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Lactobacillus hilgardii as inoculant for corn silage in Italy

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Keywords: aerobic stability, dry matter content, ensiling duration, *Lactobacillus hilgardii*.

Introduction In the last 20 years, heterofermentative lactic acid bacteria (LAB), and *L. buchneri* in particular, have been used as inocula to prevent the aerobic deterioration of silages, because of their ability to increase the production of acetic acid, which inhibits yeasts and increases aerobic stability. Silages that have a high dry matter (DM) content (>35%) have lower concentrations of acetic acid than wetter silages and thus are often more prone to aerobic spoilage. *L. buchneri*-based inocula need a long conservation period (>90 d) to be efficacious (Driehuis et al. 1999) and have resulted to be less efficient in high DM content corn silages (Comino et al. 2014). Owing to the farmers' need to quickly open corn silages made in late summer, due to the feeding needs of the herd or to compensate high DM losses that may have occurred in the previous year's silages, early acting inocula are required to prevent aerobic deterioration. The aim of this study was to evaluate the effect of a new strain of *L. hilgardii* on the aerobic stability of whole crop corn silage affected by different DM contents and different ensiling durations.

Material and Methods The effect of *L. hilgardii* was evaluated in 5 different trials. Five corn fields (from 27 to 42% DM) were harvested as whole plants and not treated (C) or inoculated with *L. hilgardii* CNCM I-4785 (LH) [theoretical application rate of 300,000 cfu/g fresh matter (FM)]. The fresh forages were ensiled in 20-L plastic silos with five replications for each treatments at a density ranging from 669±31 kg FM/m^3 to 529±21 kg FM/m^3 and opened after 15, 30, 100 and 250 days of conservation. At opening the silages were analyzed for DM content, pH, fermentative profile and microbial count. The DM content was determined at 60°C for 72 h, fermentative profile was characterized in the acid extract by HPLC. Yeast and mold counts were obtained using the pour plate technique on Yeast Extract Glucose Chloramphenicol agar. After each opening, the silages were subjected to an aerobic stability test by continuously measuring the temperature during air exposure. Aerobic stability was defined as the number of hours the silage temperature remained stable before increasing more than 2°C above room temperature. Data were analyzed via analysis of variance, utilizing inocula and DM content as the fixed factors, with their significance reported at a 0.05 probability level, using the General Linear Model of the Statistical Package for Social Science (v 24.0, SPSS Inc., Chicago, Illinois, USA).

Results and Discussion

The LH inoculum influenced the fermentation profile by reducing the lactic acid content and by increasing the acetic acid content of silages after 100 and 250 d of conservation (data not shown). At 100 d of ensiling, the lactic-to-acetic acid ratio decreased in LH silages, except for the wetter silage (Figure 1). The 1,2-propanediol level was under the detection limit (<0.01 g/kg DM) in 4 out of 5 control silages, whereas it was detected in 3 out of 5 LH silages as already reported by Assis et al. (2014). The aerobic stability of the silages increased as the ensiling duration increased, regardless of the treatments (Table 1). This effect is mainly explained by the reduction of yeast count as illustrated in Figure 2, where the higher the yeast count, the lower the aerobic stability. Furthermore, Figure 2 showed the effect of lactic-to-acetic acid ratio and ensiling duration on yeast count, and the higher the ratio the higher the yeast count. According to our results, Reis et al. (2018) found that, after 19, 60 and 103 d, the lactic-to-acetic acid ratio was always smaller in LH treated than in the control silages, due to the higher production of acetic acid by the inoculum.Yeast count under detection level were only observed after 100 d of ensiling, especially in LH silages. Interestingly, the yeast count was reduced by LH starting from 15 d onwards in all trials. After 30 and 100 d of fermentation, LH increased the aerobic stability of the silages, by a mean value of 15 h (+18.6%) and 29 h (+27.2%), respectively. The

yeast count was reduced by LH, from 15 d onwards in all the trials. Dry matter content of the forages influenced this pattern as reported by Comino et al. (2014).

Table 1. Aerobic stability and yeast count after 4 ensiling periods for different DM corn silages

DM (%)	Aerobic stability 2°C (h)								Yeast count (log_{10} cfu/g)							
	15 d		30 d		100 d		250 d		15 d		30 d		100d		250 d	
	C	LH	C	LH	C	LH	C	LH	C	LH	C	LH	C	LH	C	LH
27	56	57	137	154	182	209	221	247	3.47	3.05	2.37	2.04	1.73	1.05	0.50	0.50
34	65	81	72	96	97	119	102	124	3.90	3.57	3.72	2.56	3.17	1.16	2.17	1.41
36	69	66	83	97	76	77	184	200	4.26	3.96	3.50	3.22	3.71	3.78	0.88	1.02
40	-	-	48	58	89	91	52	103	-	-	4.04	4.28	4.29	4.10	4.72	3.41
42	51	53	58	67	96	191	-	-	5.11	5.00	4.49	4.23	4.00	2.80	-	-
Treat	NS		**		***		NS		**		**		**		NS	
DM	***		***		***		***		***		***		***		***	
Treat*DM	*		NS		**		NS		NS		**		*		NS	

C = control; DM = Dry matter; LH = *L. hilgardii*

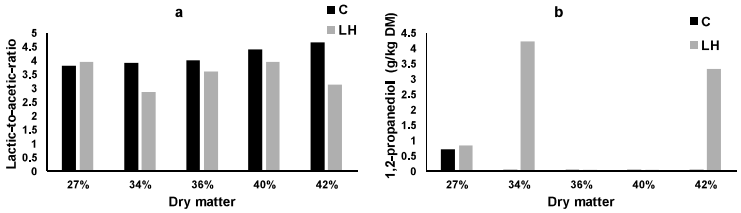

Figure 1. Lactic-to-acetic acid ratio (a) and 1,2-propanediol (b) of the treated and untreated corn silages with different DM contents after 100 d of ensiling (C = control; LH = *L. hilgardii*).

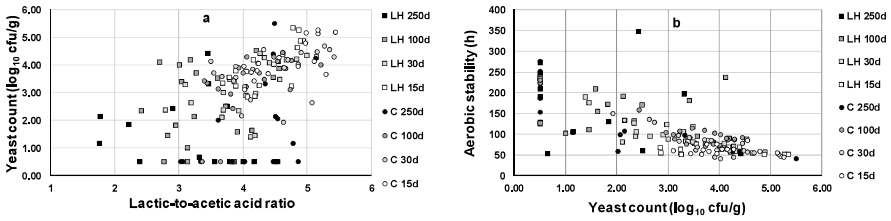

Figure 2. Scatter plot of lactic-to-acetic ratio and yeast count (a) and scatter plot of the yeast count and aerobic stability (b), as affected by the treatments and ensiling duration (C = control; LH = *L. hilgardii*).

Conclusion The new strain of *L. hilgardii* had positive effects on improving the aerobic stability of early opening corn silages as it increases the acetic acid content and reduces the yeast count. However, the effect of *L. hilgardii* is influenced by the DM content of the silages.

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