

APPLICATION OF SOME LACTIC ACID BACTERIA STRAINS TO IMPROVE FERMENTATION AND AEROBIC STABILITY OF MAIZE SILAGE**JUDIT PETER SZUCS, AGNES SULI, TIMEA SULI ZAKAR, ELIZABET BERE CZ, MATE PEK**

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ABSTRACT

The object of the trial was to study the effect of some lactic acid bacteria strains on the fermentation and aerobic stability of whole plant maize silages.

The whole plant maize raw material was 32% DM, in soft cheddar stage of grain ripeness. It was ensiled in 4.2 litre capacity glass micro-size silos in 5 replicates /each treatment and stored on constant air conditioned room temperature (22 °C) during 95 days. The average packing density of raw material was 211 kg DM/m³.

The applied treatments: 1. Untreated control maize, 2. *Enterococcus faecium* 100,000 CFU/g fresh maize (FM), 3. *Lactobacillus plantarum* 50,000 CFU/g FM + *Enterococcus faecium* 50,000 CFU/g FM, 4. *Lactococcus lactis* 100,000 CFU/g FM, 5. *Lactobacillus plantarum* 50,000 CFU/g FM + *Lactococcus lactis* 50,000 CFU/g FM, 6. *Lactobacillus plantarum* 100,000 CFU/g FM.

Aerobic stability study: Applied Honig (1990 system).

The main experiences are the following: Applied lactic acid bacteria strains improved the quality of fermentation of maize in general compare to untreated control one.

Lactic acid bacteria strains significantly stimulated lactic acid production and decreased propionic and butyric acid production. The origin of ammonia decreased also under influence of lactic acid bacteria strains in anaerobic conditions.

Enterococcus faecium and *Lactococcus lactis* are not able to protect the maize silages against the aerobic deterioration with the applied dosage.

Lactobacillus plantarum itself produced the most favourable fermentation characteristics and protected the aerobic stability of silage the most effectively (during 4 day) compare to all other treated maize silages

Keywords: aerobic stability, fermentation, lactic acid bacteria strains, *Lactobacillus plantarum*, maize silage

INTRODUCTION

Bacterial inoculants are expected to ensure a more efficient fermentation phase as well as reduce the risk of aerobic deterioration when silages are exposed to air. Bacterial inoculants can improve fermentation characteristics first of all by speeding up the fall of pH and lowering ammonia levels because of reducing proteolysis into the silo. Various inoculant strains have been found to produce bacteriocins and other compounds that inhibit other bacteria and fungi improving their success (MUCK, 2013). Bacterial inoculants containing homofermentative lactic acid bacteria (LAB), in most of the cases *Lactobacillus plantarum*, secondly *Enterococcus faecium*, *Lactococcus lactis* are often added to silage because they very quickly produce large quantities of lactic acid, which lowers the pH of the silage (LI AND NISHINO, 2011; MARCIŃÁKOVÁ ET AL., 2008).

However, some bacterial inoculants often have no effect or can even make the aerobic stability of silages worse (LIN ET AL., 1992; PAHLOW AND HONIG, 1994; MUCK, 2013) because of the reduction of acetic acid (LINDGREN ET AL., 1990; McDONALD ET AL., 1991; RUSER AND KLEIMAN, 2005) and also because yeasts metabolize lactic acid to produce alcohol (MUCK, 2013).

Recently, the aerobic stability of different silage crops has been markedly improved through inoculation with a heterolactic bacterium eg. *Lactobacillus buchneri*, *Lactobacillus brevis*. Acetic acid produce from heterofermentative LAB inhibits the

proliferation of yeasts in silage (FILYA ET AL., 2002; RUSER AND KLEIMAN, 2005). Improvements in aerobic stability have been reported in corn silage (RANJIT AND KUNG, 2000; LI AND NISHINO, 2011, 2013).

Many additives have been developed to improve the ensiling process and nutritive value of silages. At present biological additives containing lactic acid bacteria strains are preferred because they are non-toxic, non-corrosive to machinery, do not present environmental hazards and are regarded as natural products, and therefore bacterial inoculants have been very popular, over the last 20 years (DAVIES, 2010). However their application cannot replace professional silage management.

MATERIAL AND METHOD

Experimental ensilage procedure

The applied treatments:

- T1. Untreated control maize FM (fresh weight)
- T2. *Enterococcus faecium* 100,000 CFU/g FM maize,
- T3. *Lactobacillus plantarum* 50,000 CFU/g FM
+ *Enterococcus faecium* 50,000 CFU/g FM
- T4. *Lactococcus lactis* 100,000 CFU/g FM
- T5. *Lactobacillus plantarum* 50,000 CFU/g FM
+ *Lactococcus lactis* 50,000 CFU/g FM
- T6. *Lactobacillus plantarum* 100,000 CFU/g FM maize

The inoculants were individually prepared for application by suspending the number of grams to be applied in distilled water and then evenly applying 2 ml of suspension of bacterias / kg of forage (fresh weight). The same amount of distilled water was applied to the untreated maize as well. The maturity of whole plant maize raw material was in soft cheddar stage of grain ripeness and the dry matter content was 32%. The harvested chopped maize was ensiled in 4.2 litre capacity air-tight sealed glass micro-size silos in 5 replicates /each treatment and stored on constant air-conditioned room temperature (22 °C) during 95 days. The average packing density was 211 kg DM/m³.

Chemical analysis

DM, pH, lactic-, acetic-, propionic- and butyric acids, ammonia-N and ethanol content of silages were analysed according to the internationally recognised methods.

Examination of aerobic stability

Determination of aerobic stability of silages was monitored by System Völkenrode (Honig, 1990) principle.

It is based on monitoring temperature rise due to increased microbial activity of samples exposed to air.

Procedure

For the determination minimum 100 g samples are weight into a tins. Temperature sensors are placed into the centre of the samples and the whole unit is insulated with an outer layer of polystyrene on the sides and at bottom and top. The temperature is registered hourly by a computer program. From the values of each day a mean value is calculated. The room temperature is taken as the reference temperature about 22 °C, and substrated from the silage temperatures. The measurement is continued for 7 days.

Evaluation

The time a silage is supposed to be stable is given till the registration unit shows a temperature rise of 3 °C above ambient temperature.

Statistical analysis

Full statistical analyses was using an internationally recognised statistical procedure.

We processed data by means of IBM PC computer with the aid of Microsoft Excel program. As method of mathematical statistics, we used the method of comparison of calculated mean values and significance.

The raw material was analysed before ensilage, and the fermentation products of maize silages were analysed on 95th day after ensiling.

RESULTS AND DISCUSSION

Raw material

The maturity of ensilaged silomaize was at the beginning of soft cheddar stage of grain ripeness. The chemical composition of these plant was similar to the standard average. DM content 32%, which showed easy ensiling of raw material. There was no significant difference between all treatments, neither control nor treated samples, so the further differences in any other parameters were not originated from different DM content.

1. Control silage

After 95 days of storage pH of maize silage was 3.70. Significantly less ($P = 0.1\%$) lactic acid (3.20%) more butyric acid (0.08) % was produced compare to the treated silages. The highest amount of NH_3 (37.4 mg % DM) was detected in control silage compare to different strains of lactic acid bacteria treated silages which was significant in case of T4 ($P = 5\%$), T5 and T6 silages ($P = 0.1\%$ respectively) see *Tables 1 and 2*.

The untreated control silage was stable until 3 and half day. The samples started to get warmer on 8th hours and achieved 3 °C warmer temperature compare to ambient on 86th hours (therefore lost stability). Pick temperature was 30 °C on 136th hours (*Table 3*).

2. Enterococcus faecium treated silage,

There were significant differences among pH and some fermentation products compare to the control silage:

There was higher pH 3.72 ($P = 5\%$) and lactic acid content 4.4% ($P = 0.1\%$) less acetic acid 1.41% (non significant), butyric acid 0.01% ($P = 1\%$) ethanol 1.8% (ns) and ammonia 34 mg % DM (ns).

The silage was stable less then 3 day only after opening the micro silos on aerobic condition. Getting warmer of silage started from 9th hour, and suddenly rised the temperature with 3 °C higher than the ambient (which indicated the lost of stability) on 71th hour (15 hours earlier than the control).

3. Lactobacillus plantarum + Enterococcus faecium treated silage

The 3.75 pH and 5.11% lactic acid content of silage is significantly higher ($P = 0.1\%$) to the control silage. Less acetic- and butyric acid, ethanol and ammonia content are not significant compare to control one.

Aerob stability was relatively better than the control.

The stability lasted until 86th hour exposure to air but got worm 3 hours later (11th hour) and the temperature of forages were rising slowly to pick 28 °C until 174th hour which was 38 hours later than the control.

4. *Lactococcus lactis* treated silage

3.77 pH and 5.12% lactic acid content are significantly higher ($P = 0.1\%$); the same acetic less butyric acid content and higher ethanol production are not significant, while the smaller 27 mg % DM ammonia level is $P = 5\%$ significant with control.

The deterioration of silage started in the 3rd hour while its stability lasted in the 79th hour (7 hours earlier than the control). Pick temperature (33.2 °C) was achieved in the 123th hour (13 hours earlier than control).

5. *Lactobacillus plantarum* + *Lactococcus lactis* treated silage

Higher pH (3.77) and lactic acid production (5.40%) and lower acetic acid production (1.26%) were significantly different ($P = 0.1\%$) with control. Also differed significantly the lower butyric acid production (0.006 %) in $P = 1\%$ level, and considerably high ethanol (3%) in $P = 5\%$ level. Ammonia production was only half (56%) of control, which is significant on $P = 0.1\%$ level.

Aerobic stability was kept only 89 hours (3 hours longer compare to control). Silage started to deteriorate from 11th hours (3 hours later) and 37.9 °C pick temperature was achieved on the 115th hours of aerobic stability test (21 hours later than the control silage).

6. *Lactobacillus plantarum* treated silage

Parameters of fermentation and their significance to the control are the following: higher pH (3.75) $P = 0.1\%$; higher lactic acid (5.19 DM %) $P = 0.1\%$ lower acetic acid (1.37 DM %) $P = 10\%$; No butyric acid $P = 1\%$, Half of ethanol content (0.91 DM %) $P = 5\%$; Half of ammonia content (18.1 mg % DM) $P = 0.1\%$ level of significance.

These fermentation characteristics are the best among all other silages, not only in comparison with control, but also with the other *Lactobacillus* bacterium treated silages as well.

The aerobic stability test showed the best results also:

Aerobic stability was kept until 95th hour (4 day) (9 hours longer than control). Silage deterioration started on 12th hour (4 hours later than the control).

The pick temperature 25.7 °C was the lowest, and recorded on 190th hour (54 hours later than control) which means low rising of temperature, slow deterioration of silage.

Table 1. Fermentation product of maize silages on DM basis

Parameters	Mean of treatments (n=5)					
	T1	T2	T3	T4	T5	T6
Dry matter %	31,15	31,92	31,34	31,67	32,22	32,04
Lactic acid %	3,17	4,45	5,10	5,11	5,40	5,18
Acetic acid %	1,46	1,42	1,38	1,47	1,26	1,37
Butyric acid %	0,08	0,03	0,06	0,04	0,01	0,00
Propionic acid %	0,24	0,08	0,18	0,05	0,08	0,13
Ethanol %	1,89	1,78	2,35	2,44	3,01	0,92
Ammonia mg % DM	37,40	33,80	31,80	26,9	20,90	18,00

Table 2. Statistical evaluation of some parameters of the fermentation on 95th day

Parameters	n	Treatment												t*	Level of signif.	Diff. of signif. (p%)
		T1 (Control)		T2		T3		T4		T5		T6				
		Mean	s	Mean	s	Mean	s	Mean	s	Mean	s	Mean	s			
pH	5	3,69	0,01	3,72	0,02	3,75	0,01	3,77	0,01	3,77	0,02	3,75	0,01	t ₁₋₂ =2,42 t ₁₋₃ =5,27 t ₁₋₄ =7,65 t ₁₋₅ =8,25 t ₁₋₆ =5,84	5% 0,1% 0,1% 0,1% 0,1%	0,020 0,040 0,037 0,037 0,037
Lactic acid DM%	5	3,17	0,36	4,44	0,33	5,11	0,55	5,12	0,45	5,40	0,47	5,19	0,45	t ₁₋₂ =4,61 t ₁₋₃ =6,63 t ₁₋₄ =7,06 t ₁₋₅ =8,07 t ₁₋₆ =7,30	0,1% 0,1% 0,1% 0,1% 0,1%	1,036 1,036 1,036 1,036 1,036
Acetic acid DM%	5	1,46	0,16	1,41	0,04	1,38	0,06	1,47	0,05	1,26	0,02	1,37	0,07	t ₁₋₂ =0,81 t ₁₋₃ =1,42 t ₁₋₄ =0,19 t ₁₋₅ =3,81 t ₁₋₆ =1,73	ns** ns ns 0,1% 10%	- - - 0,195 0,089
Butyric acid DM%	5	0,077	0,03	0,012	0,01	0,056	0,07	0,044	0,04	0,006	0,01	0,001	0,000	t ₁₋₂ =3,19 t ₁₋₃ =1,00 t ₁₋₄ =1,61 t ₁₋₅ =3,29 t ₁₋₆ =3,72	1% ns ns 1% 1%	0,057 - - 0,061 0,057
Ethanol DM%	5	1,89	0,49	1,78	0,77	2,35	0,19	2,44	0,33	3,01	0,75	0,91	1,29	t ₁₋₂ =0,23 t ₁₋₃ =0,92 t ₁₋₄ =1,15 t ₁₋₅ =2,37 t ₁₋₆ =2,08	ns ns ns 5% 5%	- - - 0,971 0,971
Ammonia mg%	5	37,4	4,74	34,02	5,85	31,78	1,46	26,94	3,26	20,9	5,00	18,10	10,95	t ₁₋₂ =0,89 t ₁₋₃ =1,38 t ₁₋₄ =2,72 t ₁₋₅ =4,29 t ₁₋₆ =5,01	ns ns 5% 0,1% 0,1%	- - 7,945 14,464 14,464

*Critical level of „t”: P_{10%}:1,71; P_{5%}:2,06; P_{1%}:2,8; P_{0,1%}:3,75

** ns : non significant

Table 3. Summary of aerobic stability test in fermentation study

Treatment	Get warm	Finish of aerob stability	1 st Pick temperature		2 nd Pick temperature	
	hour	hour	hour	°C	hour	°C
T1	8	86	136	29.9	--	--
T2	9	71	149	28.5	--	--
T3	11	86	174	28.0	--	--
T4	3	79	123	33.2	--	--
T5	11	89	95	37.9	182	38.1
T6	12	95	190	25.7	--	--

CONCLUSIONS

- Applied lactic acid bacteria strains improved the quality of fermentation of maize silages in general compare to untreated control one.
- Lactic acid bacteria strains significantly stimulated lactic acid production and decreased propionic and butyric acid production.
- The origin of ammonia decreased also under influence of lactic acid bacteria strains in anaerobic conditions.
- *Enterococcus faecium* and *Lactococcus lactis itself* are not able to improve the protection of the aerobic stability of maize silages with the applied dosage.
- *Lactobacillus plantarum* applied itself produced the most favourable fermentation characteristics of silage and protected the most effectively the aerobic stability of maize silage (during 4 day) compare to all other lactic acid bacteria strains.

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