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Factors affecting the numbers of expected viable lactic acid bacteria in inoculant applicator tanks

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ABSTRACT

The application of correct numbers of viable microorganisms to forages at the time of ensiling is one of the most important factors affecting the probability of a beneficial effect from an inoculant. The objective of this study was to determine relationships between numbers of expected lactic acid bacteria (LAB) from silage inoculants in application tanks and various factors that might affect their viability. The pH and temperature of inoculant–water mixes were measured in applicator tanks (n = 53) on farms in Wisconsin, Minnesota, South Dakota, and California during the corn harvest season of 2012. Samples were collected on-farm and plated on de Man, Rogosa, and Sharpe agar to enumerate LAB and establish the number of viable LAB (cfu/mL). Expected numbers of LAB were calculated from the minimum label guarantees for viable bacteria and mixing rates with water. In addition, the pH of the inoculant–water mixes at sampling, the ambient temperature at sampling, and the length of time that the samples had been in the tank were measured and obtained. The log difference between the measured and expected numbers of LAB was calculated and expressed as $\Delta D - E$ in log scale. Ambient temperature at sampling had no relationship with time in the tank or $\Delta D - E$. Most (83%) of the inoculants had been mixed with water in the applicator tanks for <10 h. For these samples, a negative linear correlation ($R^2 = 0.36$) existed between time that the inoculant–water mixes were in the applicators tanks and $\Delta M - E$. The pH of the inoculant–water mixes was also negatively correlated ($R^2 = 0.28$) with time in the applicator tank, but pH was not related to $\Delta M - E$. The temperatures of the inoculant–water mixtures were negatively correlated with $\Delta M - E$ ($R^2 = 0.39$). Seven of 8 samples whose ΔD-E were at least −0.95 or more lower than expected (equivalent of about 1 or more log concentration less than expected) had water temperatures above 35°C. These data support our previous laboratory findings and suggest that high temperatures of inoculant–water mixes have the potential to negatively affect the final application rate of some inoculants, which may affect their overall effectiveness to improve silage fermentation.

Key words: lactic acid bacteria, inoculant, silage

INTRODUCTION

Lactic acid bacteria (**LAB**) are commonly used as inoculants to improve the fermentation and aerobic stability of silages (Stokes and Chen, 1994; Muck, 2010). The microbial inoculants are typically mixed with water, held in tanks on the forage harvester, and applied to forages before ensiling. To maximize their potential effectiveness, the correct amount of viable organisms must be evenly applied and distributed onto the forage mass during application. Utilizing inoculants that meet minimum label guarantees for live LAB, correct mixing and application, and proper storage before and during application can ultimately influence the probability of an inoculant affecting the ensuing fermentation. The temperature of the inoculant–water mix and the length of time that the mix is held in the applicator tank could affect the actual application rate of the additive. In the field, inoculant tanks have the potential to absorb heat from solar radiation and from the forage harvester. In a laboratory study, Mulrooney and Kung (2008) reported that the viability of several microbial inoculants was markedly decreased when the temperature of the water that they were in was above 35°C. Thus, the primary objective of this study was to measure the number of LAB in inoculant tanks in the field and determine if a relationship existed between the expected numbers of viable bacteria in the tanks and the temperature of the water that they were stored in or the length of storage time.

MATERIALS AND METHODS

Samples of the inoculant–water mixtures were collected from 53 applicator tanks in Wisconsin, Minnesota,

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South Dakota, and California during the normal corn silage harvest season in September 2012. Temperature ranged from 18.3 to 36°C and humidity ranged from 27 to 96%. Samples were collected from a variety of different types of tanks [6 tanks built into the chopper 2 barrel tanks used with an Ag Bagger (Ag Bag Systems, St. Nazianz, WI), 2 Pioneer low-volume applicators (DuPont Pioneer, Johnston, IA), 5 high-volume, chopper-mounted, clear applicators (manufacturers unknown), and 38 Dohrmann low-volume applicators (Dohrmann Enterprises, Waite Park, MN)]. Choppers were both self-propelled and pull-type choppers. A variety of inoculants from various manufacturers were used (Table 1). To sample the inoculant–water mixes from the applicator tanks, the contents of the tanks were thoroughly mixed for about 20 to 30 s, and samples were collected with a sterile pipette and placed in a sterile collection cup. Tenfold serial dilutions were prepared aseptically in sterile 1/4 strength Ringers solution (Oxoid BR0052G; Oxoid Ltd., Cambridge, UK). Serial dilutions were spread-plated within 5 min of collection on prepoured de Man, Rogosa, and Sharpe agar (CM 3651, Oxoid, Basingstoke, UK) for the enumeration of LAB. Plates were incubated at room temperature (20–23°C) for 3 to 5 d before enumeration of LAB. To account for differences in inoculant concentrations, mixing rates, application rates, and minimum label guarantees, numbers of LAB are reported as the difference between the measured bacterial counts in the tank and the expected bacterial counts calculated from mixing rate and minimum label guarantee of LAB for the various products. This difference was denoted as $\mathbf{\Delta M - E}$ (log cfu/mL). This calculation was made using the original inoculant containers to obtain the minimum label guarantee for LAB. A theoretical value of 0 for $\Delta M - E$ was the result of the measured concentration of LAB from the application tank matching the theoretical calculation based on minimum label guarantee and mixing rate with water. A $\Delta M - E$ value greater than 0 would be obtained if the determined concentration of LAB was greater than expected based on minimum label guarantee, and values less than 0 would mean that determined values were less than expected. Calculation of $\Delta M - E$ was made to eliminate bias because the final applica-

Table 1. The inoculants, distributors, and bacterial species that were enumerated in various inoculant tanks

No. of samples	Inoculant name	Distributor or manufacturer	Bacterial species
3	11C33	Pioneer, Johnston, IA	*Lactobacillus buchneri* *Lactobacillus plantarum* *Enterococcus faecium*
2	Bag Bugs	Walluski Western Ltd., Astoria, OR	*Lactobacillus plantarum* *Enterococcus faecium* *Pediococcus pentosaceus*
4	Biomax LB	Chr. Hansen, Milwaukee, WI	*Lactobacillus buchneri* *Enterococcus faecium* *Lactobacillus plantarum*
6	Biotal Plus	Lallemand Animal Nutrition, Milwaukee, WI	*Pediococcus pentosaceus* *Lactobacillus plantarum* *Propionibacterium freudenreichii*
3	Buchneri 500	Lallemand Animal Nutrition, Milwaukee, WI	*Lactobacillus buchneri* *Pediococcus pentosaceus*
15	Crop-N-Rich MTD1	Vita Plus, Madison, WI	*Lactobacillus plantarum*
2	FermenAider WS	Bio-Vet Inc., Barneveld, WI	*Lactobacillus plantarum* *Pediococcus pentosaceus* *Pediococcus acidilactici* *Lactobacillus brevis* *Lactobacillus casei*
2	Prime SI	VFS, Bakersfield, CA	*Pediococcus pentosaceus* *Lactobacillus plantarum*
3	Promote SI	Cargill Inc., Brookville, OH	*Lactobacillus plantarum* *Pediococcus acidilactici*
2	Feedtech Custom Chop F20	DeLaval, Vernon Hills, IL	*Lactococcus lactis* *Pediococcus pentosaceus* *Enterococcus faecium* *Lactobacillus plantarum*
6	Sil-All 4 × 4 WS 10X	Alltech, Milwaukee, WI	*Lactobacillus plantarum* *Pediococcus acidilactici* *Pediococcus pentosaceus* *Lactobacillus acidophilus*
5	Stage 2	Vita Plus, Madison, WI	*Lactobacillus buchneri* *Pediococcus pentosaceus*