et al.*, 2008).The coexistence of the host and microbial gut communities seems to be immunologically driven, and we are only beginning to understand the complex ways in which they adapt to each other (Winkler *et al.*, 2007). Host-level selection of specific members of a microbiota has been demonstrated under laboratory conditions by reciprocal transplantations of gut microbiota from one host species to germ-free recipients of a different species: groups of bacteria were expanded or contracted in the recipient host to resemble its "normal" microbiota through a process that may have been influenced by diet (Rawls *et al.*, 2006). Our results support the hypothesis that each animal's rumen microbiota is host-specific and that in adult animals it is very resilience to experimental induced changes such as the transfer of rumen contents from one animal to another. Whether this specificity explains different digestive response when the rumen ecosystem is challenged with high or low quality diets needs to be further investigated.

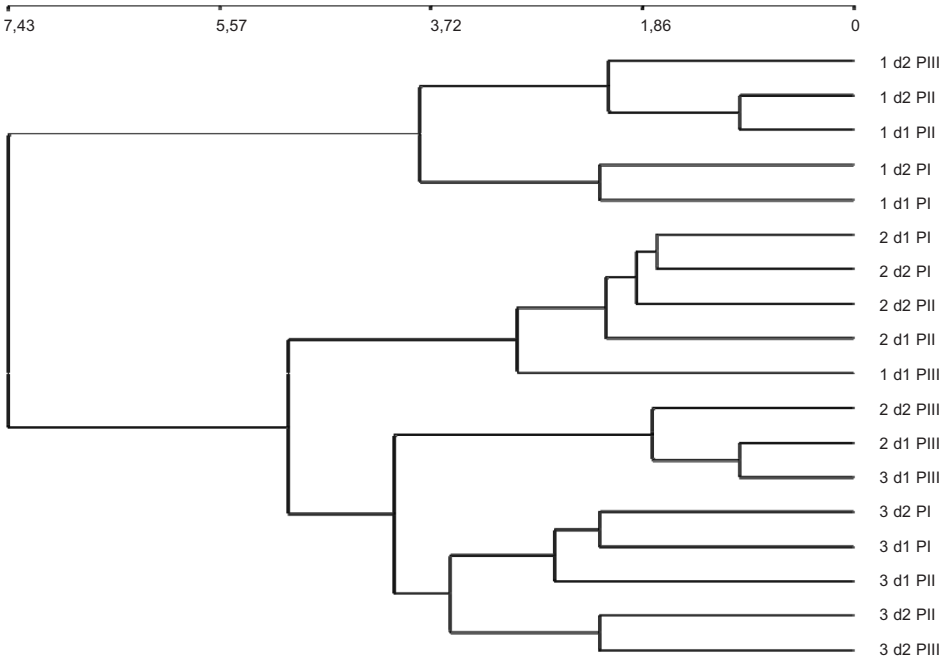

Fig. 2. Dendrogram illustrating the effect of animal (1, 2 and 3), period (I, II and III) and sampling day (d1, d2) on total bacteria (16s rRNA gene) t-RFLP patterns. The scale bars shows Bray-Curtis distances.

IV – Conclusions

Our results show that within an established flock of goats each animal harbours its own bacterial community and that there seems to be a day-to-day stability over the course of a couple of weeks, which has important implications for designing experimental sampling protocols.

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