Research Article

Marek Selwet*

Influence of inoculation with Lactobacillus on fermentation, production of 1,2-propanediol and 1-propanol as well as Maize silage aerobic stability

https://doi.org/10.1515/biol-2020-0038
received November 25, 2019; accepted April 21, 2020

Abstract: The aim of this study is to determine the influence of a commercial bacterial inoculant (L1) and a preparation (L2) containing three Lactobacillus strains capable of producing 1,2-propanediol and short-chain fatty acids on maize silage aerobic stability improvement. The research showed that during 90-day ensilage, the applied preparations significantly reduced the content of DM, water-soluble carbohydrates (WSCs), pH and DM recovery ($P < 0.05$). The concentration of lactic acid (LA), acetic acid (AA) and propionic acid (PA) in the inoculated samples increased significantly ($P < 0.05$). 1,2-Propanediol and 1-propanol were not found in control silages (without additives). The addition of L1 and L2 significantly ($P < 0.05$) increased the concentration of these substances. The L1 and L2 mixtures significantly extended ($P < 0.05$) the silage aerobic stability.

Keywords: Lactobacillus buchneri, aerobic stability, silage

1 Introduction

Lactic acid-fermenting bacteria are used as a major microbiological modifier that could improve the silage chemical composition [1]. These bacteria may increase the dry matter recovery rate [2] and improve the hygienic state of silage, which is determined by the content of moulds and yeasts [3]. It is noteworthy that acetic acid produced by heterofermentative lactic acid bacteria may alter the yeast metabolism and improve the silage aerobic stability. However, heterofermentative bacterial strains do not metabolise lactic acid efficiently as they consume large amount of energy in this process. It is a disadvantage, which causes a greater loss of nutrients [4]. Therefore, it seems reasonable to use adequate mixtures of lactic heterofermentative and homofermentative strains depending on the ensiled plant material [5]. Alternatively, enzyme preparations can be used, but they are more expensive and it is more difficult to prepare them [6]. There is a considerable divergence in the results of the latest research concerning the type of additives used. They are divided into different groups: ‘homolactic’ (homofermentative bacteria), ‘hetero’ (heterofermentative bacteria), ‘combo’ (homofermentative plus heterofermentative bacteria) and ‘chemical’ (chemical additives) [7]. Heterofermentative bacterial inoculants ferment water-soluble carbohydrates into organic acids, especially lactic acid, which quickly acidifies silage and inhibits the undesirable bacteria growth. Heterolactic bacterial inoculants ferment water-soluble carbohydrates into antifungal acids, such as acetic and propionic acids, which inhibit the growth of spoilage-causing fungi. Commercially available inoculants contain one or both types of lactic acid bacteria. So far, few studies have simultaneously compared several commercially available inoculants with chemical additives [8]. Recently, there have been numerous studies on the use of the selected lactic acid bacterial strains, mainly Lactobacillus buchneri. According to the results, this heterofermentative strain improves the silage aerobic stability. Apart from that, it anaerobically degrades lactic acid to acetic acid and 1,2-propanediol. Probably, 1,2-propanediol is an intermediate metabolite, which becomes degraded into 1-propanol and propionic acid by Lactobacillus diolivorans [9]. Lactobacillus reuteri can synthesise covalam, which is a coenzyme for diol dehydratase – the enzyme catalysing 1,2-propanediol conversion into 1-propanol and propionic acid [10]. During co-fermentation, the synthesis of acetic acid, 1,2-propanediol and propionic acid is stimulated by bacterial strains from these species. They improve the aerobic stability of renewable feed silage. The research results were used to make bacterial preparations stimulating the ensilage of renewable raw materials [11].

The aim of this study is to determine the influence of a commercially available bacterial preparation and a
mixture of *L. buchneri* strains on 1,2-propanediol and 1-propanol, the chemical composition and aerobic stability of maize silages.

## 2 Materials and methods

### 2.1 Plant material

Maize (*Zea mays* L.) SAN cultivar (FAO 240) from the Hodowla Roślin in Smolice Ltd/Sp. z o.o. IHAR Group was ensilage. Type of use: medium-early three-line hybrid (TC) with advantages of a grain hybrid for CCM and silage. The features of maize were as follows: high yields of total dry matter and dry matter of cobs, high resistance to *Fusarium* stem rot and root lodging, tolerant to smut, long-lasting green leaves and stems and the height of 270 cm. Plant density is 150,000 per ha. Maize was harvested in October at the end of silage maturity (BBCH 83). It was cut/harvested at a height of 40 cm. Before ensiling, it was cut into 2–3 cm chaff. Maize was grown in monoculture.

### 2.2 Bacterial preparations used to silage inoculation

L1 is a commercially available preparation containing in lyophilisate the following cultures: *Lactobacillus plantarum* K KK 593/p, *L. plantarum* C KK 788/p, *Lactobacillus brevis* KKP 839 and *L. buchneri* KKP 907. The producer recommended a dose of 5 g t⁻¹ of ensiled material. The concentration of bacteria in 1 g of the preparation was 10⁹ cfu g⁻¹.

L2 is a mixture (lyophilisate) of three strains: *L. buchneri* ATCC 4005, *Lactobacillus dioliovorans* LGM 19667, and *L. reuteri* ATCC 23272 (DSMZ). A dose of 5 g t⁻¹ of ensiled material was used. The concentration of bacteria in 1 g of the mixture was 10⁹ cfu g⁻¹.

### 2.3 Ensilage and determination of aerobic stability

Silages were prepared in PVC laboratory micro silos with a capacity of 4 dm³ equipped in a closure allowing removal of gaseous products. The average temperature during ensiling was 20 ± 1°C. During the aerobic stability test, samples were aerated for 7 days at 20°C. After this period, changes in microorganism counts and silage selected chemical parameters were investigated. After 90-day ensilage, moist samples weighing 85 g were removed from micro silos and placed in 500 mL plastic containers with 4 mm holes enabling air circulation. The temperature was measured with a temperature reader (Hotmux DDC Corporation, Pennsauken, NJ, USA) every 5 min at 2 h intervals. Stability was defined as the time necessary to raise silage temperature by ≥2°C relative to the ambient temperature. The number of replications was 5.

### 2.4 Microbiological and chemical analyses

Lactic fermentation bacteria were cultured on MRS Agar (Oxoid). Incubation time was 48 h at 35°C. Yeasts and moulds were cultured on OGYE Agar (Oxoid) with oxytetracycline (oxytetracycline-glucose-yeast-extract agar). Incubation time was 5 days at 25°C.

Lactic acid, acetic acid, propionic acid, ethanol, 1-propanol and 1,2-propanediol concentrations were measured with a gas chromatograph equipped with an FID detector, 2 m long 80/100 Chromosorb® WAW glass column (Supelco), i.D. 2 mm with GP 10% SP–1,200/1% H₃PO₄ filling and Varian 8200 CX autosampler. Hydrogen was used as the carrier gas (flow 30 cm³ min⁻¹) with oven temperature of 120°C, injection temperature of 250°C and detector temperature of 300°C. Fluka acid standards were used.

The basic composition of feeds was determined in accordance with AOAC [12]. pH values were measured with a pH meter (Hann Instruments) in a suspension prepared from 20 g of silage and 180 cm³ of demineralised water, which was homogenised for 10 min.

### 2.5 Statistical analysis

The GLM SAS procedure package was used for statistical calculations [13]. Differences between the means were tested using Tukey’s test.

## 3 Results

Basic chemical composition and counts of lactic acid bacteria, yeast and mould in the ensiled maize forage are presented in Table 1.

Table 2 presents the chemical composition and counts of microorganisms after 90-day ensilage. Dry matter concentration and WSC in silages treated with bacterial
The content of 1,2-propandiol and 1-propanol in the combination with L2 was relatively 62% and 75%, respectively, greater than in the combination with L1.

Table 3 lists changes in silages exposed to oxygen. Inoculation with L1 and L2 strains significantly \((P < 0.05)\) reduced the growth of pH in silages. AA and PA concentrations in the samples with Lactobacillus strains were significantly \((P < 0.05)\) greater than in the control sample, but the LA content was significantly \((P < 0.05)\) reduced. LAB counts in the inoculated silages were significantly \((P < 0.05)\) greater, whereas yeast and mould counts were lower \((P < 0.05)\) than in the control samples. No 1,2-propandiol or 1-propanol was found in the control samples. The content of 1,2-propandiol and 1-propanol in the combinations with L1 and L2 was the same as in silages before the aerobic stability test.

### Table 3: The chemical composition and count of microorganisms in maize silage after the aerobic stability test

| Parameters                     | Control | L1     | L2     |
|-------------------------------|---------|--------|--------|
| pH                            | 6.2a    | 5.01b  | 4.99b  |
| LA % DM                       | 5.1a    | 5.8b   | 4.6b   |
| AA % DM                       | 1.7a    | 3.5b   | 4.9a   |
| PA % DM                       | 0c      | 0.8b   | 1.0a   |
| 1,2-Propanediol % DM          | 0c      | 0.5h   | 1.5b   |
| 1-Propanol % DM               | 0c      | 0.2b   | 0.8a   |
| LAB log CFU g\(^{-1}\)         | 8.4a    | 7.13b  | 6.15c  |
| Mould log CFU g\(^{-1}\)      | 7.17a   | 6.18b  | 5.02c  |

LA = lactic acid, AA = acetic acid, PA = propionic acid, LAB = lactic acid bacteria.

\(^{a,b,c}\) Means marked with different letters in a row are different at \(P < 0.05\).

Figure 1 shows temperatures taken during measurements of the silage aerobic stability. L1 and L2 mixtures significantly \((P < 0.05)\) extended the aerobic stability. The silage temperature in control samples increased by 2°C within 72 h. The inoculated silages were characterised by longer stability, i.e., 103 h for L1 and 102 h for L2.

### 4 Discussion

According to the study by Rezende et al. [14], when silages are exposed to air, considerable changes in their chemical composition (significant increase in pH) occur and their temperature increases considerably during exposure to oxygen.
Many strains of *Lactobacillus* can be used to improve the silage aerobic stability of maize. In our experiment, the effect of a commercially available preparation was compared with a preparation containing heterofermentative strains, namely, *L. buchneri*, *L. dioliovorans* and *L. reuteri*. Zielińska et al. [15] and Muck et al. [16] described the synergistic effects of the combination of various *Lactobacillus* strains and their improvement of silage stability. It seems very important that these strains can metabolise 1,2-propanediol into propionic acid and 1-propanol. According to the scientific reports, *L. buchneri* [17], *L. dioliovorans* [18] and *L. reuteri* [10,19,20] exhibit these properties.

Driehuis et al. [21] and Jungbluth et al. [22] observed that *L. buchneri* strains increased acetic acid and 1,2-propanediol concentrations and decreased lactic acid content in silages. Our research findings were similar. However, it is noteworthy that too high concentrations of acetic acid may affect the silage taste.

Oliveira et al. [23] observed that when one strain or a mixture of *Lactobacillus* strains were applied to silages, they reduced pH values and WSC concentrations. The same observations were made in our research. However, contrary to the results of our research, Oliveira et al. also found a lower concentration of acetic acid in samples with *Lactobacillus* strains. Similar results were recorded when the concentration of lactic acid in the inoculated samples was greater than in the control samples. At the same time, these authors concluded that the observed effects depended on the type of plant ensiled.

When the aerobic stability of silages was checked, the concentration of acetic acid was found increased in inoculated samples (concentration of bacteria in 1 g of preparation was $10^9$ cfu g$^{-1}$). Basso et al. [24] noted similar results but at a concentration of $5 \times 10^5$ cfu g$^{-1}$. According to the study by Ranjit and Kung [25], acetate production by *Lactobacillus* can be continued during exposure to oxygen. Acetic acid concentration tended to increase, whereas the content of lactic acid was found to decrease in inoculated silage samples subjected to aerobic incubation. In consequence, pH decreased because acetic acid exhibited higher pK$_a$ values than lactic acid [9]. Inoculants used in our research did not affect changes in the CP content. Silva et al. [26] used *L. buchneri* strains and noted an increase in the CP level compared with that in the control sample. Similar results were reported by Bumbieris et al. [27], who observed that the CP content in inoculated samples (7.47%) was greater than in the control samples (6.87%). *Lactobacillus* strains used in our study reduced yeast and mould counts. The L2 preparation exhibited a stronger fungistatic effect. It is noteworthy that the production of substances inhibiting fungal growth, including acetic and propionic acid, may largely depend on the phase of their growth as well as temperature, chemical composition and pH of the substrate [28].

![Figure 1: Variation in the temperature of maize silages during the aerobic stability test.](image-url)
5 Conclusions

The performed research showed that silages inoculated with Lactobacillus strains revealed better aerobic stability than control samples due to higher acetic and propionic acid concentrations, which reduced pH as well as yeast and mould counts. At the same time, silages inoculated with heterofermentative strains of Lactobacillus had a higher content of 1,2-propanediol and 1-propanol. This fact may indicate that mixtures of these bacterial strains are excellent inoculants that improve the aerobic stability of silage.

Conflict of interest: The authors state no conflict of interest.

References

[1] Kleinenschmit DH, Kung Jr L. Ameta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. J Dairy Sci. 2006a;89:4005–13.

[2] Dehghani MR, Weisbjerg MR, Hvelplund T, Kristensen NB. Effect of enzyme addition to forage at ensiling on silage chemical composition and NDF degradation characteristics. Livest Sci. 2012;150:51–58.

[3] Dorszewski P, Grabowicz M. The mycological status of green forages and silages from a mixture of legumes with grasses and whole crop maize. Folia Pomer Univ Technol Stetin Agric Aliment Pisc Zootech. 2017;33:33–40.

[4] Filya I. The effect of Lactobacillus buchneri, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. J Appl Microbiol. 2010;95:1080–6.

[5] Kleinischmit DH, Kung Jr L. The effects of Lactobacillus buchneri 40788 and Pediococcus pentosaceus R1094 on the fermentation of corn silage. J Dairy Sci. 2006b;89:3999–4004.

[6] Silva TH, Takiya CS, Vendramini THA, de Jesus EF, Zanferari F, Rennó FP. Effects of dietary fibrolytic enzymes on chewing time, ruminal fermentation, and performance of mid-lactating dairy cows. Anim Feed Sci Tech. 2016;221:35–43.

[7] Moral G, Daniel JLP, Kleinischmit C, Carvalho CPA, Fernandes J, Nussio LG. Additives for grain silages: a review. Slovak J Anim Sci. 2017;50:42–54.

[8] Queiroz OCM, Arriola KG, Daniel JLP, Adesogan AT. Effects of 8 chemical and bacterial additives on the quality of corn silage. J Dairy Sci. 2013;96:5836–43.

[9] Choińska R, Giryń H, Bartosiak E, Kotyrba D. Effect of dry mass and bacterial silage inoculant on the content of organic acids in silages. Postepy Nauki i Technologii Przemysłu Rolno-Spożywczego. 2013;68:35–14.

[10] Toraya T. The structure and mechanism of action coenzyme B12 dependent diol dehydratase. J Mol Catal B Enzym. 2011;10:87–106.

[11] Zielinska K, Fabiszewска A, Swiatek M, Szymanowska-Pawlowska D. Evaluation of the ability to metabolize 1,2-propanediol by heterofermentative bacteria of the genus Lactobacillus. Electron J Biotechnol. 2017;26:60–63.

[12] AOAC. International. Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International. 21st ed. Arlington, VA, USA; 2019.

[13] SAS, User’s Guide: Statistics, Version 9.1 ed. SAS Inst. Inc., Cary, NC, USA; 2002.

[14] Rezende AV, Rabelo CHS, Rabelo FHS, Nogueira DA, Faria Jr DCNA, Barbosa LA. Perdas fermentativas e estabilidade aeróbica de silagens de cana-de-açúcar tratadas com cal virgem e cloreto de sódio. R Bras Zootec. 2011;40:739–46.

[15] Zielinska K, Miecznikowski A, Stecka K, Stefańska I, Fabiszewska A, Kupryš-Caruk M, et al. Badania nad poprawą aktywności biologicznej preparatów bakterijnych do kiszenia roślin wysokoskrobionych. Sprawozdanie z realizacji tematu BST o symbolu: 500-01-ZF-05. Poland: IBPRS w Warszawie; 2015.

[16] Muck RE, Nadeau EMG, McAllister TA, Contreras-Govea FE, Santos MC, Kung Jr L. Silage review: recent advances and future uses of silage additives. J Dairy Sci. 2017;101:3980–4000. doi: 10.3168/jds.2017-13839.

[17] Zielinska K, Fabiszewska A, Stecka K, Swiatek M. A new strain of Lactobacillus buchneri A, composition, a multi-component preparation for starch-rich plant preservation, their use and a method for plant preservation. EP 2 785826, 2014.

[18] Charley R, Kung Jr. Treatment of silage with Lactobacillus diiovorans. US 2005/0281917 A1, 2005.

[19] Hammers WP, Hertel C. Genus Lactobacillus beijerinck 1901, 212AL. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K, Whitman WB, editors. Bergey’s Manual of Systematic Bacteriology. Vol. 3, 2nd edn. New York, NY: Springer; 2009. pp. 465–90.

[20] Sun Z, Yu J, Dan T, Zhang W, Zhang H. Phylogenesis and Evolution of lactic acid bacteria. In: Zhang H, Cai Y, editors. Lactic Acid Bacteria, Fundamentals and Practice. New York, NY: Springer Science+Business Media; 2014. p. 1–101.

[21] Drieuhaus F, Oude Elferink SJ, van Wikselaar PG. Fermentation Characteristics and aerobic stability of grass silage inoculated with Lactobacillus buchneri, with or without homofermentative lactic acid bacteria. Grass Forage Sci. 2001;56:330–43.

[22] Jungbluth KH, Trimborn M, Maack GK, Büscher W, Li M, Cheng H, et al. Effects of three different additives and two different bulk densities on maize silage characteristics, temperature profiles, CO2 and O2-dynamics in small scale silos during aerobic exposure. Appl Sci. 2017;7:5545. doi: 10.3390/app7080545.

[23] Oliveira AS, Weinberg ZG, Ogundade IM, Cervantes AAP, Arriola KG, Jiang Y, et al. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. J Dairy Sci. 2017;100:4587–603.

[24] Basso FC, Bernardes TF, Toledo AP, Roth P, Lodo BN, Berchielli TT, et al. Fermentation and aerobic stability of corn silage inoculated with Lactobacillus buchneri. R Bras Zootec. 2012;41:1789–94.
[25] Ranjit NK, Kung Jr L. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and stability of corn silage. J Dairy Sci. 2000;83:526–35.

[26] Silva NC, Santos JP, Ávila CLS, Evangelista AR, Casagrande DR, Bernardes TF. Evaluation of the effects of two *Lactobacillus buchneri* strains and sodium benzoate on the characteristics of corn silage in a hot-climate environment. Grassl Sci. 2014;60:169–77.

[27] Bumbieris Jr VH, Guimarães VAP, Fortaleza APS, Massaro Jr FL, Moraes GJ, Meza DAR. Aerobic stability in corn silage (*Zea mays* L.) ensiled with different microbial additives. Acta Sci Anim Sci. 2017;39:357–62.

[28] Fabiszewska AU, Zielińska KJ, Wróbel B. Trends in designing microbial silage quality by biotechnological methods using lactic acid bacteria inoculants: a minireview. World J Microb Biot. 2019;35:76. doi: 10.1007/s11274-019-2649-2.