



PROCEEDINGS OF THE VI INTERNATIONAL SYMPOSIUM ON FORAGE QUALITY AND CONSERVATION

EDITORS

LUIZ GUSTAVO NUSSIO
ÉRICA BENJAMIN DA SILVA
KÉSIA DA SILVA OLIVEIRA
VIVIANE CARNAVAL GRITTI
PEDRO AUGUSTO RIBEIRO SALVO
GUSTAVO G. DE SOUZA SALVATI
DANNYLO OLIVEIRA DE SOUSA



VI INTERNATIONAL SYMPOSIUM ON FORAGE QUALITY AND CONSERVATION

November 07-08th, 2019
The Beira Rio Hotel, Piracicaba, SP - Brazil

University of São Paulo - "Luiz de Queiroz" College of
Agriculture – Department of Animal Science
Av. Pádua Dias, 11 – PO box 09 – Zip Code 13418-900
Piracicaba, SP
Phone: +55 (19) 3429-4176
Fax: +55 (19) 3429-4215
E-mail: isfqc2017brazil@gmail.com

Thursday – November 07th, 2019

- ▶ 07:40-08:10 – Registration
- ▶ 08:10-08:30 – Opening Ceremony
- ▶ 08:30-09:10 – Interpretation of chemical, microbial, and organoleptic components of silages (Limin Kung Jr. – University of Delaware, USA)
- ▶ 09:10-09:50 – Present and future of microbial inoculants for silages (Lucas Mari – Lallemand, Brazil)
- ▶ 09:50-10:20 – Coffee Break
- ▶ 10:20-10:35 – Volunteered paper
- ▶ 10:35-10:50 – Volunteered paper
- ▶ 10:50-11:30 – Chemical additives for silages: When to use and what options we have (Horst Auerbach – KONSIL Europe GmbH, Germany)
- ▶ 11:30-11:40 – Sponsortime
- ▶ 11:40-12:30 – Poster exhibition
- ▶ 12:30-14:00 – Lunch
- ▶ 14:00-14:40 – Strategies to explore the potential of corn hybrids (Thiago Bernardes – Federal University of Lavras, Brazil)
- ▶ 14:40-14:50 – Sponsortime
- ▶ 14:50-15:20 – Coffee Break
- ▶ 15:20-16:00 – Recent advances and future technologies for silage harvesting (Brian Luck – University of Wisconsin, USA)
- ▶ 16:00-16:40 – Profiling of metabolome and bacterial community dynamics in silages (Xusheng Guo – Lanzhou University, China)

**PROCEEDINGS OF THE
VI INTERNATIONAL
SYMPOSIUM
ON FORAGE QUALITY
AND CONSERVATION**

How selective are the culture media used in silage microbiology?

P. Drouin¹ and F. Ferrero²

¹Lallemand Animal Nutrition, Lallemand Specialities Inc., Milwaukee, United States, email: pdrouin@lallemand.com. ²Department of Agricultural, Forest, and Food Sciences, University of Turin, Italy, email:francesco.ferrore@unito.it

Keywords : culture media, selectivity, microbiome, silage

Introduction. Silage microbiology generally performed plate counts in order to evaluate the number of cells present in the tested samples. Microbial counts are performed following standard methods often developed for the food industry and adapted to the silage environment. For lactic acid bacteria (LAB), silage literature reports the use of the Mann-Rogosa-Sharp (MRS) selective media as the main media used by research groups. This media was used either directly, by adding antifungals, or acidified. The plates could then be incubated under aerobic or anaerobic conditions. Other culture media like Rogosa were also used. When testing for yeast and mold (Y-M), the number of selective culture media reported by silage literature is higher. A literature survey reports more than ten media and additives combination. To test the difference between selective media for LAB and Y-M, we analyzed microbiota of five samples following selection pressure from five LAB selective media as well as eight Y-M selective media.

Materials and methods. To test plate counts and microbiota diversity, five samples were collected from bunker silos. Composite samples from a corn and an alfalfa-grass bunker were collected, one from the center of the bunker and a second from the top-right shoulder (side). The last sample consisted of a total mix ration (47% corn silage – 8% alfalfa-grass silage). All five samples were serially diluted and plated on each culture media (see Table 1 from the descriptions). After incubation at 28 °C, counts were performed and all colonies were scrapped from the agar surface following addition of one milliliter of sterile dilution buffer for corn-center (CC), corn-side (CS), alfalfa-center (AC), alfalfa side (AS), TMR. Samples were stored at -80 °C until extraction of the DNA. For microbiota analysis, DNA was extracted using the Zymo Fecal/Soil miniprep DNA isolation kit from the suspension and fresh feed samples. The DNA was then sequenced from bacteria diversity (V4-V5 region) and fungal diversity (ITS) according to the methodology described in Drouin et al. (2019).

Results and discussion. LAB counts for the samples were within the expected values in relation with the type of substrates (silage or TMR), with values of 4.9, 7.8, 5.1, 8.3, 7.1 log₁₀ CFU/g for CC, CS, AC, AS, and TMR samples, respectively, for counts on MRS. For Y-M media, differences in counts were observed between yeast CFU and molds counts. Cell-counts differed significantly between the selective plates for both LAB and Y-M media.

After amplicons meta-sequencing, the results confirmed the basic trends observed following plate counts. To show some of the differences, Table 1 present the results of alpha diversity compute by the Shannon diversity index. For the different culture media within each type of sample, the selective pressure, measured by the diversity index, was generally similar. For example, Shannon values for the center of the corn silage bunker ranged from 1.08 to 1.57. In this case, diversity was similar to the silage sample. Still, no general trends could be drawn

between the different samples. Generally, the LAB culture media were highly selective, while Y-M selective media showed higher diversity compared to the values from the silage samples. This trend also differed between the origin of the samples of Y-M, with higher selective pressure by the culture media for the samples from the side of the bunkers compared to the center.

Selectivity against LAB was similar between Rogosa, MRS-acetate, MRS-cycloheximide, and MRS-nystatin. For these media, *Acetobacter* represents 30-40% of the relative abundance when incubated under aerobiosis. Anaerobic incubation was restrictive to the *Acetobacter*. MRS with no additive also allowed *Pseudomonas* to grow. The *Acetobacter* population was specific to the side section of the corn bunker. Rogosa media the most selective against *Lactobacillus*.

In relation with fungal abundance of species, the DRBC media provided the closest similarity between the fresh samples and the plate suspensions. Czapek was enriched in the yeast *Dipodascus* and MEA (no additive) was enriched in the mold *Mucor*.

Table 1. Culture media and diversity results for the five composite samples of silage and TMR.

Selective media	Incubation condition	Alpha diversity (Shannon diversity index)				
		Corn silage		Grass-alfalfa silage		TMR
		Center	Side	Center	Side	
16S - Fresh sample		1.06	4.33	4.61	3.89	4.39
MRS	aerobic	1.57	3.44	0.47	2.29	2.65
MRS-acetate	aerobic	1.34	2.80	NA	2.38	2.24
MRS-cycloheximide	aerobic	1.55	2.84	0.20	2.60	2.08
MRS-nystatin	aerobic	1.65	2.98	0.57	2.35	2.06
Rogosa	aerobic	1.08	2.39	NA	1.34	2.54
MRS	anaerobic	1.44	2.50	n.d.	n.d.	n.d.
MRS-acetate	anaerobic	1.44	2.30	n.d.	n.d.	n.d.
MRS-cycloheximide	anaerobic	1.58	2.33	n.d.	n.d.	n.d.
MRS-nystatin	anaerobic	1.53	2.16	n.d.	n.d.	n.d.
ITS - Fresh sample		3.27	1.89	3.19	3.20	3.12
MEA	aerobic	4.60	2.34	4.63	0.33	4.51
MEA-neomycine-streptomycine	aerobic	No CFU	2.13	No CFU	0.30	2.51
MEA-lactate	aerobic	No CFU	2.27	No CFU	0.40	2.10
PDA	aerobic	4.65	2.35	4.47	0.01	1.55
Sabouraud	aerobic	4.72	2.62	4.64	0.58	4.17
YGC	aerobic	No CFU	No CFU	NA	0.19	3.02
Czapek	aerobic	3.24	3.16	No CFU	0.05	3.88
DRBC	aerobic	No CFU	2.21	5.23	0.82	3.41

Conclusion. LAB selectively by MRS-acetate media was good and in relation with the corresponding silage samples. Utilization of Rogosa or incubation under anaerobic conditions allow selecting against *Acetobacter*. For Y-M, the media offering the best matching against diversity of the original samples was DRBC.

References. Drouin, P. 2019. J. Dairy Science. in press.