

## INTRODUCTION

Ensiling is an ancient method used to preserve the nutritive value of forages by packing and storing forage in airtight conditions. Silage fermentation occurs naturally under anaerobic conditions (the absence of oxygen). Plants contain native lactic acid bacteria (LAB), and when silage is placed under anaerobic conditions, these LAB produce lactic acid, reducing the pH to a level in which other bacteria cannot survive. However, LAB are not the only microorganisms on plants. Bacteria like clostridia and enterobacteria, as well as yeast and molds, also are present on plants and compete with LAB for sugars. In addition, native LAB populations on plants are not the same from crop to crop. Native LAB levels are generally lower in alfalfa (*Medicago sativa* L., 10<sup>5</sup> colony forming units [cfu]/g fresh matter) and greater on perennial grasses (10<sup>6</sup> cfu/g fresh matter), corn (*Zea mays* L., 10<sup>7</sup> cfu/g fresh matter), and sorghum (*Sorghum bicolor* [L.] Moench, 10<sup>7</sup> cfu/g fresh matter) (Pahlow et al., 2003). Environmental conditions also have an effect on native LAB levels. Bacteria are more abundant in warmer than in cooler temperatures, with higher levels at certain maturity stages (Pahlow et al., 2003). In alfalfa, native LAB were higher in warmer temperatures, after a longer wilting time, and when rainfall occurred during wilting (Muck, 1989). Moreover, native LAB are low in the standing crop, increasing exponentially after chopping in both corn and alfalfa (Lin et al., 1992).

Use of silage additives is recommended to preserve the nutritive value of the crop when circumstances could compromise proper fermentation

**Table 1. Fermentation Reactions by Lactic Acid Bacteria (Muck, 2008)**

Type of Fermentation	Reaction
Homofermentative	1 6-C Sugar → 2 Lactic Acid
Heterofermentative	1 6-C Sugar → 1 Lactic Acid + 1 Acetic Acid + CO <sub>2</sub>
	1 6-C Sugar → 1 Lactic Acid + 1 Ethanol + CO <sub>2</sub>
	1 Lactic Acid → 1 Acetic Acid + CO <sub>2</sub>

(Contreras-Govea & Muck, 2006). Silage microbial inoculants are one type of available additive, and have been classified as stimulators of fermentation (Kung et al., 2003). These inoculants contain LAB that complement the native LAB population, helping to ensure a better fermentation (Muck, 2008; Contreras-Govea & Muck, 2006). The objective of this publication is to provide information about silage microbial inoculants, how they work, and the conditions under which they should be applied for better success in hot weather conditions.

## SILAGE MICROBIAL INOCULANTS

Silage microbial inoculants are selected LAB that are applied to dominate the naturally occurring fermentation processes of crops in the silo. They are divided into two groups depending on how they ferment plant sugars: homofermentative LAB and heterofermentative LAB (Table 1). Homofermentative bacteria, such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Pediococcus* spp., and *Enterococcus* spp., mainly produce lactic acid. Heterofermentative bacteria, such as *Lactobacillus buchneri*,

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produce lactic acid, acetic acid, ethanol, and carbon dioxide (Table 1). Generally, lactic acid is preferred in the silo because it is a stronger acid than acetic acid (Muck, 2008). Lactic acid reduces pH faster, thereby reducing plant respiration and enzyme activity and inhibiting other bacteria. However, acetic acid is a better inhibitor of yeast, and maintains better aerobic stability than lactic acid.

### **HOMOFERMENTATIVE LACTIC ACID BACTERIA EFFECT ON SILAGE QUALITY**

Homofermentative LAB are the most common inoculants on the market. Initially, the main goal of using these inoculants was to preserve the quality of the ensiled plants as near to original levels as possible. Homofermentative bacteria accomplish this goal by decreasing pH, reducing dry matter losses to a minimal level (2–3%), reducing proteolysis (the breakdown of protein) and ammonia formation, and increasing lactic acid and dry matter digestibility (Muck & Kung, 1997). A fast decline in pH can also inhibit clostridial bacteria that produce butyric acid, a product of a bad fermentation that causes malodor. In addition, homofermentative bacteria have the potential to improve animal performance. A review of research studies reported that these inoculants improved weight gain in beef cattle and milk production in lactating cows in 50% of the trials (Kung & Muck, 1997). When the inoculated silage produced a positive effect, the average increase in weight gain was expected to be 5%, while milk production increased 3% (Kung & Muck, 1997). The cause of this improvement in performance is not clear. *In vitro* studies suggest that inoculated silages improve rumen microbial growth, which has been observed even when the inoculants had little effect on silage fermentation (Muck, 2008). However, the *in vitro* studies also showed that not all inoculants work equally, which could indicate a strain-specific effect (Muck, 2008).

### **HETEROFERMENTATIVE LACTIC ACID BACTERIA EFFECT ON SILAGE QUALITY**

The main purpose of heterofermentative inoculants is to improve aerobic (the presence of oxygen) stability by reducing the level of yeast in the silage

(high levels of yeast can cause heating). *Lactobacillus buchneri* is the main heterofermentative LAB used in forage crops in the U.S. (Muck, 2008). *Lactobacillus buchneri* produces more acetic acid than homofermentative bacteria (Kleinschmit & Kung, 2006). These bacteria are more effective in corn than alfalfa or small-grains because *L. buchneri* is less abundant in corn than alfalfa (Lin et al., 1992). Because of the lower levels of *L. buchneri* and other heterofermentative bacteria in corn, acetic acid, a yeast inhibitor, is normally lower in corn than in alfalfa, making corn more susceptible to aerobic stability problems, mainly when the silo is open in summer with high temperatures. One issue with *L. buchneri* is that they grow slower than other bacteria in the silage; therefore, the natural homofermentative bacteria will promote the initial fermentation, and later *L. buchneri* will convert lactic acid to acetic acid. Because of this, when *L. buchneri* is used, it is recommended that a minimum of 45–60 days elapse before opening the silo in order to ensure good aerobic stability (Muck, 2008).

Recently, some heterofermentative LAB, such as *L. buchneri*, *L. reuteri*, *L. crispatus*, and *L. brevis*, have been reported to produce ferulate esterases (Nsereko et al., 2008). Ferulate esterases are enzymes that increase cell wall degradation, releasing more soluble carbohydrates from plants for fermentation or use by rumen bacteria. The advantage of these new LAB strains is that, in addition to improving aerobic stability, they increase silage digestibility and—potentially—animal performance (Nsereko et al., 2008). At this moment, more research is needed to support these findings about these new strains.

### **COMBINING HOMOFERMENTATIVE AND HETEROFERMENTATIVE INOCULANTS**

The potential advantages of combining both types of LAB are having a fast initial pH reduction controlled by the homofermentative bacteria and a good aerobic stability later that is controlled by heterofermentative bacteria producing more acetic acid. Few laboratory studies have been conducted mixing both types of bacteria. In one study, *L. plantarum*, *L. buchneri*, and a mix of both were compared with uninoculated silage in sorghum and

**Table 2. Silage Characteristics of Corn and Sorghum Fermented with Homofermentative LAB, Heterofermentative LAB, or a Combination of Both (Filya, 2003)**

Crop	Treatment	pH	Lactic Acid (%)	Acetic Acid (%)	Yeasts (log <sub>10</sub> cfu/g)
Corn	Uninoculated	3.72	4.04	1.27	3.86
	<i>L. buchneri</i> (heterofermentative)	4.13	2.76	3.89	< 2.00
	<i>L. plantarum</i> (homofermentative)	3.64	7.94	0.33	4.45
	Both	3.80	5.55	3.17	< 2.00
Sorghum	Uninoculated	3.87	4.86	0.96	4.18
	<i>L. buchneri</i> (heterofermentative)	4.26	2.54	4.30	< 2.00
	<i>L. plantarum</i> (homofermentative)	3.75	9.39	0.62	4.73
	Both	3.88	6.18	3.49	< 2.00

corn (Filya, 2003). The combination of both LAB types had a complementary effect, producing more lactic acid than heterofermentative bacteria and more acetic acid than homofermentative (Table 2). Moreover, yeast population was lower in the mixture of both LAB than in homofermentative bacteria, which indicated a potentially better aerobic stability.

Similar effects were reported by Kleinschmit and Kung (2006), who compared uninoculated corn silage to silage inoculated with a combination of *L. buchneri* and *Pediococcus pentosaceus*. In this study, yeast also was lower in the inoculated corn silage and aerobic stability was better than the uninoculated silage.

### WHY DO INOCULANTS NOT WORK SOMETIMES?

There are several potential reasons why these products sometimes fail:

**Natural bacteria population.** It was mentioned in the introduction that natural LAB populations differ among crops. Generally, corn and sorghum have greater native bacteria populations than alfalfa. To have an impact on fermentation, the inoculant application rate must be at least 10% of the native LAB population.

**Low sugar content of the crop.** Water-soluble carbohydrates are the main food source for LAB. Crops with low sugars, like alfalfa and warm-season grasses, are more challenging for LAB to reduce pH and achieve good fermentation. It has been suggested that in crops like alfalfa, sugar content may limit the effect of inoculants (Muck & Kung, 1997).

**Dry matter content.** Lactic acid bacteria do not move; they need water to move and intake sugars. Therefore, high dry matter content and low moisture will affect the activity of both the natural LAB and the inoculants. In contrast, very wet plant material will promote native bacteria to grow, affecting the impact of the inoculants on fermentation.

**Crop specificity.** Some LAB strains are crop-specific. In other words, they grow best on the crop they were selected from, such as corn, alfalfa, or sorghum. Some inoculant strains grow well on a wide range of crops.

### HOT WEATHER EFFECT ON INOCULANTS

Temperature has an effect on plant and biochemical processes before and during ensiling (Muck et al., 2003). Most of the studies of microbial inoculants for silage have been conducted in temperate weather conditions where temperatures are mild at

the time of ensiling. However, with temperatures around or above 100°F at the time of harvest and ensiling, as in many areas of New Mexico, some issues must be considered at the time inoculants are applied. McDonald et al. (1966) reported that ensiling grasses at a temperature of 107°F resulted in clostridial fermentation and lower amounts of lactic acid than ensiling grass at 68°F. Similarly, Adesogan (2006), who ensiled corn in Florida, found that corn ensiled at 107°F had lower lactic and acetic acid concentrations and higher pH and ammonia concentrations than corn ensiled at a cooler temperature. Under these high temperature conditions, fermentation tended to be more heterolactic than homolactic. He also reported that high temperatures reduced LAB populations, but increased clostridial bacteria because they have a higher temperature for optimal growth than LAB. Therefore, warmer conditions should be more favorable for clostridial fermentation (Muck et al., 2003). The time that inoculants are exposed to high temperatures also has an effect on their viability. In one study, six inoculants were incubated for six hours at four different temperatures ranging from 85–115°F. Viability of the inoculants declined with increasing temperatures. Some inoculants were more tolerant of high temperatures than others (Mulrooney & Kung, 2008). Under hot weather conditions, it is important to store the inoculants at a reasonable temperature (around 85°F) prior to the time of application. It is also important to fill, pack, and seal the silo as soon as possible to reduce respiration and the heating it causes, and to quickly achieve an anaerobic environment.

### **APPLICATION OF INOCULANTS**

There is a minimum application rate of inoculants that should be used to increase the likelihood of impacting fermentation. Adesogan (2006) conducted a study with two commercial inoculants to compare the effect of two application rates on fermentation of corn. Inoculants were applied at a recommended rate ( $1.0 \times 10^5$  cfu/g fresh forage) and at double the recommended rate ( $2.0 \times 10^5$  cfu/g fresh forage). He concluded that there was no benefit on fermentation from inoculating at double the recommended rate. In alfalfa, an application rate of

100,000 cfu/g fresh forage was recommended when native bacteria populations were lower than  $1.0 \times 10^6$  cfu/g fresh forage, a common condition in alfalfa (Muck, 2008).

Always follow the inoculant manufacturer's recommendations. This pertains to storage of the inoculant as well as application. Dry or wet inoculants work equally well (Kung et al., 2003). However, keep in mind that dry inoculants need to be moistened by plant juices in the silo before the LAB begin to grow; a wet inoculant may start working sooner on a crop ensiled on the dry side. Finally, these bacteria cannot move around. They depend on the producer doing a good job of mixing the product with the crop (Muck, 2008).

### **SELECTION OF MICROBIAL INOCULANTS**

There are many inoculants on the market, and not all LAB strains work equally well. Some LAB strains are crop-specific, and you should therefore select an inoculant that is specific for the crop you want to ensile. If you want to improve silage quality, homofermentative LAB are a better option. If you are looking for better aerobic stability, *L. buchneri* is a better option than *L. plantarum*. If you want both silage quality and aerobic stability, a combination of both *L. buchneri* and *L. plantarum* could be the best option. Always ask the inoculant manufacturer or salesperson for information to support the product.

### **SUMMARY**

It is clear that microbial inoculants enhance silage fermentation by speeding the decrease in pH, increasing lactic acid concentration, and improving aerobic stability and—potentially—animal performance. Sometimes microbial inoculants do not always improve fermentation due to competition from other microbes, availability of sugars, moisture levels, or crop specificity. However, good inoculants will work the majority of the time if directions are followed. Always keep in mind, however, that microbial inoculants are not the solution to mistakes made during the ensiling process.

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## Notes

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