Biological Inoculants in Forage Conservation

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Abstract
3rd generation biological inoculants –containing lactic acid bacteria and enzymes – are prefered nowadays in order to coordinate the fermentation in such a way that they increase lactic acid production by leaps and bounds at the beginning of the fermentation and improve the quality and stability of silage during the fermentation and feeding. The quality of raw material (maturity of plant, chop lenght, spreading of inoculant uniformly) and the proper filling, compacting, covering and wrapping have a great influence on the effectiveness of the inoculant. The mycotoxin content of malfermented silages is an undesirable risk factor. The authors established, that the *Lactobacillus buchneri* and enzymes containing inoculant protected better the carotene content of low, medium- and high wilted lucerne haylages ($P<0,05$) compare to untreated ones Aerobic stability experiment by Honnig 1990 method was carried out with medium wilted (36 % DM) lucerne haylage which was treatedtreated before ensilage with , the dosage of 10^5 CFU/g Pediococcus acidilactici, $1,5x10^5$ CFU/g Lactobacillus buchneri and cellulase and hemicellulase enzimes (20 000 CMC /g) remained stabyle, unspoiled after 9 days exposure to the air, while the untreated haylages spoiled after 2;4;or 7days on aerobic condition. The different Lactobacillus plantarum strains (50.000 CFU of *Lactobacillus plantarum* DSM 16568 + 50.000 CFU of *Lactobacillus plantarum DSM 4784/ g FM* of maize applied together were able to improve the aerobic stability of silomaize silage.

Keywords: biological inoculant, carotene content, aerobic stability.

1. Introduction

The most important silage plants of Hungary are: lucerne, silomaize and grass.
The natural fermentability of fresh silo plants may be improved by the usage of some kind of silage additives. Nowadays, the spreading of biological preservatives can be observed. The rason for this is the restriction of the market of carbohydrate-based additives, and the chemicals of selective effectability on microbes (organic and inorganic acids and their salts, as well as additives contain nitrate or nitrite, formaldehyde etc.) had to be repressed beacause of their effects on public and labour health e.g. danger of pesticide residue.
In these days there are more than one hundred bilogical preservatives on the market in Europe. In the majority of them the lactic acid bacterium culture consists of not only one but more strains of lactic acid bacteria. In most cases, the strains are selected resolutely based on their characteristics. In the most effective preservatives strains are built upon each other. Almost all of the inoculants contain the Lactobacillus plantarum.
Silage additives produce variable results on aerobic deterioration of silages. A high concentration of lactic acid cannot provide aerobic stability for sure. Recently the heterofermentative *L. buchneri* is regarded to be the most promising lactic acid bacteria for increasing aerob stability. Applied by itself it may show a negative effect by reducing the speed of fermentation, but its combination with homofermentative lacto acid bacteria does compensate this disadvantage [1]. According to Driehuis, Elferink, Ruser and Kleiman [2, 3, 4, 5] it takes effect on stability in the 2nd phase: during the 1st phase lactic acid originates from sugar and in the 2nd phase acetic acid and 1, 2 propandiol are generated from lactic

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acidemphatize the role of propionic acid originating from 1,2 propandiol and 1 propanol in stability. (1,2 propandiol and 1 propanol are not found in untreated silage). L.buchneri may produce other yet unindentified metabolites with antifungal activity.

Fort he succesful operation of starter culture it is necessary that there be enough fermentable water soluble carbohydrate (WSC) int he forage according to Pahlow and Honig [6]. The minimal amount of water soluble carbohydrate is 3%.

Today, the third generation of biological preservatives is on the market, which differs from biological preservatives used during the previous years in that it contains also some kind of ensyme preparation besides liofilized (freezy-dried) bacterium culture.

The task of these ensymes is to complete the non fermentable carbohydrate resource of the plant ensilaged by the degradation of polysaccharids (cellulose, hemicellulose, starch), which are found in the plant but are not fermentable by lactic acid bacteria, to fermenting carbohydrate [7, 8, 9, 10, 11, 12, 13].

The cellulase and hemicellulase can be found nearly in all of the preservatives. Most of the preparations contain also pentozanase. In some preservatives, also xilanase, ß-glucanase, galactomannase or pectinase can be found. Relatively many of the preparations containing also the examined ensyme, include the amilase degrading starch.

The circumstance that the cellulase, hamicellulase and amilase originating from microorganisms do not have identical conditions for optimal activity, offers good possibility for the development of biological preservatives. For instance, while the cellulase originating from Trichoderma reseei has the functional optimum between 4,8-5,2 and its optimal temperature is around 55-65^{o}C, the optimal pH of the cellulase extracted from Trichoderma viride is between 4 and 5 and its most favourrable domain of temperature is between 40 and 50^{o}C. . At the same time, during ensilage we strive so that the temperature in the silage will not exceed 30^{o}C. Similar differences can be found regarding the optimal activity conditions between the amilases extracted from different microorganisms [14].

From the results of experiments carried out so far, we can draw the conclusion that the effectivity of third generation biological preservatives depends ont he dry matter content of green ensilaged forage. The question is complex since the inoculation of lactic acid bacteria is most effective in forages of 30-35% of dry matter content. The ensymes degrading crude fiber are more active in the environment of lower (under 30%) dry matter content [9]. These data clearly refer to the fact that the biological preservative containing ensymes that break down fiber, degrades the crude fiber of green lucerne with original moisture content of 18,7% more effectively than that of wilted lucerne with higher (31,7%).dry matter content [15, 16].

In the case of third generation biological preservatives containing inoculation culture and cell wall degrading ensyme-preparation we are forced to make a compromise in respect on the dry matter content of the ensilaged plant. When ensilaging green lucerne the advisable dry matter content the lactic acid bacteria are already able to activate well and the cell wall degrading ensymes are able to function satisfyingly.

The difference between the enviromental conditions prevailing in the silo and the optimal activity circumstances of the ensymes may be corrected for a certain extent by increasing the dosage of the ensyme. Of course, the price of the ensymes sets limits to this process.The efficiency of the ensyme preparations may be improved also by the way that several ensymes completing the activity of each other, would take place in each preparation in respect of the effectivity of the degrading of fiber. The chemical composition of the fiber does matter, that is why the ensyme-composition of the ensyme preparations should match also to this condition [17].

2. Materials and methods

Carotene content of lucerne haylages:

Different degree of wilting was applied on fresh cut lucerne: slightly wilting 28% DM , medium, 40% DM and hard 52% DM. The wilted row materials were treated with 10^5 CFU/g FM (colony forming units / *fresh material) Lactobacillus plantarum* containing biological inoculant dosage of. 10^5 CFU/g FM Another experiment medium wilted (36%DM) lucerne was ensilage with 3 rd generation preservative, the dosage of 1x10^5 CFU/g FM Pediococcus acidilactici, 1,5x10^5 CFU/g FM Lactobacillus buchneri plus cellulase and hemicellulase enzimes(20 000 CMC /g FM)

The samples made in microsize silos, 4.2 litre capacity of srewed glass containers.The carotene content of haylages was observed and compared to the untreated control.

Examination of carotene. After dissoluting of the sample with petrol-petrolaether we filtered the solution on chromatographic column then we photometrically tested the filtration on 540 micrometer wavelenght.

Aerobic stability of lucerne- and silomaize silages:

The **lucerne** originated from 3rd cut in early flowering maturity. The medium wilted Lucerne was applied with Lalsil Dry inoculant containing *Pediococcus acidilactici* (CNCM MA 18/5M), *Lactobacillus buchneri* (NCIMB 40788) and cellulase and hemicellulase enzimes. The treated and untreated control forages were roundbaled and wrapped with plastic folia 200 pcs/each. The aerob stability was determined 4 different times after ensiling using the standard of Honig system [18]. The bales for observation were selected randomly on 45th 60th 67th and the 122nd days and the samples were exposed to air during 7, 9, or 14 days at 22-24,5 ^{o}C room temperature. The registration of the temperature of the samples was on the basis of one hours by computer.

The harvested **silomaize** was in hard cheddar stage of maturity and stored in clamp silo. The applied treatment*:1.*Untreated control 2.Inoculated with BIOMAX 5 1 gram per tonne fresh weight of maize to apply 50.000 CFU of *Lactobacillus plantarum* DSM 16568 + 50.000 CFU of *Lactobacillus plantarum* DSM 4784/ g FM of maize. The determination of aerobic stability made by system of Völkenrode [18]. Principle is based on monitoring temperature wich rises due to increased microbial activity of samples exposed to air.

3. Results and discussion

Carotene content of lucerne haylages:

The carotene content of lucerne haylages see on table 1. and the carotene protection on figure 1.

It has proven that all treated silages contain more carotene than that of untreated ones.

The difference of significance is p > 0,05 compare to the control The protection of carotene was considerably better with 3rd generation preservative and with *LB plantarum* on hard wilted lucerne

Table 1. Carotene content of lucerne haylages

Treatment	Wilting	n	Carotene mg/DM kg	Significance
Lb. plantarum	Slightly	5	188	p > 0,05
Untreated control	28 % DM		170	
Lb. plantarum	Medium	5	150	p < 0,05
Untreated control	40 % DM		137	
Lb. plantarum	Hard	5	116	p < 0,05
Untreated control	52% DM		89	
Lb. plantarum			104	p < 0,05
Pedicoccus acidilactici	Medium	5	153	
Lb. buchneri + enzymes	36 % DM			p < 0,05
Untreated control			128	

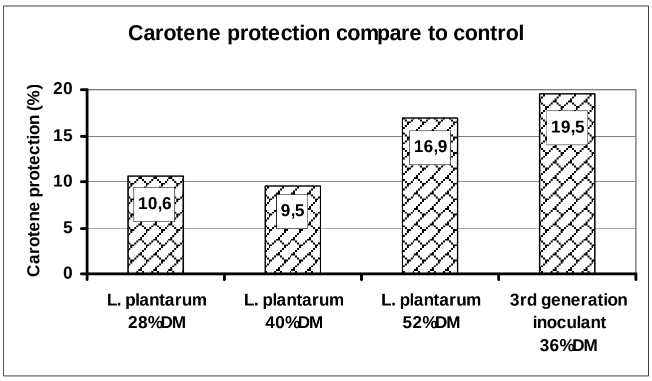

Figure 1. Carotene protection compare to control

Aerobic stability of lucerne- and silomaize silages

Lucerne haylages: The considerable deterioration of silage starts when the temperature of the sample is higher with 2 ˚C (at least) than the ambient temperature.

1st observation: After 45 days of ensilage the control samples were undamaged until 5th day exposed to air but after than get worm and spoiled for the 7th day. The temperature of Lalsil Dry treated samples have no changed, which means that 45 days fermentation resulted a stable silage. The undisturbed 60 days fermentation resulted a stable silages made with Lalsil Dry or without any treatment in *2nd observation. In the 3rd observation* the first phase of determination proved the conclusion of 2nd *observation*: all samples remained undamaged during 1 week. The spoilage of control silages started on the 2nd week, and culminated on 9th - 11th days. While the Lalsil Dry treated samples remained undeteriorated during 14 days exposed to air. *4th observation:* After 122 days of ensiling the stability of Lalsil Dry treated silages remained, there was no any deterioration during 9 days exposed to air. On the contrary with this the control silages started to deteriorate rapidly: 1st sample from 2nd day, 2nd sample from 4th day while the 3rd sample after 1 week exposed to air (Figure 2), This result seems to be confirmed by less pH and ammonia content of treated samples in the end of the aerobic stability experiments(Figure 3).

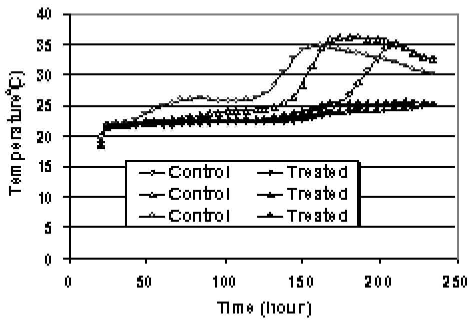

Figure 2. Aerobic stability of lucerne silages

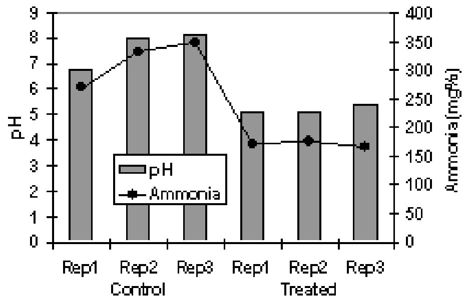

Figure 3. Ammonia and pH of lucerne silages

Silomaize silages: The average aerobic stability of untreated control and the two different strains of *Lactobacillus plantarum* treated silomaize silages see on Figure 4.

The aerobic stability of control silages were between 48-96 hours (the deterioration started after 2 days of opening on 20°C ambient room temperature in aerob conditions). The 7th day samples pH was raising for 4,1, the silages contained a very small amount of alcohol (evaporated until than), and high NH_3 (42 mg%/FM means 114mg/DM), the weight loss was 1,5 %.

The spoilage was proved by the result of microbiological analyses and sensory test as well.

The chemical compositon and nutritive value_of BIOMAX 5 treated silage was similar to the control, but the aerobic stability of the silage was very good. The samples remained totally undamaged during the 7 days experiment.

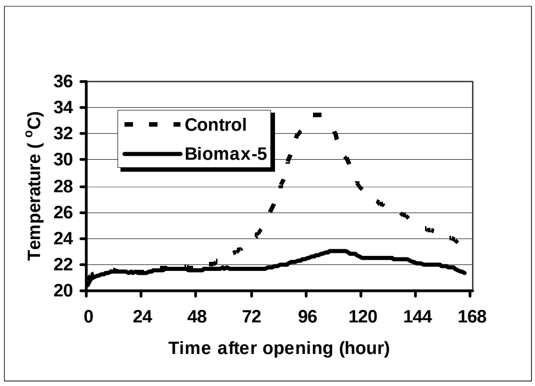

Figure 4. Aerobic stability of silomaize silages

4. Conclusions

The applied biological preservatives namely the *Lb. plantarum* and the 3rd generation preservative which contains LB and enzymes protected the carotene of the lucerne haylages.

The aerobic stability of Lalsil Dry treated lucerne silages are strong. There was no deterioration neither 7 days nor 14 days exposed to air while the untreated samples get spoiled after 5-7 days and totally moulded for 14 days.

The different *Lactobacillus plantarum* strains applied together was able to improve the aerobic stability of silomaize silage.

Nevertheless in these days several considerable objections have arisen regarding the effectiveness of 3rd generation biological inoculants – containing lactic acid bacteria and enzymes-, the perspectives they offer cannot be controverted.

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