

New Inoculants Containing Lactic Bacteria Applied in Forage Ensiling

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Abstract

In a first study, the capacity of lactic bacteria to accumulate biomass in different culture media and temperatures was tested and the biosynthesis parameters were established. In the second study, the strains producing the highest quantity of biomass and determining the most rapid pH drop in culture medium were conditioned in solid supports. The obtained solid products containing lactic bacteria were used to inoculate different types of forages. Ensilage was carried out in laboratory silos made from O₂-impermeable plastic flasks, vacuumed using a vacuum pump. The experiment was 2 x 2 factorial with two types of forage (alfalfa and sorghum), each of them inoculated and not inoculated with lactic bacteria. The evolution of lactic bacteria, pH value, and the concentration in volatile acids was verified. In the third experiment, lactic bacteria were used to inoculate silages in farm conditions. The obtained results recommend the tested strain for the improvement of preserving conditions and nutritive value in ensiled forages.

Keywords: inoculants, lactic bacteria, silage.

1. Introduction

The silage inoculants consist in viable microorganisms able to transform low molecular mass sugars from vegetable substrate in lactic acid [1]. The pH drop in ensiled forages as a result of lactic acid accumulation, together with the absence of oxygen as a result of compression of forage, lead to the inhibition of undesired microbiota. This way, the degrading processes are stopped and the nutritive value of the silage will be preserved [2,3].

The aims of this work are:

- to study the capacity of some lactic bacteria from the Collection of Industrial Microorganisms of Animal Science and Biotechnology Faculty from Timisoara to grow in cost effective and available media and;

- to obtain lactic bacteria inoculants and to test these products for their capacity to improve the preservation conditions in ensiled forages.

2. Materials and methods

Bacterial strains

Three lactic bacteria strains were used: *Lactobacillus plantarum* CMIT2, *Lactobacillus acidophilus* CMIT3 and *Enterococcus faecium* CMIT4.

Culture conditions

The strains were preserved in MRS+CaCO₃ 1% and cultivated in different conditions:

- different temperatures
- in MRS (control) and in whey resulted from enzymatic milk curdling.

Obtaining cells-excipient mixtures:

Lactic bacteria were grown in static conditions (microaerophilic microorganisms) in MRS medium, at 37°C for 20 hours. Batch cultures of *Lactobacillus acidophilus*, *Lactobacillus*

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plantarum, *Enterococcus faecium*, and a combined culture of the three of them was done until exponential phase (log) was achieved. The fresh cultures of lactic bacteria were mixed with calcium carbonate:

1. 100 mL from the combined culture was mixed with 100 grams of calcium carbonate. The wet mixture was dried at 40°C in a dark ventilated chamber. Samples were taken from mixture before and after drying for CFU on MRS-agar plates. The dry mixtures were introduced in plastic bags, air-tight sealed and preserved in a refrigerator.

Samples were taken at 30, 60, 90, 180 days of storage in refrigerator for analysis of viability in real time of lactic bacteria in dry and wet products, on organic (wheat bran, CMC) and mineral (zeolite, calcium carbonate, ceramics) excipients.

The effect of lactic bacteria in laboratory silage

Two types of forages were inoculated with lactic bacteria: alfalfa, forage difficult to ensilage, due to the low content in low molecular mass sugar and high content in proteins, with pH buffer effect; and sorghum, forage easy to ensilage, due to the high content in low molecular mass sugar (higher than sugar in corn), and low content in proteins.

Forages were inoculated with 5×10^5 lactic bacteria/ gram forage. After inoculation, the ensiling was carried out in two liters capacity plastic flasks, vacuumed using a vacuum pump. The flasks were air-tight sealed. Probes not inoculated were made for each type of forage (control). In the control for alfalfa 4% molasses was added, to increase the content in sugar. The flasks were storage at 28°C. Two flasks (inoculated and not inoculated) were opened at 2, 4, 7, and 14 days of storage. The evolution of lactic bacteria, pH value and acidity was determined. The number of lactic bacteria was determined by counting the colonies developed in MRS plates under microaerophilic conditions. Acidity in silage was determined by titration of water extract with NaOH 0,1N, considering 1ml NaOH = 0,009008 g lactic acid.

The effect of lactic bacteria in silage in farm conditions

The biosynthesis of lactic bacteria biomass was carried out in 100 liters of whey inoculated with 5% of lactic bacteria culture in MRS, in static conditions, 16 hours at 42°C. The obtained culture

of lactic bacteria in whey was diluted with warm water (40 - 45°C) and applied at the ensilage of a mixture of corn stalk and sugar beet husks. After 1, 2, 4, 7, 14 and 90 days of ensilage, probes were tacked from the silage and pH, CFU, ammoniac nitrogen and organoleptic qualities were determined.

3. Results and discussion

In the first stage of this work, lactic bacteria strains were cultivated in MRS and whey and evolution of pH (figure 1) and turbidity (figure 2) was determined. Since the turbidity in whey is due to protein particles in suspension, the evolution of turbidity couldn't be used as a proof parameter for bacterial grow. The pH drop was considered as an evidence of lactic bacteria activity. Also, after 20 hours of culture in whey, the number of lactic bacteria was determined using the CFU method (Table 1).

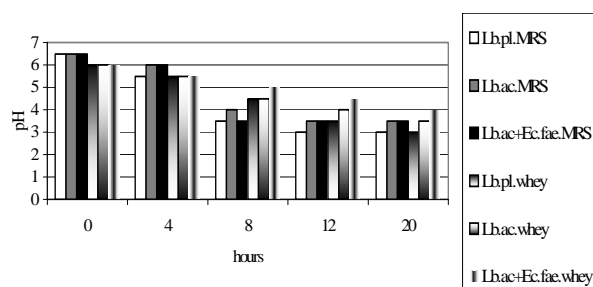


Figure 1. Evolution of pH in lactic bacteria cultures in MRS and whey.

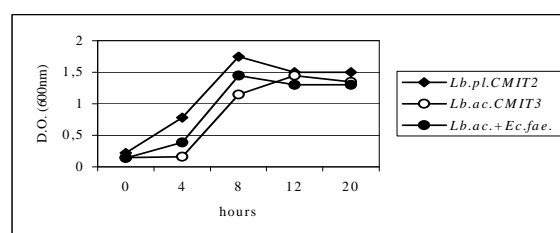


Figure 2. Evolution of turbidity in lactic bacteria cultures in MRS

Table 1. The number of lactic bacteria cultivated in whey

Strain	CFU after incubation
<i>Lb. plantarum</i> CMIT2	$4,95 \times 10^8$
<i>Lb. acidophilus</i> CMIT3	$2,2 \times 10^8$
<i>Lb. acidophilus</i> + <i>Ec. faecium</i>	$4,2 \times 10^8$

It is obvious that *Lb. plantarum* CMIT2 is able to grow in whey as good as in MRS. To demonstrate that the pH drop in whey is not determined by preexistent microflora, the whey was sterilized and concomitant cultures was carried out. It was found that *Lb. plantarum* CMIT2 has a good development in septic whey, even better as in MRS. The nutritive components were degraded during the sterilization of whey and lactic bacteria growth was affected. Also, it is important to observe that the preexistent microflora in whey not inoculated had a poor growth and the pH value is the same after 24 hours of incubation. This means that microflora in whey is not acidophilic, therefore not acidolactic.

The most propitious temperature for *Lb. plantarum* CMIT2 was determined (Table 2). The tested strain shows a good growth at high temperatures, this quality can be used to inhibit the contaminants in culture media.

Table 2. Growth parameters of *Lb. plantarum* CMIT2 at different temperatures

Temperature(°C)	37	42	44	46
pH	3,5	3,5	4	4
D.O.1 –D.O.2*	1,74	2,31	1,70	0,67
Acidity (g%)	1,27	0,89	0,74	0,44

* difference between optical density at 600 nm measured at time 0 and optical density at 600 nm after 12 hours of incubation in MRS.

In the second stage of this work, lactic bacteria strains were separated from culture medium and conditioned with calcium carbonate. The obtained white powder was preserved at room temperature and viability of lactic bacteria was monitored.

The following quantities of lactic cells biomass were obtained: *Lb. plantarum* CMIT2 - 2,79 g / 100 ml culture; *Lb. acidophilus* CMIT3 - 2,82 g / 100 ml culture; *Lb. sp. CMIT1* + *Ec. faecium* CMIT4 - 2,88 g / 100 ml culture. Figure 3 shows the viability of lactic bacteria in dry powder preserved at room temperature.

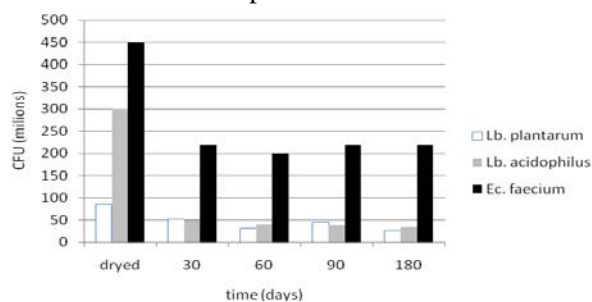


Figure 3. Viability of lactic bacteria in dry product for use as silage inoculant

The results indicate in the case of the dry product that the lactic bacteria are viable in the studied period, and, more important, their number is constant after 30 days of storage. This fact enables the user of this product to base on a specific number of living lactic bacteria when apply the product to inoculate a silage. The inoculants will be dosed to reach a concentration of 5×10^5 lactic bacteria/ gram forage.

Effect of lactic bacteria in laboratory minisilages

Results obtained in alfalfa haylage

Alfalfa laboratory silage flasks prepared as in *Material and methods* were opened after 2, 4, 7 and 14 days of storage and pH, CFU, and acidity was verified. The results are shown in figure 4, 5, and 6. The control consists in alfalfa mixed with molasses 4%, and the experimental probes consists in alfalfa inoculated with *Lb. plantarum* CMIT2. The maximum accumulation of lactic bacteria (fig. 4) in inoculated probes and in control as well is after four days of ensilage. The number of lactic bacteria in inoculated probes is 90% higher then in control.

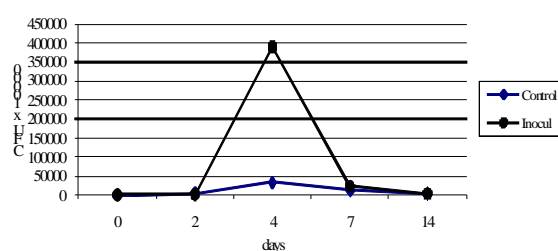


Figure 4. Evolution of lactic bacteria in alfalfa laboratory silage.

The pH value decrease in a higher rate in control, due to the higher content in sugar from molasses (fig. 5).

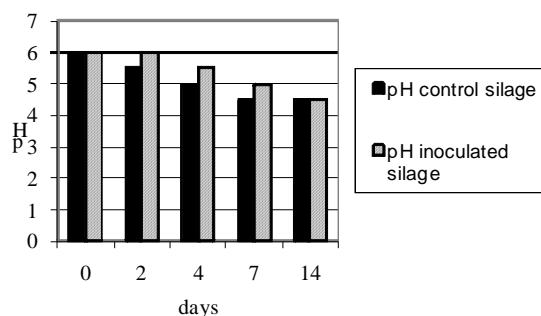


Figure 5. Evolution of pH in alfalfa laboratory silage.

As shown in figure 6, acidity in inoculated probes maintains higher than acidity in control (alfalfa and molasses).

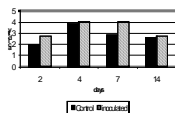


Figure 6. Evolution of acidity in alfalfa laboratory silage

The presence of ammonia was not detected. Knowing that alfalfa is forage that raises difficulties at the ensilage due to the low content in sugars, the results obtained in this experiment are positives. Although the inoculated probes contains less sugar than control because molasses was not added in the probes, observing the parameters of ensilage the followings can be concluded:

- the growth curve of lactic bacteria is approximately the same and the number is always higher in inoculated probes;
- pH value follows a parallel evolution, after 14 days of storage pH values are equal (4,5);
- acidity is higher in inoculated probes, therefore the nutritive qualities of forage are preserved better than in control.

Results obtained in laboratory sorghum silage

In this experiment, the experimental probes are represented by chopped sorghum inoculated with *Lb. plantarum* CMIT2 and the control consists in chopped sorghum. Both inoculated probes and control were preserved in anaerobic conditions as described in *Material and methods*. The plastic flasks were opened after 1, 2, 4, 7, and 14 days of storage at 28°C. The results show that in inoculated probes the number of lactic bacteria is 10% higher than in control (fig. 7).

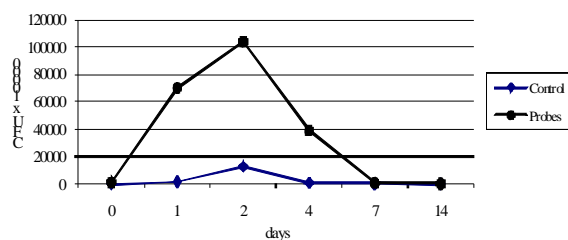


Figure 7. Evolution of lactic bacteria in sorghum laboratory silage.

The evolution of pH shows lower values in first four days in inoculated probes and equal values after 14 days of ensilage (fig. 8).

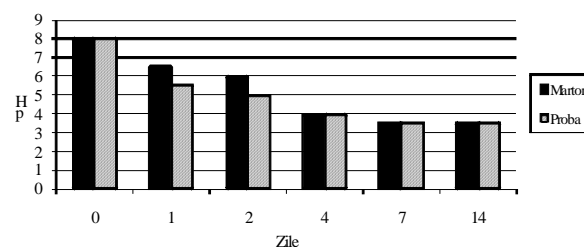


Figure 8. Evolution of pH in sorghum laboratory silage

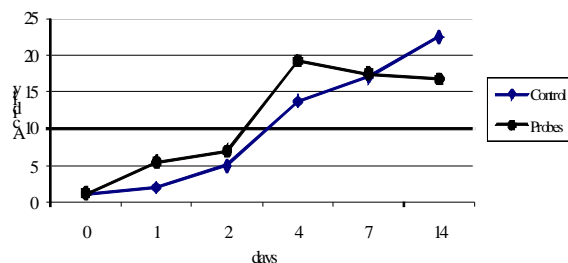


Figure 9. Evolution of acidity in sorghum laboratory silage

Regarding the evolution of acidity (fig. 9) in sorghum laboratory silage, results leads to the following conclusions:

- the acidity in control increases, reaching the highest value after 14 days;
- the acidity in inoculated probes increases in the first four days and remains constant until the 14-th day;
- comparing the evolution of acidity and the lactic bacteria growth curve, we can conclude that a higher number of lactic bacteria (day two) leads to a higher concentration of volatile acids (day

four), which leads to the death of lactic bacteria (days 4, 7, 14).

Concluding, a lower pH in the first part of ensilage and a higher acidity leads to a better preservation of nutritive qualities in inoculated silage.

The effect of lactic bacteria in farm condition silage

An inoculant consisting of *Lb. plantarum* CMIT2 grown in whey was used to inoculate 70 tones of corn talks mixed with sugar beet husks. The obtained silage was preserved in good conditions, the presence of ammonia and butyric acid was not detected, the pH value decreased to 4 (fig. 10) and the lactic bacteria reached the highest number after two days of ensilage (fig. 11).

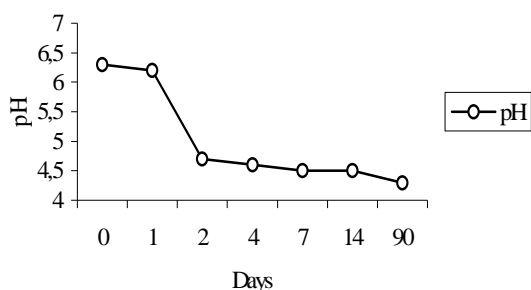


Figure 10. Evolution of pH in inoculated silage in farm conditions.

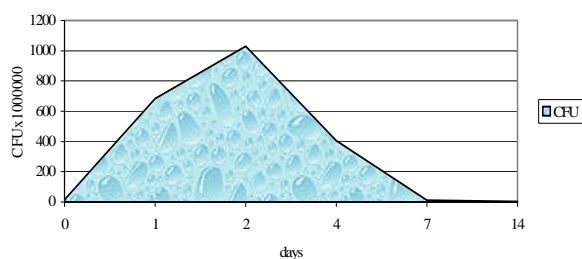


Figure 11. Evolution of lactic bacteria in inoculated silage in farm conditions

The inoculation of the silage promoted a proper dynamics of fermentation, at low temperatures, eliminating the risk of caramelizing, phenomenon often met at the ensilage of this kind of forage. At

the maturation of the silage, good fermented forage was obtained, with typical characteristics for a high quality silage: sour odor and flavor, yellow-green color. The forage has a good palatability, and administrated to lactating dairy cows with good results at the transition from the summer feeding to the winter ration.

4. Conclusions

The lactic bacteria from the Collection of Industrial Microorganisms of Animal Science and Biotechnology Faculty from Timisoara were cultivated in cost-effective and available substrate (whey) with good results. The growth of lactic bacteria in whey was comparable with the growth in the special and expensive medium, MRS.

Calcium carbonate can be used to obtain dry products containing lactic bacteria, which remain viable in high concentration, up to 2.2×10^8 cells / gram of dry product after 180 days of storage.

Drying of the inoculants keeps a constant number of viable cells, an important parameter in the administration of these products as silage inoculants or feed additives.

The silage inoculant was tested at the preservation of a forage that raises difficulties at the ensilage (alfalfa) and a forage easy to ensilage (sorghum). Also, the efficiency of the inoculant was tested in farm conditions and a good quality ensiled forage was obtained.

The results leads to the conclusion that the inoculant can be applied with positive results for the improvement of preserving conditions of the forages and the improvement of nutritive qualities of ensiled forage.

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