doi: 10.26114/pja.iung.459.2021.45.03

Polish Journal of Agronomy
2021, 45, 21–27

The effect of inoculation with *Lactobacillus* spp. on the production of 1,2-propanediol and 1-propanol, hygienic quality and aerobic stability of maize silage

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Abstract. The aim of the study was to determine the influence of a commercial bacterial inoculant PK (*Lactobacillus plantarum* K KKP/593/p, *L. plantarum* C KKP/788/p, *L. brevis* KKP 839, *L. buchneri* KKP 907) and a ML preparation (*L. buchneri* ATCC 4005, *L. diolivorans* LGM 19667, *L. reuteri* ATCC 23272) on the concentration of 1,2-propanediol and short-chain fatty acids (acetic acid, propionic acid) on maize silage aerobic stability prolongation. The silage was prepared in laboratory microsilos with a capacity of 10 dm^3. The basic composition of the feed was determined according to the AOAC. The measure of oxygen stability was the time needed to raise the silage temperature by ≥2 ˚C about the ambient temperature.

The research showed that during 120-day ensilage, the applied preparations significantly ($p<0.05$) reduced the content of dry matter, water-soluble sugars and pH. The concentration of lactic acid, acetic acid and propionic acid in the inoculated samples increased significantly. 1,2-propanediol and 1-propanol were not found in control silages. The addition of PK and ML significantly increased the concentration of these substances. The number of lactic acid bacteria in silage with biological additives was significantly higher, and the number of yeasts and mould fungi was lower compared to the control. The applied mixtures of PK and ML significantly extended the time of aerobic stability of silages.

Keywords: bacterial preparations, silage, *Lactobacillus* spp.

INTRODUCTION

Lactobacillus bacteria constitute the largest group of lactic acid bacteria. They can be utilised as the main microbiological modifier to improve the chemical composition of silage (Kleinschmit, Kung Jr., 2006a). The addition of these bacteria may result in changes in the dry matter content (Dehghani et al., 2012) and improve the hygienic quality of silage, the indicator of which is the content of bacteria, moulds and yeast (Dorszewski, Grabowicz, 2017). It should be noted that acetic acid produced by heterofermentative lactic acid bacteria might affect yeast metabolism and improve the aerobic stability of silage. A disadvantageous process is the ineffective metabolism of lactic acid by heterofermentative *Lactobacillus* strains, which consume a large amount of energy during the process. This is associated with greater nutrient loss in silage (Filya, 2010). Thus, it seems reasonable to use mixtures of heterofermentative strains as well as lactic homofermentation in appropriate combinations adapted to the ensiled plant material (Kleinschmit, Kung Jr., 2006b). An alternative may be enzymatic formulations; however, their cost is higher and preparation methods are more cumbersome (Silva et al., 2016). Silage additives can be divided into different groups: (i) "Homofermentative", (ii) "Hetero" (heterofermentative bacteria), (iii) "Mixed" (homofermentative and heterofermentative bacteria), and (iiii) "Chemical" (chemical preservatives) (Morais et al., 2017). In the initial stage of ensiling, during the fermentation of water-soluble sugars (mainly into lactic acid), lactic homofermentative bacteria quickly acidify the environment, thus inhibiting the development of undesirable microorganisms, predominantly bacteria (e.g. *Clostridium* spp. responsible for butyric acid fermentation). In contrast, during the metabolism of carbohydrates, in addition to lactic acid, lactic heterofermentative bacteria produce also short-chain fatty acids, such as acetic and propionic acids, which inhibit the growth of fungi responsible for spoilage of silage, particularly after opening a pile. Commercially available preparations contain one or more types of lactic acid bacteria (Queiroz et al., 2013); however, the choice of preparations for utilisation in agricultural practice remains a matter of debate.

Numerous studies conducted over the years on the use of selected strains of lactic acid bacteria, mainly *L. buchneri*, demonstrate the beneficial effects of this heterofer-

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mentative strain on improving the aerobic stability of silage (Selwet, 2020). Additionally, the *L. buchneri* strain is characterised by the ability to anaerobically degrade lactic acid into acetic acid and 1,2-propanediol. It is believed that 1,2-propanediol is an intermediate metabolite, which is degraded to 1-propanol and propionic acid by *L. diolivorans* bacteria (Choińska et al., 2013). Both acetic and propionic acids have i.a. antifungal activity. Moreover, *L. reuteri* bacteria are able to synthesise cobalamin (vitamin B_{12}), which is a coenzyme for propanediol dehydratase, an enzyme that converts 1,2-propanediol to 1-propanol and propionic acid (Toraya, 2011). The desired metabolites, such as acetic acid, propionic acid and 1,2-propanediol, are synthesised as a result of co-fermentation of bacteria belonging to the above species. Hence, research results obtained by many authors may contribute to obtaining optimal mixtures of lactic acid bacteria strains, which could improve the aerobic stability of silage (Zielińska et al., 2017a).

The aim of the conducted research was to compare the effects of a commercial bacterial preparation containing four strains of the genus *Lactobacillus* and a non-commercial mixture of three bacterial cultures of the genus *Lactobacillus* on the increase in the concentration of 1,2-propanediol and 1-propanol, chemical composition and population of lactic acid bacteria, yeast and moulds. An important parameter was the determination of the synergistic effects of mixtures of bacteria of the *Lactobacillus* genus on extending the aerobic stability of maize silage intended for animal feeding or energy purposes.

MATERIALS AND METHODS

Ensiled plant material

Maize (*Zea mays* L.) of the Kresowiak cultivar (FAO 240) obtained from the Plant Breeding Smolice was ensiled. Type of use: medium-early three way cross hybrid predominantly intended for silage and CCM, semi dent grain type, stay green agronomic profile. Functional characteristics: high yields of total dry matter and cob dry matter, high smut tolerance, height: 270 cm. Planting density: 90 000 ha^{-1}. Harvest time during full milk maturity of kernels containing approximately 40% of dry matter (BBCH 75). The maize was cut at the height of 40 cm. Prior to ensiling, it was ground into chaff with a length of 3 cm. Maize was grown in monoculture.

Preparations used in experiment

PK – commercial preparation contained the cultures of *L. plantarum* K KKP /593/p, *L. plantarum* C KKP /788/ p, *L. brevis* KKP 839 and *L. buchneri* KKP 907. The dose recommended by the producer was 5 g t^{-1} of the ensiled material. The concentration of bacteria in the preparation was 10^{10} cfu g^{-1} (Lactosil, Polsil).

ML – mixture of 3 cultures: *L. buchneri* ATCC 4005, *L. diolivorans* LGM 19667, *L. reuteri* ATCC 23272 (DSMZ). A dose of 4 g t^{-1} of the ensiled material was used. The concentration of bacteria in the mixture was 10^{10} cfu g^{-1}.

The silage method and determination of aerobic stability

The silage was prepared in laboratory microsiloses with a capacity of 10 dm^3. The microsiloses were made of PVC and contained a closure enabling the discharge of gaseous products (the number of repetitions for each combination was 20). Preparations were added as aqueous suspension (water in control treatments). The average temperature during ensilage was 18 ˚C ± 1 ˚C.

During the aerobic stability test, samples were aerated for 180 h at a temperature of 18 ˚C ± 1 ˚C. Moist samples weighing 85 g were removed from the microsiloses after 120 days of silage and placed in plastic containers with a volume of 500 cm^3. The containers exhibited holes with a diameter of 5 mm, which enabled air circulation. Temperature was measured every 5 min at 2 h intervals using a temperature reader (Hotmux DDC Corporation, Pennsauken, NJ, USA). Stability was defined as the time needed to raise the silage temperature by ≥2 ˚C in relation to the ambient temperature. Each combination was performed in 5 replications. After 180 h the changes in the number of microorganisms and selected chemical parameters of the silage were examined.

Microbiological and chemical analyses

The number of lactic acid bacteria was determined on the MRS agar (OXOID) in accordance with PN-EN 15787:2009. Yeast was grown on the YPD agar (SIGMA), while moulds were determined on the OGYE agar (OXOID) with the addition of oxytetracycline (oxytetracycline-glucose-yeast-extract agar). Incubation time of 5–7 days at 24 ˚C.

The concentration of lactic acid, acetic acid, propionic acid, ethanol, 1-propanol and 1,2-propanediol was determined using a gas chromatograph equipped with an FID detector, a 2 m long glass column (80/100 Chromosorb WAW) (SUPELCO), I.D. 2 mm packed with GP 10% SP - 1200/1%H_3PO_4 and a Varian 8200 CX autosampler. The carrier gas was hydrogen (flow rate of 30 cm^3 min^{-1}). The furnace temperature was 120 ˚C, the injection temperature was 250 ˚C, while the detector temperature was 300 ˚C. Fluka acid standards were used for comparison.

The basic composition of the feed was determined according to AOAC (2019). The pH values were established using the Hann Instruments pH meter in a suspension prepared from 10 g of silage and 190 cm^3 of distilled water. The suspension was homogenised for 20 min.

Statistical analysis

Statistical calculations were performed using the GLM SAS (2002) package of procedures. The differences between the means were evaluated using the Tukey's test at the significance level of $\alpha = 0.05$.

RESULTS

The obtained results are a continuation of research carried out in previous years, which concerned the effect of bacteria of the *Lactobacillus* genus on the increase in the concentration of 1,2-propanediol and 1-propanol as well as the extension of the aerobic stability of maize silage of the SAN (FAO 240) cultivar from the Plant Breeding Smolice (Selwet, 2020). The chemical composition and populations of lactic acid bacteria, yeasts and moulds in maize green forage intended for ensilage are summarised in Table 1. Table 2 presents the chemical compositions and populations of microorganisms after 120 days of ensilage. In the silage treated with PK and ML bacterial inoculants, a significantly ($p<0.05$) lower concentration of dry matter and water-soluble sugars was found compared to the control samples. Silage with the addition of *Lactobacillus* strains (PK, ML) exhibited lower pH values. The concentration of lactic, acetic and propionic acids was considerably higher in samples with microbiological modifiers (PK, ML). No significant effect of inoculation (PK, ML) on the content of ethanol and crude protein in the tested silage was determined. The employed mixtures of *Lactobacillus* (PK, ML) bacteria strains resulted in a significant increase in the number of lactic acid bacteria and a reduction in the populations of yeast and moulds compared to the control. In control silage, no traces of 1,2-propanediol and 1-propanol were found. The PK and ML additives considerably increased the concentrations of these substances. Compared to the combination with PK, in the combination with ML, the content of 1,2-propanediol and 1-propanol was 160% and 261% higher, respectively.

Table 1. The chemical composition and count of microorganisms in maize green forage before ensilage.

Dry matter [g kg^{-1}]	403
pH	5.61
Crude protein [g kg^{-1}]	93
Water soluble carbohydrates [g kg^{-1}]	75
Lactic acid bacteria [log cfu g^{-1}]	6.11
Yeast [log cfu g^{-1}]	7.10
Mould [log cfu g^{-1}]	6.00

Changes that occurred in silage exposed to oxygen for a period of 180 h are presented in Table 3. A significant effect of inoculation with the PK and LM preparations on the reduction of the pH increase in the investigated silage was noted. The concentration of acetic acid and propionic acid in the samples with the addition of *Lactobacillus* strains was significantly higher than in the control sample, while in ML treatments the concentration of lactic acid was significantly lower. Compared to the control sample, the number of lactic acid bacteria in silage containing biological additives was significantly higher, whereas the populations of yeasts and moulds were reduced. No traces of 1,2-propanediol and 1-propanol were found in the control

Table 2. The effect of inoculation with different *Lactobacillus* strains on the quality, chemical composition and count of microorganisms in silage.

Parameters	Treatments					
	control	SD	PK	SD	ML	SD
Dry matter [g kg^{-1}]	396 a	3.6	369 b	2.5	373 b	3.1
pH	4.95 a	0.2	4.00 b	0.1	4.00 b	0.1
Crude protein [g kg^{-1} dry matter]	92.7 a	1.9	92.9 a	1.3	92.8 a	1.3
Water soluble carbohydrates [g kg^{-1} dry matter]	42.5 a	9.7	37.01 b	6.4	31.92 b	5.1
Lactic acid [% dry matter]	5.02 b	1.2	6.91 a	1.1	7.01 a	0.9
Acetic acid [% dry matter]	1.12 b	0.2	2.72 a	0.1	3.19 a	0.2
Propionic acid [% dry matter]	0.00 c	…	1.10 b	0.1	1.51 a	0.1
1,2-propanediol [% dry matter]	0.00 c	…	0.62 b	0.1	1.61 a	0.2
1-propanol [% dry matter]	0.00 c	…	0.23 b	0.01	0.83 a	0.01
Ethanol [% dry matter]	0.89 a	0.1	0.81 a	0.01	0.87 a	0.01
Lactic acid bacteria [log cfu g^{-1}]	6.92 a	1.6	8.42 b	0.5	8.71 b	0.6
Yeast [log cfu g^{-1}]	5.62 a	1.4	4.17 b	0.9	4.01 b	0.7
Mould [log cfu g^{-1}]	5.27 a	0.9	5.20 a	0.5	4.62 b	0.4

Means marked with different letters in a row are different at $p<0.05$. SD – standard deviation

PK – *Lactobacillus plantarum* K KKP/593/p, *L. plantarum* C KKP/788/p, *L. brevis* KKP 839, *L. buchneri* KKP 907); ML – *L. buchneri* ATCC 4005, *L. diolivorans* LGM 19667, *L. reuteri* ATCC 23272)

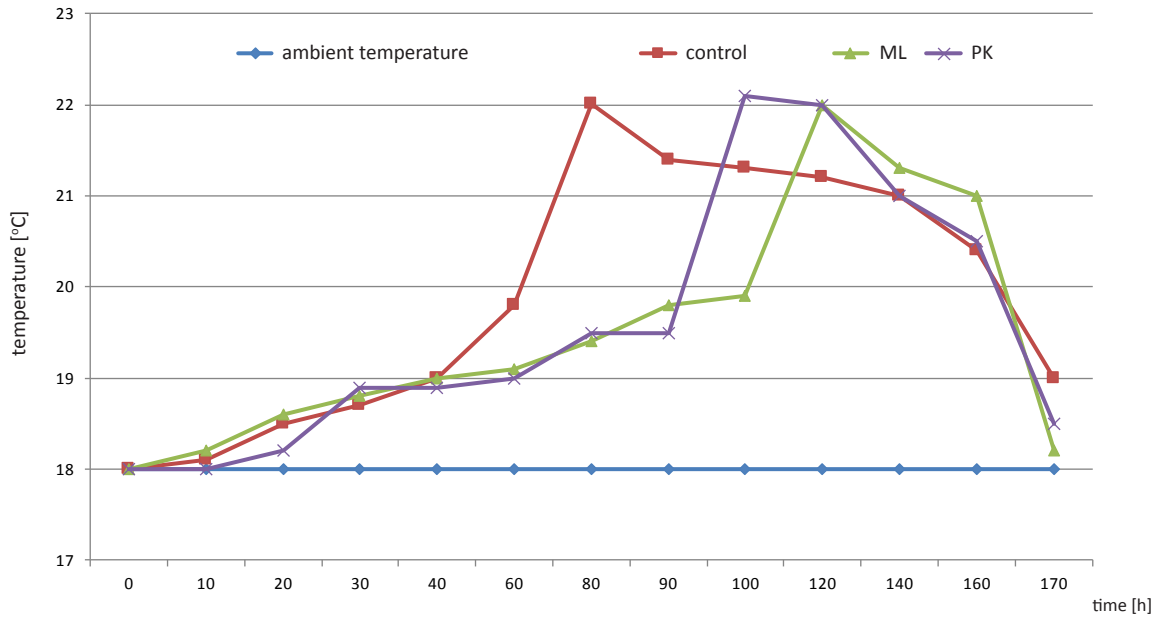
samples. The determined content of 1,2-propanediol and 1-propanol in the combination containing PK and ML was at the same level as in the silage before the aerobic stability test.

The results of the temperature measurements obtained during the evaluation of the aerobic stability of silage are shown in Fig. 1. The utilised PK and ML additives had a significant effect on extending the period of aerobic stability. In control samples, the silage temperature increased by 2 °C within 62 h. Inoculated silage was characterised by a longer stability period, which for PK was 92 h, while for ML, 100 h.

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

Parameters	Treatments					
	control	SD	PK	SD	ML	SD
pH	6.10 a	0.2	4.99 b	0.2	4.81 b	0.1
Lactic acid [% dry matter]	4.99 a	0.6	5.11 a	0.8	4.37 b	0.8
Acetic acid [% dry matter]	1.71 c	0.4	3.61 b	0.6	5.01 a	0.9
Propionic acid [% dry matter]	0.00 c	…	0.78 b	0.89	1.01 a	0.93
1,2-propanediol [% dry matter]	0.00 c	…	0.59 b	0.2	1.62 a	0.4
1-propanol [% dry matter]	0.00 c	…	0.20 b	0.1	0.80 a	0.13
Lactic acid bacteria [log cfu g^{-1}]	6.31 b	0.12	8.21 a	0.13	8.29 a	0.11
Yeast [log cfu g^{-1}]	8.31 a	0.2	7.20 b	0.15	6.18 c	0.13
Mould [log cfu g^{-1}]	7.51 a	0.34	6.21 b	0.25	5.07 c	0.14

Means marked with different letters in a row are different at $p<0.05$.
SD – standard deviation
PK – *Lactobacillus plantarum* K KKP/593/p, *L. plantarum* C KKP/788/p, *L. brevis* KKP 839, *L. buchneri* KKP 907); ML – *L. buchneri* ATCC 4005, *L. diolivorans* LGM 19667, *L. reuteri* ATCC 23272



PK – *Lactobacillus plantarum* K KKP/593/p, *L. plantarum* C KKP/788/p, *L. brevis* KKP 839, *L. buchneri* KKP 907;
ML – *L. buchneri* ATCC 4005, *L. diolivorans* LGM 19667, *L. reuteri* ATCC 23272

Figure 1. Variation in the temperature of silages during the aerobic stability test.

DISCUSSION

To enhance the aerobic stability of maize silage, numerous strains of bacteria of the *Lactobacillus* genus can be used. In this experiment, the activity of a commercially available preparation (PK) was compared with that of a preparation containing the described heterofermentative strains of *L. diolivorans*, *L. buchneri* and *L. reuteri* (ML). The synergistic effects of the combinations of various strains of *Lactobacillus* bacteria on the improvement of silage stability were described in the works by Zielińska et al. (2015) and Muck et al. (2017).

According to Rezende et al. (2011), silage subjected to oxygen exposure underwent significant changes with respect to its chemical composition and exhibited a higher heat-up rate.

What seems very important is the ability of bacteria of the *Lactobacillus* genus to synthesise 1,2-propanediol. As previously reported in the literature, such properties are characteristic of the new *L. buchneri* KPP 907 p strain (Zielińska et al., 2014; Zielińska et al., 2017b). The occurrence of strains capable of decomposing 1,2-propanediol, such as *L. diolivorans* sp. nov. (Charley, Kung Jr, 2005), as well as of the synthesis of vitamin B_{12} (e.g. *L. reuteri*) was also noted in maize silage (Hammes, Hertel, 2009; Toraya, 2011; Sun et al., 2014). While studying the aerobic stability of maize silage after inoculation with *L. buchneri*, *L. plantarum* and *L. rhamnosus* strains, Driehuis et al. (2001) and Jungbluth et al. (2017) noted an increase in the concentration of acetic acid and 1,2-propanediol as well as a decrease in the content of lactic acid in silage. These results have been confirmed in own research. However, it should be noted that excessively high acetic acid concentrations might negatively affect the taste properties of silage and decrease the feed intake by animals.

Oliveira et al. (2017) noted that the treatment of maize silage with one strain or a mixture of *L. plantarum, L. rhamnosus* and *Pediococcus pentosaceus* strains decreased the pH and concentration of water-soluble sugars, which has also been confirmed by own research. The above authors also determined a reduction ($p<0.01$) in the level of acetic acid in samples with the addition of strains of these bacteria. Nonetheless, these results have not been confirmed by own research. Dissimilar results were obtained for the increase in the lactic acid concentration in the inoculated samples compared to control samples. Concurrently, the above-mentioned authors indicated that such inoculation effects depended on the type and developmental stage (BBCH) of the ensiled plant.

During the silage aerobic stability test carried out as part of our own research, an increase in the concentration of acetic acid in the samples with microbiological additives was noted (the number of bacteria in 1 g of the preparation was 10^{10} cfu g^{-1}). Similar results were obtained by Basso et al. (2012) in the case of maize silage; however, with the number of *L. buchneri* 40788 bacteria of approximately $5x10^5$ cfu g^{-1}. According to Ranjit and Kung Jr (2000), the production of acetate by *Lactobacillus* spp. continued under aerobic exposure. In such silage, an upward trend in the concentration of acetic acid and a decrease in the content of lactic acid in inoculated samples were observed. Concurrently, a decrease in the pH was noted because acetic acid exhibits higher pKa values compared to lactic acid (Choińska et al., 2013). The microbiological modifiers used in own research did not change the crude protein content. On the other hand, following the application of two *L. buchneri* strains in maize silage, Silva et al. (2014) noted an increase in the level of crude protein compared to control. Similar results can be found in the study by Bumbieris et al. (2017), who noted higher crude protein content in maize silage samples with the addition of *L. buchneri* CCT 3746 (7.47%) compared to control samples (6.87%). The utilised strains of bacteria of the *Lactobacillus* genus reduced the number of yeasts and moulds. The ML preparation exhibited a stronger fungistatic effect. It should be noted, however, that the production of fungistatic substances, including acetic acid and propionic acid, might largely depend on the fungal growth phase, substrate temperature, chemical composition and pH (Fabiszewska et al., 2019).

CONCLUSIONS

1. The results of the study show that silage samples inoculated with strains of bacteria of the *Lactobacillus* genus were characterised by better aerobic stability compared to the control samples.
2. The use of a mixture of ML strains (*L. buchneri* ATCC 4005, *L. diolivorans* LGM 19667, *L. reuteri* ATCC 23272) resulted in a better stabilising effect compared to the control samples and the commercial PK preparation (*L. plantarum* K KKP/593/p, *L. plantarum* C KKP/788/p, *L. brevis* KKP 839, *L. buchneri* KKP 907). These silage samples contained higher concentrations of acetic and propionic acids and exhibited a lower pH than in control treatments and smaller numbers of yeasts and moulds.
3. Silage samples with the addition of heterofermentative strains of the *Lactobacillus* genus (ML) contained higher concentrations of 1,2-propanediol and 1-propanol in relation to the control samples as well as those treated with the PK preparation, which could suggest that mixtures of these strains played the role of excellent bacterial inoculants that improved the aerobic stability of silage.
4. Silage samples with the addition of three selected strains of bacteria of the *Lactobacillus* genus proved to display the longest aerobic stability period.

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received – 19 February 2021
revised – 19 April 2021
accepted – 10 May 2021