

## How selective are the culture media used in silage microbiology?

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**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 log<sub>10</sub> 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	
<b>16S - Fresh sample</b>		<b>1.06</b>	<b>4.33</b>	<b>4.61</b>	<b>3.89</b>	<b>4.39</b>
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.
<b>ITS - Fresh sample</b>		<b>3.27</b>	<b>1.89</b>	<b>3.19</b>	<b>3.20</b>	<b>3.12</b>
MEA	aerobic	4.60	2.34	4.63	0.33	4.51
MEA-neomycine-stretomycine	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.