ENSILING WITH A NOVEL *LENTILACTOBACILLUS BUCHNERI* SUBSP. *SILAGEI* STRAIN 30673 ENHANCES AEROBIC STABILITY OF MAIZE SILAGE THROUGH YEAST SUPPRESSION

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ABSTRACT

Maize silage is susceptible to aerobic spoilage during feed-out due to its high starch and residual sugar content leading to DM and feed quality losses. Heterofermentative lactic acid bacteria can be employed to improve silage stability during air exposure. This study evaluated the effects of a novel *Lentilactobacillus buchneri* subsp. *silagei* strain NCIMB 30673, on fermentation characteristics and aerobic stability of maize silage. Whole-crop maize at 37% DM was ensiled either untreated or inoculated at 1.0×10^5 CFU/g and stored for 28 or 99 days. At 28 days, both treatments achieved stable pH. The inoculated silage showed enhanced fermentation with higher lactic and acetic acid concentrations however both treatments exhibited high yeast levels, resulting in lower aerobic stability. After 99 days, treated silage exhibited a more pronounced impact on fermentation, characterised by increased acetic acid concentration, reduced yeast populations, and significantly improved aerobic stability compared with the control. These results demonstrate that *L. buchneri* subsp. *silagei* NCIMB 30673 effectively enhances aerobic stability of whole plant maize silage through yeast suppression, making it a promising candidate as silage inoculant.

Keywords: aerobic stability, inoculant, *Lentilactobacillus buchneri* subsp. *silagei*, yeast suppression

INTRODUCTION

Lentilactobacillus buchneri is an obligately heterofermentative lactic acid bacterium (LAB) widely used in silage making (Pahlow *et al*., 2003). In contrast to homofermentative LAB, which primarily promote rapid acidification through lactic acid (LA) production, *L. buchneri* is distinguished by its ability to anaerobically convert LA into acetic acid (AA) and 1,2-propanediol (1,2-PDOL) (Oude Elferink *et al*., 2001). Acetic acid functions as a potent antifungal compound, inhibiting the lactate-assimilating yeasts responsible for initiating aerobic spoilage. (Danner *et al*., 2003). Maize silage, owing to its high starch and residual sugar content, is particularly susceptible to aerobic deterioration during the feed-out phase, resulting in dry matter (DM) losses and reduced feed quality (Tabacco *et al*., 2011). Consequently, *L. buchneri* is widely employed as a silage inoculant to improve aerobic stability after silo opening through enhanced acetic acid production. Recent taxonomic advancements have identified two distinct subspecies within the *L. buchneri* group: *L. buchneri* subsp. *buchneri* and the newly characterised *L. buchneri* subsp. *silagei* originally isolated from rice grain silage (Tanizawa *et al*., 2020). *Lentilactobacillus buchneri* subsp. *silagei* strain NCIMB 30673 was isolated from aerobically stable whole-crop barley silage. This study aimed to evaluate the effectiveness of this strain in improving fermentation characteristics and enhancing the aerobic stability of maize silage.

MATERIAL AND METHODS

Whole plant maize was harvested and chopped in field and transported to the laboratory for ensiling. Forage (37% DM) was either left untreated (CON) or inoculated with *Lentilactobacillus buchneri* subsp. *silagei* strain NCIMB 30673 (LB) at a target application rate of 1.0×10^5 CFU/g of fresh forage. Samples were ensiled using 1.8 L glass jars at a target packing density of 180kg DM/m^3 and subsequently stored for 28 and 99 days at a constant 20 ± 1 °C. Samples were ensiled with three (day 28) or five (day 99) replicate silos. Upon opening, silages were homogenized, and representative subsamples were analysed for DM (oven drying at 60°C until constant weight), microbial populations, pH, and fermentation profiles. Volatile fatty acids and alcohols were quantified via high-performance liquid chromatography (HPLC) equipped with a refractive index detector. After silo opening, aerobic stability was assessed at 20 ± 1 °C by recording the internal temperature at 15-minute intervals using Squirrel data loggers (Grant). Stability was defined as the duration required for the silage temperature to rise 2 °C above ambient. Statistical analyses were performed in R (version 4.3.1) using ANOVA followed by Tukey's Honestly Significant Difference (HSD) test, with significance defined at $P < 0.05$.

RESULTS AND DISCUSSION

By day 28, both CON and LB- silages achieved a stable pH of 3.81, however LB silages exhibited more extensive fermentation characterized by higher total fermentable acids and alcohols products (TFP) ($P = 0.039$; Table 1). *L. buchneri*-treated silage had significantly higher LAB population compared to CON ($P < 0.001$; Table 1), which resulted in elevated levels of LA ($P = 0.053$; Table 1). After 28 days of fermentation AA concentrations remained relatively low across both treatments (11.07 in CON and 15.03 g/kg DM in LB) but were significantly higher in treated silages ($P = 0.037$; Table 1). The accumulated levels of AA, however, were insufficient to inhibit the undesirable yeasts which remained high in both CON and LB silages ($P = 0.926$; Table 1). Consequently, both treatments showed lower aerobic stability, with spoilage occurring within 1.15 days for CON and 1.23 days for LB-treated silages ($P = 0.864$; Table 1).

By day 99, silages treated with LB displayed more pronounced impact on fermentation. While the population of LAB remained higher than in CON ($P < 0.001$; Table 1), the levels of LA declined and were significantly lower than in untreated maize ($P < 0.001$; Table 1). In contrast, the concentration of AA in LB silages more than doubled relative to those at day 28, reaching 35.01 g/kg DM whereas CON silages showed minimal increase (14.86 g/kg DM, $P < 0.001$; Table 1). The increased AA concentration in the LB-treated silages was likely responsible for the lower yeast numbers recorded relative to the untreated control (0.66 log10 compared to 4.04 log10 CFU/g in LB and CON respectively; $P < 0.001$; Table 1). Consequently, this resulted in a significant improvement in aerobic stability ($P < 0.001$) and a significant reduction in aerobic losses ($P < 0.001$; Table 1) in LB relative to CON.

https://doi.org/10.11118/978-80-7701-093-1-0096

Tab. 1: Fermentation parameters, microbial groups and aerobic stability in maize silages

Parameter	Day 28				Day 99			
	CON	LB	SEM	P-value	CON	LB	SEM	P-value
pH	3.81	3.81	0.01	1	3.84	4.02	0.01	<0.001
DM, g/kg	329.08	325.26	6.60	0.703	328.60	316.49	3.32	0.012
DM loss, %	4.8	5.41	0.14	0.044	4.48	5.43	0.07	<0.001
LA, g/kg DM	44.66	58.69	3.64	0.053	60.8	41.46	1.30	<0.001
AA, g/kg DM	11.07	15.03	0.91	0.037	14.86	35.01	0.78	<0.001
1,2-PDOL, g/kg DM	<LOD	0.37	0.03	<0.001	0.58	27.86	0.80	<0.001
Ethanol, g/kg DM	6.36	8.64	0.44	0.022	7.74	9.11	0.31	0.020
TFP, g/kg DM	62.78	83.91	4.95	0.039	85.64	113.78	2.65	<0.001
LAB, log10 CFU/g	7.77	8.43	0.03	<0.001	7.98	9.00	0.08	<0.001
Yeasts, log10 CFU/g	6.29	6.23	0.41	0.926	4.05	0.66	0.25	<0.001
Moulds, log10 CFU/g	0.60	0.60	–	–	0.60	0.66	0.04	0.347
AS, days	1.15	1.23	0.31	0.864	2.11	6.88	0.35	<0.001
Aerobic losses, % FM	2.29	2.35	0.20	0.824	2.57	1.76	0.10	<0.001

CONCLUSION

The application of LB significantly altered the fermentation profile and aerobic stability of maize silage. The greatest impact was realised after 99 days of fermentation where the metabolic conversion of LA to AA resulted in a successful suppression of yeasts, thereby enhancing aerobic stability relative to CON. These findings demonstrate that LB is a promising candidate as silage inoculant for improving aerobic stability.

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