

Inhibition of Clostridia growth by *Lactiplantibacillus plantarum* and *Lactococcus lactis*

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INTRODUCTION

A broad range of lactic acid bacteria were screened for their ability to inhibit clostridia growth. Two new isolated strains, *Lactiplantibacillus plantarum* (LP) DSM 34271 and *Lactococcus lactis* (LC) DSM 34262, showed a good effect against clostridia in agar diffusion tests. Based on these results, the two newly isolated lactic acid bacteria were inoculated in slightly wilted grass materials to investigate their effect on quality parameters in the ensiling process.

MATERIAL & METHODS

Agar Diffusion Test for Studying Clostridia Inhibition: *Clostridium tyrobutyricum* DSM 2637 spores were enclosed into RCM agar plates. Supernatants of more than 200 lactic acid bacteria (LAB) strain cultures were put into wells and incubated anaerobically at 37°C for 24 h. A clear zone shows the inhibition of clostridia [1].

Ensiling Material and Treatment: Grass material from three sources were slightly wilted (range of DM 27–36%). The chopped grass materials was ensiled as untreated control, treated with *L. plantarum* DSM 34271 or *L. lactis* DSM 34262. Lactic acid bacteria were sprayed onto the grass (2.5×10^5 cfu/g grass) and the laboratory silos were stored at 20°C. After 90 days of storage, the pH-value, fermentation patterns (HPLC) and Ammonia-N were analyzed from an aqueous extract of the silage.

RESULTS & DISCUSSION

Agar Diffusion Test for Studying Clostridia Inhibition:

The two selected lactic acid bacteria showed a clear inhibition zone (Figure 1). *L. lactis* DSM 34262 had a better effect against the test strain *C. tyrobutyricum* DSM 2637, compared to *L. plantarum* DSM 34271.

Silage Trials using slightly wilted grass material:

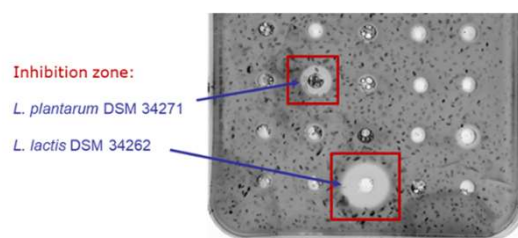
The dry matter of the grass ensiled reached from 27% to 36%. The mean pH-value in the untreated control was 4.72. Inoculated silages reached significantly lower pH values of 3.98 and 4.25, respectively. This indicates improved preservation in the treated grass. Corresponding to pH pattern, treated silage showed significantly higher contents of lactic acid of 7.65 and 5.98 g, compared to controls' 3.11 g per 100 g DM, respectively. Treated materials' lower concentrations of butyric acid and ammonia-N are consistent with pH and lactic acid pattern. Additionally, silage fermenting to higher lactic acid contents lost less weight, supposed by inhibiting spoilage microorganisms growth (Control: 2.88 g/100 g FM; Treatment: 1.36 and 1.84 g/100 g FM) (Table 1).

Table 1: Comparison of the parameters of mean values from three silage trials using wilted grass material after 90 days of storage.

Parameter	Unit	Mean Values of three Silage Trials / 90 d				
		Control	LP (DSM 34271)	p-value	LC (DSM 34262)	p-value
DM	g/100 g FM	31.0	32.1	0.159	31.5	0.451
pH – value		4.72	3.98*	<0.001	4.25*	0.013
Ammonia-N	g/100 g DM	0.30	0.15	0.064	0.22	0.192
Weigth losses	g/100 g FM	2.88	1.36*	0.004	1.84*	0.019
Lactic acid	g/100 g DM	3.11	7.65*	<0.001	5.98*	<0.001
Acetic acid	g/100 g DM	1.33	0.68	0.083	0.93	0.268
Butyric acid	g/100 g DM	0.56	0.05*	0.027	0.20	0.130

* Indicates mean values within a line are significantly different at $p < 0.05$ (Mann-Whitney-Test) in comparison to the control group.

Figure 1: Inhibition zones of investigated lactic acid bacteria



CONCLUSION

In a screening project two lactic acid bacteria strains inhibited Clostridia significantly. The two isolates, *L. plantarum* DSM 34271 and *L. lactis* DSM 34262, inhibit clearly the growth of the test strain *C. tyrobutyricum* DSM 2637 (Figure 1). Furthermore, the two selected lactic acid bacteria were tested in slightly wilted grass with high risk for clostridia contamination. The two strains were able to improve silage quality in the three independently conducted ensiling experiments (Table 1). The main effects were a significant decrease of pH with corresponding increased production of lactic acid. Additionally, a reduced activity of clostridia can be assumed to be indicated by lower amount of butyric acid formed.

References:

[1] Schoster, A., Kokotovic, B., Permin, A., Pedersen, P. D., Dal Bello, F., & Guardabassi, L. (2013). In vitro inhibition of Clostridium difficile and Clostridium perfringens by commercial probiotic strains. Anaerobe, 20, 36-41.