T310

The effect of heat stress on rumen microbial composition analyzed by sequence-specific rRNA cleavage method

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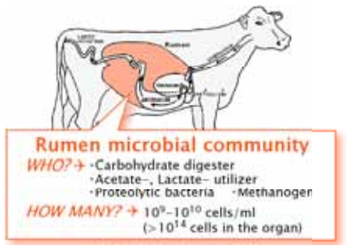
Introduction



- Heat stress and the strain are the greatest challenges to production facing dairy farmers. High ambient temperatures can reduce the milk yields of lactating dairy cows, decrease reproducibility, and delay heifer growth.
- Cows tend to change nutrient requirements to prevent the elevation of the body temperature during heat stress. Ruminal microbial community may reflect adaptation to the alteration of the dynamic characteristics of digestion and maintenance of normal rumen function.

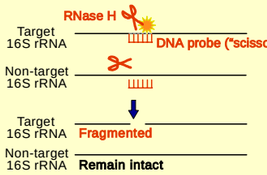
Aim of this study ✈✈✈

-- To develop a 16S rRNA-based method to determine the bacterial community in ruminal ecosystems

-- To characterize the changes in the ruminal bacterial community of dairy cows under heat stress condition

Sequence-specific rRNA cleavage method

(Uyeno et al. [2004])

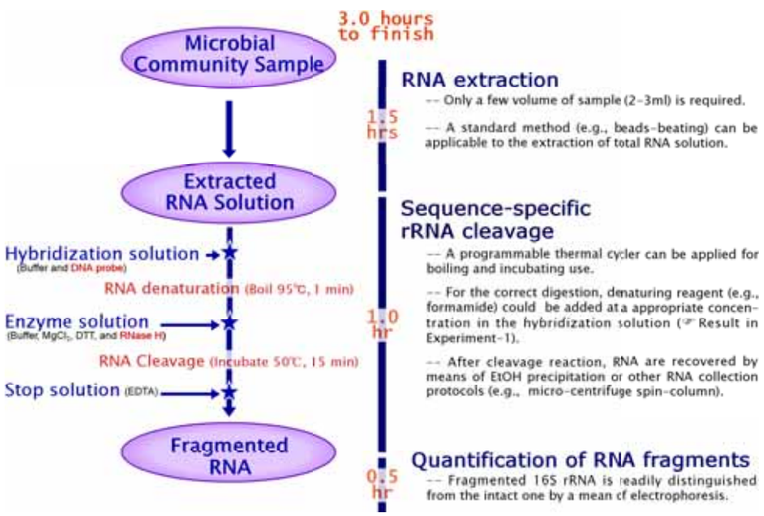


RNase H could degrade the RNA at the site where DNA probe is hybridized with the RNA molecule of the target group.

In a total RNA solution retrieved from complex microbial community, only the target RNA can be fragmented.

- we presented a novel RNA-based method for the quantitative detection of a specific group of microorganisms in complex ecosystems. This method is easy to perform and only requires a short amount of time.

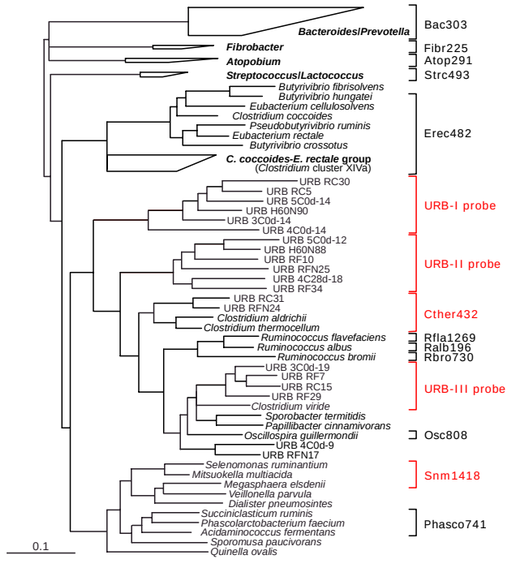
Analytical Procedure For determining bacterial population



EXPERIMENT-1

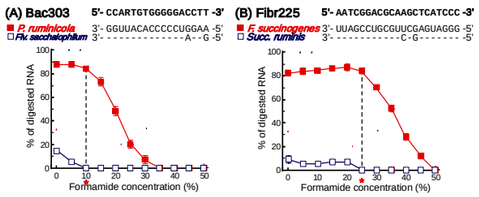
Construction of the probe set for the rumen bacterial community

Rumen bacterial community overview



✓To fully describe the rumen bacterial community, we have developed a systematic probe set, based upon a comparative 16S rRNA sequencing approach to the rumen bacterial community. We consequently made up a set of 15 probes. Newly designed probes are indicated **in red**.

Probe specificity verification



✓For each probe, a formamide concentration (indicated as * in the figure) was determined at which 16S rRNA of the non-target strain was not cleaved at all while 16S rRNA of the target strain was sufficiently cleaved.

EXPERIMENT-2

Analyzing the changes of bacterial community composition in response to heat stress

Bacterial populations of ruminal samples of heifers under heat stress condition

Probe (Target group)		RNA population (%) 1st Period (9 months) 20C	28C	33C	2nd Period (15 months) 20C	28C	33C	SEM	Effect T	P	T x P
Eub338	(*Bacteria*)	92.0	91.7	92.3	88.7	92.3	87.1	0.7			
Bac303	(*Bacteroides* / *Prevotella*)	28.9	32.9	33.7	46.4	45.2	44.3	1.6		**	
Erec482	(*C. coccoides - E. rectale* group)	6.4	7.2	9.6	9.5	12.8	12.7	0.6	**	**	
Fibr225	(*Fibrobacter*)	4.4	1.5	2.1	6.5	2.7	1.2	0.5	**		
Rfla1269	(*R. flavefaciens*)	3.5	4.1	3.3	2.0	2.5	2.3	0.2		**	
Rbro730	(*R. bromii*, *C. sporosphaeroides*)	0.8	1.8	1.2	0.6	1.3	1.0	0.1	**		
Phasco741	(*Phascolarctobacterium* and relatives)	1.0	1.1	0.8	0.7	1.7	1.5	0.1			
Ralb196	(*R. albus*)	1.3	1.4	1.5	1.4	0.8	0.7	0.1			
Snm1418	(*Selenomonas* and relatives)	0.6	1.4	1.3	0.7	1.2	1.3	0.1			
Strc493	(*Streptococcus* / *Lactococcus*)	0.7	1.1	1.8	0.2	1.1	2.1	0.2	**		
Cther432	(*C. thermocellum* subgroup)	0.7	1.1	1.2	0.7	1.2	1.3	0.1	*		
Atop291	(*Atopobium*)	3.3	3.1	4.0	2.4	4.0	3.4	0.2			
Osc808	(*Oscillospira*)	10.8	5.9	5.2	1.2	1.8	2.0	0.7	**	**	*
URB-I	(Uncultured rumen bacteria cluster-I)	2.3	2.2	1.7	2.2	2.0	1.2	0.1	*		
URB-II	(Uncultured rumen bacteria cluster-II)	1.2	2.0	1.8	1.3	1.6	1.9	0.1	*		
URB-III	(Uncultured rumen bacteria cluster-III)	1.9	2.2	2.8	3.1	2.7	1.7	0.2			*
total (The proportion of total bacterial rRNA population)		68.1 (0.74)	69.0 (0.75)	72.0 (0.78)	78.9 (0.89)	82.6 (0.89)	78.5 (0.90)	1.4 (0.02)	**		

T, temperature; P, treatment period; TxP, the interaction.
*, p<0.05; **, p<0.01.

✓*E. rectale - C. coccoides*, *Streptococcus*, *R. bromii*, *C. thermocellum*, URB-II increased
Fibrobacter, *Oscillospira*, URB-I decreased as housing temperature increased.

✓*Bacteroides*, *Oscillospira*, *R. flavefaciens*, *E. rectale - C. coccoides* group differed between periods.

✓The total amount of 16S rRNAs of targeted groups accounted for 90% max of the total bacterial 16S rRNAs.

Physiological changes observed:

(Unpublished data)

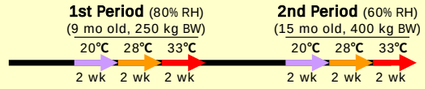
-- Lower hay intake and lower DMI
-- Lower ruminal pH
-- Higher respiration rate and body temperature
-- Lower DG

in heifers at high ambient temperature.

- As changes in diet can alter the ruminal community, the hypothesis that fibrolytic bacterial activity (e.g., *Fibrobacter*) decreases and saccharolytic bacterial activity (*Streptococcus*) increases in response to the rise of the temperature is proposed. Quantification data was consistent with this hypothesis.

Experimental design

-- Four fistulated Holstein heifers were employed
-- Treatment periods were set as:



-- Cows were fed a mixed ration diet (1.35 kg Italian ryegrass hay silage, 0.15 kg alfalfa hay cube, and 1.5kg concentrate) for ad libitum consumption twice a day (0930 and 1730 h)
-- Rumen fluid samples were obtained at the last day of each temperature condition before the morning feeding

Conclusions

- **Exposure to increased temperature can affect the composition of the ruminal microbial community in accordance with the change in the feed intake.**
  - ⇒ **The alteration of the ruminal microbial community possibly contributes to the adaptation to heat stress.**
  - ⇒ **Uncultured rumen bacteria may play a critical role in ruminal fermentation which can respond to temperature change.**
- **This RNA-based quantitative approach allows for comprehensive description of ruminal bacterial community.**
  - ⇒ **This can be applied for tracking the dynamics of the community in relation to the rumen's physiology under specific conditions.**

Reference

- Uyeno, Y. *et al.* Sequence-specific cleavage of small-subunit (SSU) rRNA with oligonucleotides and RNase H. Appl Environ Microbiol 70, 3650-3663 (2004).
- Uyeno, Y. *et al.* Evaluation of group-specific, 16S rRNA-targeted scissor probes for quantitative detection of predominant bacterial populations in dairy cattle rumen. J Appl Microbiol *in press* (2007).
- Tajima, K. *et al.* Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. Anaerobe 13, 57-64 (2007).