YEAST SUPPRESSION AND ENHANCED AEROBIC STABILITY IN GRASS SILAGE INOCULATED WITH *LENTILACTOBACILLUS BUCHNERI* SUBSP. *SILAGEI* NCIMB 30673

Kerley, A.[1], Borkowska, A.[1], Jones, P. B.[1], Smith, P.[1], Leggett, M. J.[1]

[1]Volac International Ltd, Port Talbot, United Kingdom

ABSTRACT

Heterofermentative lactic acid bacteria (LAB) inoculants are used to enhance silage aerobic stability by increasing acetic acid production, which inhibits yeasts and moulds. *Lentilactobacillus buchneri* is widely used in such products, but strain-specific effects and the speed at which they provide effective protection remain poorly defined. This study evaluated a novel candidate inoculant, *L. buchneri* subsp. *silagei* strain NCIMB 30673, for its ability to improve the early aerobic stability of grass silage. Grass (33% DM) was chopped and ensiled in triplicate 1.8 L silos for 30 or 92 days, either left untreated or inoculated with *L. buchneri silagei* at 1.0×10^5 CFU/g fresh forage. At opening, silages were analysed for microbial counts, pH, fermentation products, and aerobic stability. Data were analysed by linear models with the interaction between time and treatment as a fixed effects, followed by emmeans pairwise comparisons at each time point ($p \leq 0.05$). At 30 days, inoculation with the *L. buchneri* significantly reduced yeast populations compared with the untreated control (3.16 $\log_{10}$ CFU/g reduction, $p < 0.001$). The *L. buchneri* substantially increased acetic acid and 1,2propanediol concentrations relative to untreated silage (day 30, both $p < 0.001$), indicating rapid activity within as little as 30 days. Aerobic stability measurements suggested that inoculated silage opened at day 30 remained stable for >11 days, whereas untreated silage deteriorated earlier (3 days). By day 92, both treatments had similar yeast and acetic acid levels, likely due to naturally occurring heterofermentative bacteria present in the untreated silage. The new *L. buchneri* subsp. *silagei* strain NCIMB 30673 rapidly enhanced acetic acid and 1,2-propanediol production in grass silage and substantially reduced yeast populations by day 30 post-ensiling, indicating strong antifungal activity. These findings suggest that NCIMB 30673 is a promising candidate silage inoculant to improve aerobic stability and control spoilage yeasts, even after short ensiling periods.

Keywords: *Lentilactobacillus buchneri* subsp. *silagei*, silage inoculant, aerobic stability, yeast suppression, acetic acid production

INTRODUCTION

Epiphytic yeasts and moulds can trigger aerobic spoilage in silage, especially when silage is poorly compacted, sealed or managed (Holmes and Bolsen, 2009; Borreani *et al.*, 2007; Pahlow and Muck, 2009). This can result in dry matter loss, elevated mycotoxins, and reduced dry matter intake (Tabacco *et al.*, 2011; Richard *et al.*, 2009; Whitlock *et al.*, 2000), potentially reducing animal performance (Kung *et al.*, 1998). To combat this, it is common to inoculate forage with a heterofermentative LAB, such as *Lentilactobacillus buchneri*, which converts lactic acid to acetic acid and 1,2 propanediol during secondary fermentation (Oude Elferink *et al.*, 2001). Acetic acid exhibits strong antifungal properties as undissociated acid molecules can penetrate yeast cells to help reduce undesired growth (Danner *et al.*, 2003). Although *L. buchneri* inoculation improves aerobic stability, strain-specific effects and the speed of protection remain poorly characterised. Advances in bacterial taxonomy have enabled more precise classification within the *L. buchneri* group. Recent work by Tanizawa *et al.* (2020) identified two distinct subspecies: *L. buchneri* subsp. *buchneri* and the newly described *L. buchneri* subsp. *silagei*. In this study a novel candidate inoculant, Lentilactobacillus buchneri subsp. silagei strain NCIMB 30673, isolated from aerobically stable whole-crop barley silage, was evaluated for its ability to enhance grass silage fermentation quality and aerobic stability.

MATERIAL AND METHODS

Rye Grass was harvested and chopped in the field (South Wales, UK) and transferred to the laboratory for further processing. Forage dry matter (DM) was 33% at point of ensiling. Forage was either left untreated (CON) or inoculated with *L. buchneri* subsp. silagei NCIMB 30673 (LB) at 1.0×10^5 CFU/g fresh forage. Triplicate 1.8 L glass jars per treatment were packed at 139 kg DM/m^3 and stored for 30 or 92 days at 20 ± 1 °C. After opening, silages were thoroughly mixed, and representative subsamples were taken for analysis of dry matter (DM; oven-dried at 60 °C to constant weight), microbial counts, pH, and fermentation characteristics. Volatile fatty acids and alcohols were quantified using high-performance liquid chromatography (HPLC; Prominence, Shimadzu) with a refractive index detector. Following silo opening, aerobic stability was evaluated at 20 ± 1 °C by monitoring internal temperature every 15 minutes with Squirrel data loggers (Grant). Stability was defined as the time required for the silage temperature to increase 2 °C above ambient. Statistical analyses were conducted in R (version 4.3.1). Data were analysed by two-way linear models with time and treatment as fixed effects and their interaction, followed by estimated marginal means (emmeans) contrasts to compare treatments within each time point, with significance defined at $p < 0.05$. For statistical analysis, microbial counts below the limit of detection (LOD; 2.60 $\log_{10}$ CFU/g) were reported as 0.5 x LOD (i.e. 2.30 $\log_{10}$ CFU/g).

RESULTS AND DISCUSSION (PRELIMINARY)

Time × treatment interactions were significant (linear model, all $p < 0.01$) for LAB and yeast counts, acetic acid, 1,2-propanediol, and aerobic stability indicating that the treatment effect for these parameters was not uniform over time. No significant interactions occurred for pH ($p = 0.49$), dry matter ($p = 0.058$), or mould counts ($p = 0.2$). However, pH ($p < 0.01$) and dry matter ($p < 0.001$) were significantly affected by treatment alone.

At Day 30, LB treatment had significantly increased LAB counts (pairwise emmeans, $p = 0.015$; Table 1), reduced yeast counts by 3.16 $\log_{10}$ CFU/g ($p < 0.001$; Table 1) and increased concentrations of both acetic acid and 1,2-propanediol (both $p < 0.001$; Table 1) relative to CON. We observed a significant improvement aerobic stability in LB silage at day 30 post-ensiling (11.9 vs 3.0 days, $p < 0.001$; Table 1). We also observed elevated pH ($p = 0.030$; Table 1) and reduced dry matter ($p < 0.001$; Table 1) in LB treated silage at day 30. Together, these results point to a secondary lactic acid fermentation from LB, as reported for other *L. buchneri* strains previously (Oude

https://doi.org/10.11118/978-80-7701-093-1-0156

Tab. 1: Fermentation characteristics means ± SE and pairwise emmeans p values. CON = Untreated silage, LB = *L. buchneri* subsp. silagei strain NCIMB 30673 treated silage

Parameter	Day 30			Day 92		
	CON	LB	p value	CON	LB	*p* value
Sample number	3	3	–	5	5	–
LAB (log^{10} CFU/g)	9.37 ± 0.04	9.56 ± 0.05	0.015	9.24 ± 0.05	8.62 ± 0.02	<0.001
Yeasts (log^{10}CFU/g)	5.86 ± 0.05	2.7 ± 0.20	<0.001	2.30 ± 0.00	2.30 ± 0.00	1.000
Moulds (log^{10} CFU/g)	2.30 ± 0.00	2.30 ± 0.00	0.109	2.30 ± 0.00	2.30 ± 0.00	1.000
Acetic acid (g/kg DM)	25.9 ± 0.73	51.29 ± 0.58	<0.001	55.21 ± 4.11	53.96 ± 2.67	0.762
1,2-propanediol (g/kg DM)	6.31 ± 0.27	36.45 ± 0.12	<0.001	38.64 ± 3.33	49.21 ± 2.69	0.011
Aerobic stability (Days)	2.97 ± 0.09	11.9 ± 0.00	<0.001	16.9 ± 0.00	16.8 ± 0.08	0.315
pH	3.96 ± 0.00	4.27 ± 0.22	0.030	4.14 ± 0.01	4.33 ± 0.01	0.068
Dry matter (g/kg)	309.9 ± 0.45	299.61 ± 0.69	<0.001	297.33 ± 1.35	291.85 ± 0.97	0.002

Elferink *et al*., 2001). The rapid generation of acetic acid at concentrations sufficient to improve aerobic stability, as seen in LB silage, would be highly desirable in a silage inoculant.

The magnitude of the treatment effect had decreased significantly for most parameters by day 92, where no significant differences were observed for yeasts, moulds, acetic acid, aerobic stability or pH (Table 1). However, significant treatment effects were observed after 92 days for LAB counts, which were higher in CON ($p < 0.001$; Table 1); dry matter, which remained lower in LB silage ($p = 0.002$; Table 1); and 1,2-propanediol, which was higher in LB silage ($p = 0.011$; Table 1). The elevated levels of 1,2-propanediol and acetic acid present in CON silage after 92 days may point to the activity of a slow acting epiphytic *L. buchneri* like bacteria reaching impactful levels over the longer time period.

CONCLUSION

In this study, the rapid generation of acetic acid in LB silage resulted in significantly lower yeast populations and higher aerobic stability after only 30 days ensiling. These results mark *L. buchneri* subsp. silagei strain NCIMB 30673 as a promising candidate silage inoculant.

REFERENCES

Borreani, G., Tabacco, E., Cavallarin, L. 2007. A new oxygen barrier film reduces aerobic deterioration in farm scale corn silage. *Journal of Dairy Science.* 90, 4701–4706.

Danner, H., Holzer, M., Mayrhuber, E., Braun, R. 2003. Acetic acid increases stability of silage under aerobic conditions. *Applied and Environmental Microbiology*. 69(1), 562–567.

Holmes, B. J., Bolsen, K. K. 2009. What's new in silage management? In: Broderick, G. A. (ed.). *Proceedings of the 15th International Silage Conference*. Madison, Wisconsin, pp. 61–76.

Kung Jr, L., Sheperd, A. C., Smagala, A. M., Endres, K. M., Bessett, C. A., Ranjit, N. K., Glancey, J. L. 1998. The effect of preservatives based on propionic acid on the fermentation and aerobic stability of corn silage and a total mixed ration. *Journal of Dairy Science*. 81(5), 1322–1330.

Oude Elferink, S. J., Krooneman, J., Gottschal, J. C., Spoelstra, S. F., Faber, F., Driehuis, F. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by Lactobacillus buchneri. *Applied and Environmental Microbiology*. 67(1), 125–132.

Pahlow, G., Muck, R. E. 2009. Managing for improved aerobic stability. In: Broderick, G. A. (Ed.). *Proceedings of the 15th International Silage Conference*. Madison, Wisconsin, pp. 77–90.

Richard, E., Heutte, N., Bouchart, V., Garon, D. 2009. Evaluation of fungal contamination and mycotoxin production in maize silage. *Animal Feed Science and Technology*. 148(2-4), 309–320.

Tabacco, E., Righi, F., Quarantelli, A., Borreani, G. 2011. Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula. *Journal of Dairy Science.* 94(3), 1409–1419.

Tanizawa, Y., Kobayashi, H., Nomura, M., Sakamoto, M., Arita, M., Nakamura, Y., Ohkuma, M., Tohno, M. 2020. Lactobacillus buchneri subsp. silagei subsp. nov., isolated from rice grain silage. *International Journal of Systematic and Evolutionary Microbiology.* 70(5), 3111–3116.

Whitlock, L. A., Wistuba, T., Siefers, M. K., Brent, B. E., Bolsen, K. K., Pope, R. V. 2000. Effect of level of surface-spoiled silage on the nutritive value of corn silage-based rations. *Kansas Agricultural Experiment Station Research Reports*. (1), 22–24.

Contact Information

Adrienne Kerley: e-mail: adrienne.kerley@volac.com