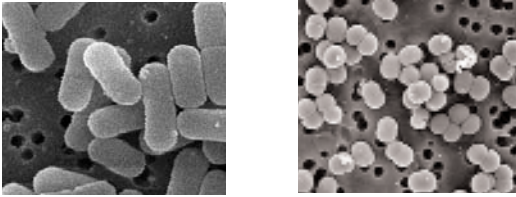


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Forage inoculants are biological products that contain a source of live, viable bacteria, sometimes combined with enzymes. The bacteria in the inoculant are applied to inoculate the freshly harvested forage, much the same as live yeasts are used to inoculate alcohol fermentations or bread. Enzymes, when present, are there to generate sugars for the inoculant bacteria to use for growth and fermentation. The bacteria grow in the forage, producing acids to drive the ensiling fermentation (see Introduction), converting the fresh forage from near neutral pH into an acidic end product (silage).



The photographs above show the two main cell morphologies of lactic bacteria. The rod-shaped bacteria are called bacilli after the Greek word "bacillus" which means rod. Shown above left are Lactobacillus buchneri 40788 cells. The spherical bacteria are called cocci after the Greek word "coccus" which means sphere. Shown above right are Pediococcus pentosaceus 12422 cells.

Inoculants are used for two primary reasons:

- 1) to stimulate or ensure a rapid fermentation (fermentation aids).
- 2) to inhibit aerobic spoilage (spoilage inhibitors).

Fermentation aids generally contain efficient (homofermentative) lactic-acid-producing bacteria (LAB) and are mainly used on low dry matter forage crops that can have low concentrations of fermentable carbohydrates and high inherent buffering capacities (e.g. grass, alfalfa, clover).

Inoculants that are designed to inhibit spoilage may contain specific LAB, e.g. *Lactobacillus buchneri*, or propionic-acid-producing bacteria. These products are designed for use on materials more prone to aerobic spoilage such as drier haylages (> 35% DM), corn and cereal silages, high moisture corn and cereal grains, and baleage.

Things to consider when comparing silage inoculants include:

- ▶ Is there ample data for the specific product in the target crop from trials conducted at independent research facilities, such as universities, verifying their claims? Are these data statistically analyzed and published in reputable journals and research meetings? These trials should validate efficacy of the product at the application rate it is being sold at and should validate any and all claims made for the product. Without data to validate specific product claims, buyer beware!
- ▶ Is the product manufactured to quality control standards and does the manufacturer have accreditation to show that manufacturing procedures are independently reviewed?
- ▶ Is the product packaged appropriately? Inoculants contain dried viable products and the three enemies of these live products are heat, moisture and air. Prevention from exposure to heat comes down to following storage instructions (see p. 17) but packaging must be designed to prevent exposure of the contents to moisture and air. The use of high barrier foils is one common approach that achieves these goals, as is packaging in sealed tubs. Manufacturers should also use nitrogen flushing during packaging to minimize residual oxygen and include specific preservation agents, e.g. moisture scavengers, in the product formulation.
- ▶ Read and understand the label (Figure 7):
 - ▶ Number of bacteria, application rate, and weight: does data supplied by the company validate the recommended application rate? (Calculations may have to be done to determine the application rate of bacteria on forage [Table 5].) It is generally accepted that fermentation aids containing homolactic acid LAB should be applied at a minimum of 100,000 CFU/g forage. Rates for organisms in spoilage inhibitors vary, though the FDA have allowed that products containing *L. buchneri* 40788 can claim improvement in aerobic stability in silages and high moisture corn (HMC) stored for 60 days, provided the product is applied at a minimum of 400,000 CFU/ g for silage or 600,000 CFU/g

Figure 7: Example of a Forage Inoculant Label

| | |
|---|---|
| <p style="text-align: center;">Acme Sile Water Soluble Concentrate</p> <p style="text-align: center;">A concentrate of selected viable lactic acid producing organisms to aid in the fermentation of all silages</p> <p style="text-align: center;">GUARANTEED MICROBIAL ANALYSIS</p> <p>Total Lactic Acid Producing Microorganisms.....45.4 billion CFU/g</p> <p>(<i>Lactobacillus plantarum</i> AB12, <i>Pediococcus acidilactici</i> CD34)</p> <p>Xylanase 2,500 U/g Alpha-amylase 2,000 U/g</p> <p><small>One unit is the enzyme activity required to liberate on mg of glucose per g per minute.</small></p> <p style="text-align: center;">INGREDIENTS</p> <p>Sucrose, dehydrated <i>Lactobacillus plantarum</i> and <i>Pediococcus acidilactici</i> cultures, dehydrated <i>Trichoderma reesii</i> and <i>Aspegillus niger</i> fermentation products, and sodium silicoaluminate.</p> <p style="text-align: center;">DIRECTIONS FOR USE</p> <p>Mix one pouch (100 grams) of Acme Sile with 25 gallons of water. Apply resulting liquid to chopped forage at the rate of 1/2 gallon per ton of forage and 1 gallon for high-moisture grain. When used at a rate of 1/2 gallon per ton, the resulting product will inoculate at a rate of 100,000 CFU/g of forage. This pouch will treat 50 tons.</p> <p style="text-align: center;">RECOMMENDED STORAGE IS IN A FREEZER OR REFRIGERATOR AT OR BELOW 40F. USE WHOLE PACKETS AT ONE TIME. SHELF LIFE IS 18 MONTHS WHEN STORED AS RECOMMENDED.</p> <p style="text-align: center;">NET WEIGHT: 3.5 OZ (100g)</p> <p style="text-align: center;">Manufactured for Acme, City, State 01234</p> | <p>product form</p> <p>number of bacteria</p> <p>type of bacteria, including strain designation</p> <p>application rate</p> <p>storage instructions</p> <p>weight</p> |
|---|---|

Table 5: Calculations for the Number of Bacteria per Pack of Inoculant and Product Application Rate (CFU/g forage)

bacteria/gram x grams = bacteria in package

example:

$$45.4 \text{ billion CFU/G} \times 100 \text{ g} = 4.54 \text{ trillion CFU/package}$$

bacteria in package/tons treated x 1 ton/908,000 g =

example:

$$4.54 \text{ trillion CFU/50 tons} \times 1/908,000 = 100,000 \text{ CFU/g}$$

for HMC. In the US, for microbes to be legally included in products they must be on the list of organisms approved by the Association of American Feed Control Officials (AAFCO) (Table 6).

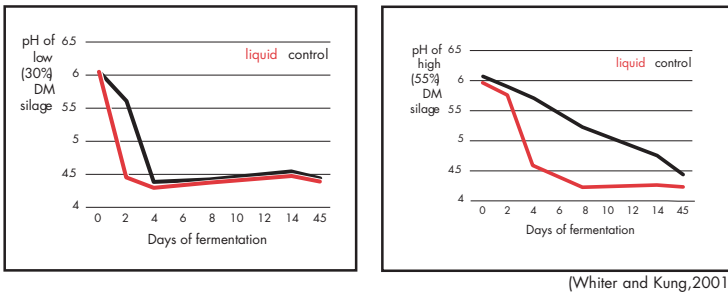
Table 6: Organisms Approved by AAFCO for Use in Animal Feed Products in the US

| | |
|---|---|
| <i>Aspergillus niger</i> | <i>Lactobacillus delbruekii</i> |
| <i>Aspergillus oryzae</i> | <i>Lactobacillus farciminis (swine only)</i> |
| <i>Bacillus coagulans</i> | <i>Lactobacillus fermentum</i> |
| <i>Bacillus lentus</i> | <i>Lactobacillus helveticus</i> |
| <i>Bacillus licheniformis</i> | <i>Lactobacillus lactis</i> |
| <i>Bacillus pumilus</i> | <i>Lactobacillus plantarum</i> |
| <i>Bacillus subtilis</i> | <i>Lactobacillus reuteri</i> |
| <i>Bacteroides amylophilus</i> | <i>Leuconostoc mesenteroides</i> |
| <i>Bacteroides capillosus</i> | <i>Pediococcus acidilactici</i> |
| <i>Bacteroides ruminicola</i> | <i>Pediococcus cerevisiae (dammosus)</i> |
| <i>Bacteroides suis</i> | <i>Pediococcus pentosaceus</i> |
| <i>Bifidobacterium adolescentis</i> | <i>Propionibacterium acidilactici</i> |
| <i>Bifidobacterium animalis</i> | <i>Propionibacterium freudenreichii</i> |
| <i>Bifidobacterium bifidum</i> | <i>Propionibacterium shermanii</i> |
| <i>Bifidobacterium infantis</i> | <i>Saccharomyces cerevisiae</i> |
| <i>Bifidobacterium longum</i> | * <i>Enterococcus cremoris</i> |
| <i>Bifidobacterium thermophilum</i> | * <i>Enterococcus diacetylactis</i> |
| <i>Lactobacillus acidophilus</i> | * <i>Enterococcus faecium</i> |
| <i>Lactobacillus brevis</i> | * <i>Enterococcus intermedius</i> |
| <i>Lactobacillus buchneri (cattle only)</i> | * <i>Enterococcus lactis</i> |
| <i>Lactobacillus bulgaricus</i> | * <i>Enterococcus thermophilus</i> |
| <i>Lactobacillus casei</i> | Yeast (as defined elsewhere) |
| <i>Lactobacillus cellobiosus</i> | |
| <i>Lactobacillus curvatus</i> | *Formerly cataloged as <i>Streptococcus</i> . |

- ▶ Levels of enzymes: if the product claims to include enzymes, guaranteed levels should be declared and they should be the same as those used in trials to validate product efficacy.

- ▶ Shelf life and storage conditions. These should be read, understood and followed. The shelf life of the inoculant is linked to the recommended storage conditions: improperly storing the product could significantly reduce its shelf life and efficacy.
- ▶ Do not use expired inoculant: **Check the expiration date!**
- ▶ Suitability of product form: dry granular application may be easier but is less effective than liquid application as crop DM increases (Figure 8). Granular inoculants should not be used in crops with DM levels above 50% (less than 50% moisture).

Figure 8: Effect of Inoculant Form on Rate of pH Drop in Alfalfa Silage



- ▶ Product stability in the applicator tank or hopper. The bacteria in liquid applied inoculants can die off quite quickly following rehydration unless the product contains a stability enhancer (Figure 9). Do not allow water with bacterial inoculants to reach temperatures above 95-100° F during use. Ask to see the rehydration stability data for any product you are considering. If liquid applied product becomes slimy, it should be discarded (bacteria have died, releasing their DNA and causing the sliminess). Granular, dry applied inoculants also die off in the hopper (Figure 10) as moisture is absorbed from the atmosphere and the temperature increases. The product flow characteristics may also suffer. Consider discarding granular inoculant left over in the hopper at the end of the day to ensure optimum product performance.

Figure 9: Stability of Liquid Applied Inoculants With and Without a Rehydration Stability Enhancer (RSE).

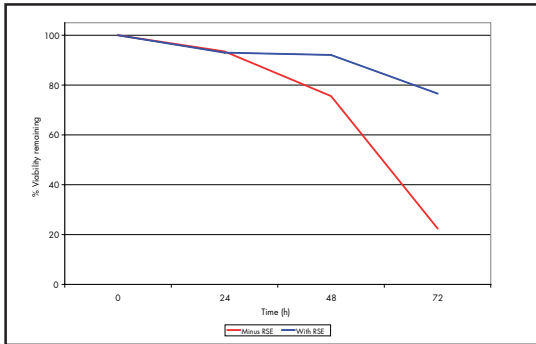
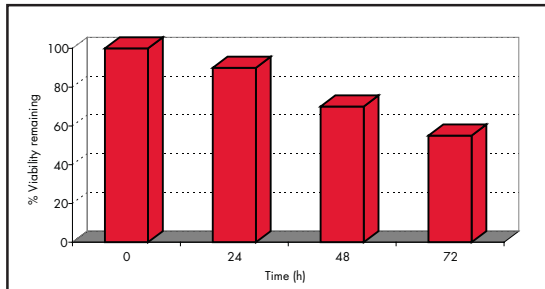


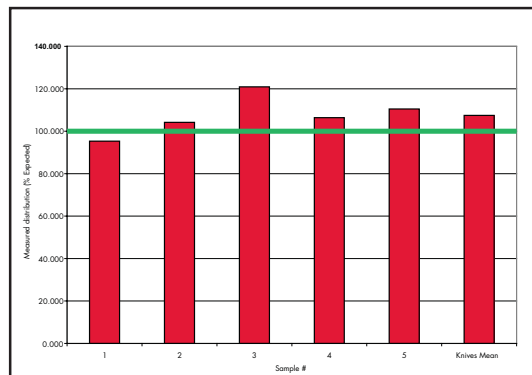
Figure 10: Stability of Granular Silage Inoculant in the Applicator Hopper



- ▶ Does the type of product match your expectations? Do you need a fermentation aid or a spoilage inhibitor? Is there independent data to show that the product can do what you are looking for? Some products require pre-incubation to achieve the correct numbers for product efficacy: consider carefully whether you are prepared for, and capable of, taking on the burden of production and quality control required for this.

- ▶ Calibrate your application rates for liquid and dry-applied inoculants. Application rates should be checked several times a day. Even distribution of the inoculants is a key factor in their ability to help the fermentation process. (Liquid flow rates for different spray nozzles are given in Appendix V.) Products are best applied at the chopper box or accelerator on the harvester. The DE1000 (Dohrmann Industries) is a low volume liquid applicator (1.28 oz per ton) which has been validated as achieving even distribution (Figure 11). The product reservoir on this system is a 10 gallon insulated tank, which helps keep the product cool to maintain viability.

Figure 11: Consistency of Product Application Rate Using DE1000 low volume liquid applicator (red bar shows actual application rates; green line shows theoretical perfect application)



It is generally accepted that using a proven, validated inoculant as part of a good forage management program will give a return on the required financial investment. The guidelines above should help you in the selection process to ensure that the product you select is applied as a live, viable product ready for the ensuing challenges that lie ahead. However, inoculants are not “magic bullets” that will make up for lax management practices: they are one tool to help as part of the overall management program.