INHIBITION OF CLOSTRIDIA GROWTH BY *LACTIPLANTIBACILLUS PLANTARUM* AND *LACTOCOCCUS LACTIS*

Schein, H.[1], Buchebner-Jance, M.[1],
Töglhofer, M.[1], Weidenholzer, E.[1]

[1]Lactosan GmbH & Co.KG, Industriestraße West 5, 8605 Kapfenberg, Austria

ABSTRACT

Clostridia are undesirable microorganisms in silage, particularly in low dry matter forage, where their growth can impair silage quality and animal performance. This study screened more than 200 lactic acid bacteria for their ability to inhibit clostridia. Two newly isolated strains, *Lactiplantibacillus plantarum* DSM 34271 and Lactococcus lactis DSM 34262, showed clear inhibitory effects against *Clostridium tyrobutyricum* in agar diffusion assays. Based on these results, both strains were evaluated as silage inoculants in three slightly wilted grass materials. Compared with untreated controls, inoculated silages exhibited significantly lower pH values, increased lactic acid concentrations, reduced butyric acid formation and lower weight losses after 90 days of storage. These findings indicate improved fermentation quality and suggest effective suppression of clostridial activity by the selected strains.

Keywords: *Lactiplantibacillus plantarum*, *Lactococcus lactis*, clostridia inhibition, silage

INTRODUCTION

Clostridia are spore-forming, anaerobic gram-positive bacteria that ferment carbohydrates, lactic acid, as well as amino acids and proteins. When clostridia drive the fermentation process, they lead to a reduction in feeding value. For feeding purposes it is very important to keep counts of clostridia spores at a low level to avoid health problems and impaired animal performance.

In a screening project, a broad range of lactic acid bacteria were evaluated for their potential to inhibit clostridial growth. Two newly isolated strains, *Lactiplantibacillus plantarum* (LP) DSM 34271 and *Lactococcus lactis* (LC) DSM 34262, demonstrated strong inhibitory activity against clostridia in agar diffusion assays. Based on these results, three ensiling trials with slightly wilted grass were conducted in which the two isolated lactic acid bacteria were compared with an untreated control regarding their influence on various quality parameters.

MATERIALS AND METHODS

The inhibition of clostridia was examined using an agar diffusion test. *Clostridium tyrobutyricum* DSM 2637 spores have been diluted to reach semi-confluent growth and enclosed into RCM agar plates. Supernatants of more than 200 strain cultures (80 µl) were put into wells and incubated anaerobically (AN) at 37 °C for 24 h. A clear zone (halo) around the well shows the inhibition of clostridia (Schoster *et al.*, 2013).

For the silage trials, grass material from three different sources with dry matter concentrations ranging from 27 to 36%, was slightly wilted. Each chopped grass material was ensiled either without treatment (control) or with *Lactiplantibacillus plantarum* DSM 34271 or *Lactococcus lactis* DSM 34262. The lactic acid bacteria were sprayed onto the grass as a liquid bacterial solution with a concentration of 2.5×10^5 cfu/g FM. A quantity of 3 kg forage was ensiled in plastic buckets to observe fermentation processes and losses after 90 days of storage at 20 °C. Forage samples were analyzed to determine forage quality (DM, pH-value, fermentation pattern and ammonia-N). Fermentation weight losses were determined based on the mass difference of the mini silos before and after ensiling.

RESULTS AND DISCUSSION

The two selected lactic acid bacteria showed a clear inhibition zone in the agar diffusion test, shown in Table 1 and Figure 1. *Lactococcus lactis* DSM 34262 had a stronger effect against the test strain *Clostridium tyrobutyricum* compared to *Lactiplantibacillus plantarum* DSM 34271.

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. Consistent with the pH pattern, lactic acid concentrations were significantly higher in the treated silages (7.65 and 5.98 g per 100 g DM) compared with the control silage (3.11 g per 100 g DM). These results indicate improved preservation in the treated grass. The lower concentrations of butyric acid and ammonia-N in the treated materials are consistent with the observed pH and lactic acid pattern. Additionally, silages with higher lactic acid concentrations exhibited lower weight losses, presumably due to the inhibition of spoilage microorganism growth (control: 2.88 g/100 g FM; treatment: 1.36 and 1.84 g/100 g FM). Values are shown in Table 2.

Tab. 1: Inhibition zones of investigated lactic acid bacteria

Strain	Incubation [C°]	Halo [mm]
L. plantarum DSM 34271	37°C / AN	1.5
L. lactis DSM 34262	37°C / AN	5.0

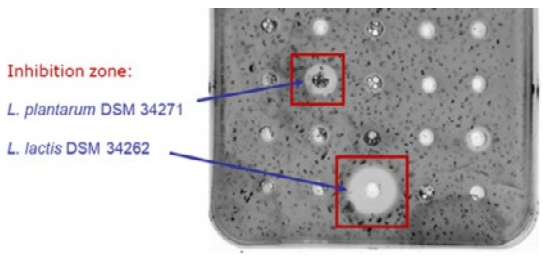

Fig. 1: Inhibition zones of investigated lactic acid bacteria

https://doi.org/10.11118/978-80-7701-093-1-0136

Tab. 2: Comparison of the parameters of mean values from three silage trials using slightly wilted grass material after 90 days of storage

		Mean values of three silage trials / 90 d				
Parameter	**Unit**	**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
Weight 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.

CONCLUSION

The present study demonstrates that the newly isolated strains *Lactiplantibacillus plantarum* DSM 34271 and *Lactococcus lactis* DSM 34262 effectively inhibit the growth of clostridia and improve silage fermentation quality. Both strains showed clear antagonistic activity against *Clostridium tyrobutyricum* in agar diffusion assays, with *L. lactis* DSM 34262 exhibiting the strongest inhibition. When applied as silage inoculants to slightly wilted grass with elevated clostridia risk, both strains significantly enhanced fermentation performance. The main effects included a pronounced reduction in pH, increased lactic acid production, lower weight losses and reduced formation of butyric acid, indicating suppressed clostridial activity. These results confirm the suitability of both strains as effective biological silage additives and highlight their practical potential to improve silage preservation and feeding quality under challenging ensiling conditions.

REFERENCES

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.

Contact Information

Hermine Schein: e-mail: schein@lactosan.at