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New trends and opportunities in the development and use of inoculants for silage

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Abstract

Inoculants are used as silage additives to improve preservation efficiency and to enhance animal performance. In most commercially available inoculants, homofermentative lactic acid bacteria (LAB) have been used because they are fast and efficient producers of lactic acid, improving natural silage fermentation. Specific LAB inoculants may also have beneficial effects on animal performance even if there is no effect on fermentation. However, these types of inoculants are not always advantageous. They do not necessarily prevent secondary fermentation by clostridia in moist silages, and they sometimes impair the aerobic stability of grass and small grain silages. Therefore, new criteria for silage inoculants should be established which consider the specific needs of the crop being ensiled. New approaches which are being taken to develop improved inoculants for silage include the following: (1) using LAB isolates which are more specific to the target crops; (2) inclusion of heterofermentative LAB to produce volatile fatty acids to inhibit yeasts and moulds upon aerobic exposure; (3) inclusion of organisms other than LAB in inoculants to inhibit detrimental microorganisms; (4) selection or engineering of LAB strains to inhibit specific microorganisms; and (5) cloning and expression of genes which would enable selected LAB strains to utilize polysaccharides in crops which are low in soluble carbohydrates. Many of these new strategies for formulating inoculants are being tested, but further research is needed to determine the most successful approaches.

Keywords: Silage; Inoculant; Lactic acid bacteria; Aerobic stability; Animal performance

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1. Introduction (The ensiling process)

Silage making is a method of moist forage preservation which is widely used all over the world, accounting for more than 200 million tons of dry matter (DM) stored annually in western Europe and the U.S. [1,2]. It is based on natural fermentation whereby lactic acid bacteria (LAB) ferment water-soluble carbohydrates (WSC) to organic acids, mainly lactic (LA), under anaerobic conditions. As a result, the pH decreases, inhibiting detrimental anaerobes, and so the moist forage is preserved. The process can be divided into four phases with distinct characteristics:

1. Aerobic, when air (oxygen) is still present between the plant particles, and the pH is 6.0–6.5. These conditions enable continuation of plant respiration, protease activity and the activity of aerobic and facultative aerobic microorganisms, such as fungi, yeasts and enterobacteria.
2. Fermentation, lasting several days to several weeks after the silage becomes anaerobic. LAB develop and become the predominant microbial population. Lactic and other acids are produced, and the pH decreases to 3.8–5.0.
3. Stable, when relatively few changes occur if air is prevented from entering the silo.
4. Feedout, when the silage is unloaded and then is exposed to air. This allows re-activation of aerobic microorganisms, mainly yeasts, moulds, bacilli and acetic acid bacteria, causing spoilage.

Each phase of the ensiling process should be controlled in order to maintain silage quality. The sensitivity of the silage to spoilage at the various stages depends on the crop ensiled, the ensiling technology and silo type. Delayed sealing which prolongs the aerobic phase may result in reduction in the WSC available for fermentation and in the LA produced in the silage ([3] pp. 152–155). During fermentation, the rate and extent of pH decline determines the degree of nutrient loss from silage. These losses in quality are caused by activity of both plant enzymes and anaerobic microbes. However, the latter is the more serious in wet forages. If the forage is too moist and pH decline is not sufficient, clostridia, which ferment lactic to butyric acid and amino acids to ammonia, might become active. This process is referred to as 'secondary or clostridial fermentation', and it results in increases in pH and loss of silage DM. On the other hand, if the DM content of the ensiled crop is too high, water activity will be reduced, and the decrease in pH will be delayed by slow LAB growth rates. In pure cultures the lower limit of water activity for LAB growth is 0.93–0.94, which theoretically is attained at 60–75% DM, depending on crop [4].

In the stable and feedout phases, the presence of oxygen is detrimental to silage quality because it enables various aerobic spoilage microorganisms to become active [5]. The microbial populations which are most frequently involved in initiation of aerobic deterioration of silages are yeasts and acetic acid bacteria which are followed by bacilli, moulds and enterococci [6–8]. Their activity results in heat production and DM losses which lower the nutritional value of the silage. Aerobic deterioration will cause an increase in pH through consumption of LA and other fermentation acids. In addition, textural changes, discoloration and other factors decreasing palatability and intake by animals may result ([3] p.

158). Some moulds may produce mycotoxins which are hazardous to the health of animals and humans alike [5].

The susceptibility of silage to aerobic deterioration is determined by physical, chemical and microbiological factors. Management (compaction, sealing, unloading rates) largely affects the movement of oxygen into silage. During feedout, air can penetrate 1–2 m behind the silage face so that exposure to oxygen may be prolonged [9–11]. Fermentation acids and low pH inhibit the rate of microbial growth, but spoilage rates are affected also by microbial numbers and the rate of aerobic microbial growth on the available substrates [12].

It is possible to use both chemical and biological additives in making silage, in order to promote adequate fermentation patterns, especially under sub-optimal conditions. Biological additives comprise bacterial inoculants and enzymes. Bacterial inoculants have advantages over chemical additives because they are safe (non-hazardous), easy-to-use, non-corrosive to farm machinery, do not pollute the environment, and are regarded as natural products. Silage inoculants containing principally lactic acid bacteria have become the dominant additives in many parts of the world not only because of convenience and safety, but because they are expected to control microbial events during silage fermentation. Their function has been to promote rapid and efficient utilization of a crop's WSC, which results in intensive production of lactic acid and a rapid decrease in pH. In addition, inoculants are also marketed as having an influence on reducing aerobic spoilage and improving animal performance.

Studies of silage inoculants focus on two important aspects: their effects on preservation (fermenta-

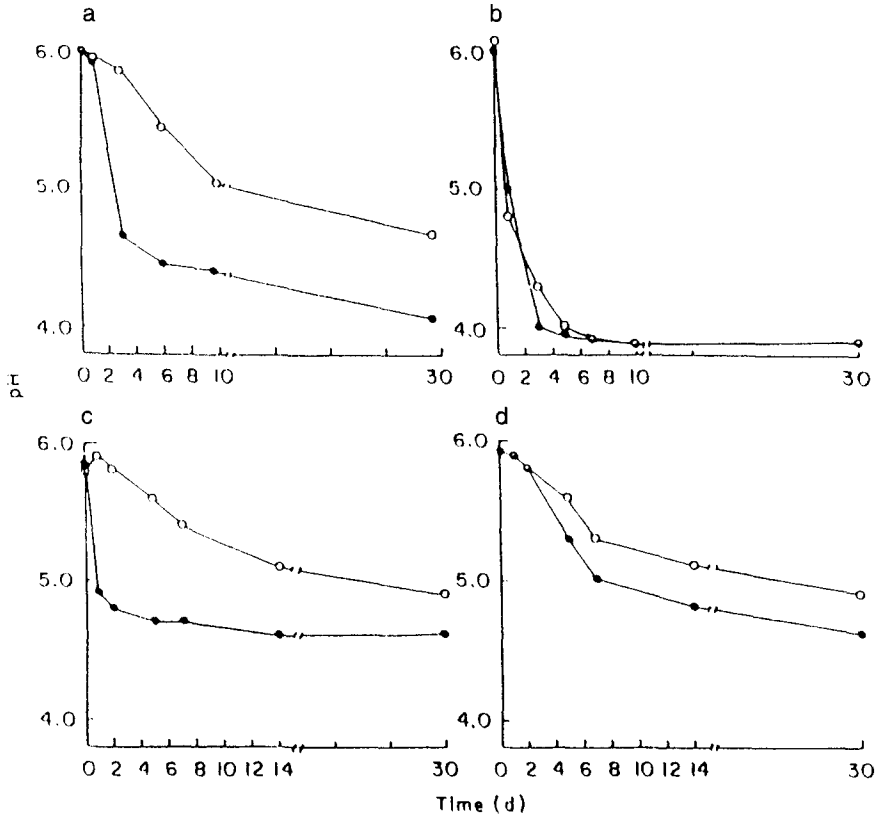

Fig. 1. The effect of an LAB inoculant (*Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus* spp.) on the rate of pH change in various laboratory silages. a, vetch; b, wheat; c, alfalfa; d, wilted alfalfa. ○, control; ●, plus inoculant. Figure reprinted, with permission, from ref. [18].

tion, respiration, effluent, DM recovery) and on the nutritional value of the silage. The objectives of this review are to evaluate the success and limitations of currently available silage inoculants regarding these aspects, to describe recent trends in research and development, and to point out needs and opportunities for further development of silage inoculants.

2. Retrospective of inoculants for silage

2.1. Current inoculant products and their effects on fermentation

The concept of using LAB cultures to enhance silage fermentation developed near the beginning of the century. Early experience with such cultures was not very successful, mainly because the added inoculants did not contain enough live bacteria [13]. It was not until freeze-drying and encapsulation techniques were developed that the use of such inoculants was possible.

Whittenbury [14] defined the criteria which a potential organism should satisfy for use as a silage inoculant: it must be competitive and grow vigorously in the silage, should be homofermentative and produce maximal amounts of LA in short time, should be acid-tolerant, and be able to grow in material of high DM and at temperatures extending to 50°C.

McDonald et al. ([3] pp. 184–236), Spoelstra [13] and Henderson [15] summarized research on silage inoculants through 1990. First, single strain inoculants usually containing *Lactobacillus plantarum* were tried because this species meets most of the criteria presented by Whittenbury and is often isolated from silages. Since the results obtained with this inoculant were not always satisfactory, mixed strain inoculants were tried that include LAB that are more active at early stages of fermentation when the pH is still above 5.0. Among the LAB most frequently used along with *L. plantarum* are *Enterococcus faecium* (which also grows rapidly under aerobic conditions), *L. acidophilus*, *Pediococcus acidilactici*, and *P. pentosaceus*. In some cases, a simple sugar is added to serve as an immediate substrate, or enzymes such as amylase or cell-wall degrading cocktails are included which would produce additional fermentable sugars. The inoculation rates of these products as stated by the manufacturers are usually 10^5–10^6 viable cells/g, which is often sufficient for the inoculant LAB to outgrow the epiphytic LAB and become the predominant population in the silage. Pahlow [16] has suggested an inoculation factor (IF) of 2 (two-fold increase in LAB) in order to achieve a consistent positive effect.

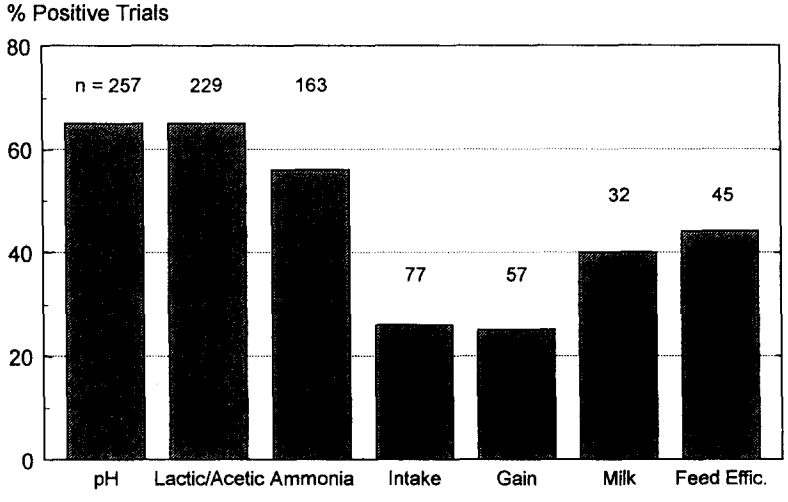

Fig. 2. Percentage of trials in published research (1985–1992) where silage inoculants significantly improved fermentation or animal performance. Number of trials per characteristic is above each bar [19].

Reports on trials with such LAB inoculants that either were or are currently available to farmers are numerous. Because research on these products has been reviewed by others, we will primarily summarize the results of those reviews before addressing new approaches in inoculant formulation.

Experiments that have reported on inoculants formulated based on the Whittenbury approach have been performed using a wide variety of strains in different containers and in different combinations with other treatments, which makes a comparison difficult. Also, it should be kept in mind that most of these trials were performed under ideal laboratory conditions in air-tight mini-silos, which might be somewhat different than farm-scale silos with regard to anaerobiosis, degree of compaction, heat transfer, and unloading rates. These factors may affect fermentation patterns (for example, when some air is present, homofermentative LAB will ferment hexoses to lactic and acetic acids, [17]) and/or final silage quality. Nevertheless, when the inoculant LAB overwhelmed the epiphytic bacteria, fermentation was affected in a reasonably consistent manner: a faster decrease in pH, lower final pH values, higher lactate:acetate ratios (obtained by increasing lactic and decreasing acetic acid production), lower ammonia nitrogen (probably from selecting LAB strains that did not ferment amino acids and from reduced protein breakdown as a result of a faster decrease in pH), and a 1–2% improvement in DM recovery (most likely from reduced heterofermentation) [3,13,15]. A typical pH response of various laboratory silages to a multi-strain LAB inoculant is given in Fig. 1, and a summary of many inoculant studies is shown in Fig. 2. Consequently, when these inoculants succeeded, fermentation was altered in a manner consistent with expectations based on the Whittenbury approach.

Table 1
The effect of LAB inoculants on the nutritional properties of low dry-matter (15–22%) grass silage and on animal performance

Source	Inoculant composition	Effect on fermentation [a]	Effect on digestibility [b]	Effect on animal performance [c]
[21]	*L. plantarum* MTD1 (10^6) [d]	+ [e] * * *		DMI + * * *, MY + * * (cows)
[22]	*L. plantarum* MTD1 (10^6)	0, both good		DMI +, MY + * * (cows)
[23]	*L. plantarum* MTD1 (10^6)	+ * *	+ /0 (wethers)	
[24]	*L. plantarum* MTD1 (10^6)	+ * *	− * *	DMI 0, LWG 0 (steers)
[25]	*L. plantarum* MTD1 (10^6)	0, both poor	0	DMI + * *, LWG + * * (growing cattle)
[26]	*L. plantarum* MTD1 (10^6)	+ * *	+ * *	DMI + * *, MY + * *, LWG + * * (cows)
[27]	*L. plantarum* MTD1 (10^6)	0	+ * *	DMI 0 (growing cattle)
[28]	*L. plantarum* MTD1 (10^6)	+ * *	+ * *	DMI 0, LWG + (beef heifers)
[29]	*L. plantarum* MTD1 (10^6)	+ * *	+ * *	DMI + *, LWG + (beef heifers)
[30]	*L. plantarum* MTD1 (10^6) [f]	+		DMI 0, MY + (cows)
[31]	*L. plantarum* MTD1 (10^6)	+ * *		DMI + * * * (wethers)
	L. plantarum 6A6 (10^6)	+ * *		DMI 0 (wethers)
	Pediococcus spp. 6A2 (10^6)	+ * *		DMI 0 (wethers)
[32]	*L. plantarum, Ent. faecium* (10^5)	+ * *		DMI + * * * (heifers)
[33]	*L. plantarum* (10^7)	+ * *		DMI +, MC + (cows)
[34]	*L. plantarum, Ent. faecium, P. acidilactici* (10^6) plus enzymes	+	0	LWG +, MY − * * (lambs)

[a] Fermentation improvement = lower final pH, faster fermentation rate, and/or higher lactate:acetate ratio.
[b] Digestibility improvement = higher DM, organic matter, crude protein and/or fiber digestibilities.
[c] DMI = dry matter intake, LWG = liveweight gain, MY = milk yield, MC = milk composition
[d] Number in parenthesis gives inoculation rate in cfu/g forage.
[e] +, 0, − designates improvement, no effect and decrease relative to the control, respectively.
*, * *, * * * = significant at $P < 0.1$, 0.05 and 0.001, respectively.
[f] Wilted to 35% DM.

2.2. The effects of LAB inoculants on animal performance

A surprising effect of inoculation has been that on animal performance. Although significant improvements in animal performance have been found less frequently than changes in fermentation, increases in performance have been substantial. Muck [19], in reviewing numerous inoculant studies for 1985–1992, reported that the incidence of significant ($P < 0.1$) positive responses for intake, liveweight gain, milk production and feed efficiency was 25, 25, 40 and 45%, respectively (Fig. 2). In positive trials increases of these parameters were 11, 11, 5 and 9%, respectively [19]. In an earlier review [13] the average increase in intake, gain and milk production was 4, 7, and 3%, respectively. The rate of statistically significant results was about one third. The reduced instances of improvement in animal performance might be related to IF. Satter et al. [20] found in their experiments with lucerne silages that an IF > 10 increased milk production by approximately 3%, whereas no improvement in milk production was observed at IF < 10. This IF value is five times higher than that needed to consistently affect fermentation as reported by Pahlow [16].

The cause of improved animal performance is unclear. The shifts in fermentation products and small improvements in DM recovery would enhance animal performance but not to the degree observed. Muck [19] reported a high degree of correlation between effects on DM digestibility and animal performance. He also noted that fiber digestibility was improved in 30% of the trials where it was measured. This is certainly unexpected in that the LAB used in inoculants are not known to have any direct activity on polysaccharides.

The effect of LAB inoculants on animal performance has been addressed in many studies. These experiments tested various inoculants in different silages, and therefore a comparison is not possible. However, a considerable number of animal experiments using a single silage inoculant comprising *L. plantarum* MTD1 have been reported, and these studies provide some insight as to how this inoculant may be improving animal performance. Summaries of recent studies with this inoculant in low DM grass silages are shown in Tables 1 and 2 [21–31]. In six out of ten studies reporting on MTD1, there was an improvement in both silage fermentation and animal performance (Table 2). However, in two studies there was an improvement in animal performance with no apparent effect on fermentation (one of which reported good quality in both control and treated silages and one with clostridial fermentation in both silages), whereas there was only one study with an improvement in fermentation but no effect on animal performance. This was surprising because precision in measuring fermentation products and pH makes it much easier to find statistically significant fermentation effects than animal performance effects. Furthermore, improvements in animal performance were poorly related to improvements in digestibility (Table 2), in contrast to what was observed in a review summarizing all inoculants [19]. These results suggest that one effect of MTD1 on animal performance may be a probiotic effect, the mechanism of which is as yet unclear. This suggestion is bolstered by a recent study [31] where MTD1 was compared with two other LAB strains. All three strains improved fermentation similarly, but only MTD1 had a positive effect on animal performance, which indicates that this phenomena might be strain specific.

Recently, Keady and Steen [28,29] also measured silage degradabilities and analyzed the rumen liquor of calves fed MTD1-inoculated grass silage. Inoculation did not have any effect on DM degradability at any flow rate; however, nitrogen degradability increased, and the authors suggest that this might be related to liberation of slowly degradable nitrogen associated with modified cell-wall structure. With regard to rumen liquor content, the inoculant treatment increased the total volatile fatty acid (VFA) concentration and tended to decrease butyrate and

Table 2
Summary of studies in Table 1 with *L. plantarum* MTD1 inoculant applied to grass silages: interaction of effects on animal performance with effects on fermentation and digestibility

		Fermentation improvement		Digestibility improvement	
		No	Yes	No	Yes
Animal performance	No	1	1	1	2
Improvement	Yes	2	6	1	3

increase propionate contents. This also might be related to nitrogen metabolism in the rumen.

How similar MTD1 is to other inoculants with regard to animal performance effects is not known. For other inoculants such a broad dataset could not be constructed from recent literature. However, significant increases in DM intake were observed also by Sharp et al. [32] who used a different inoculant in low DM grass silage comprising strains of *L. plantarum* and *Ent. faecalis* applied at 10^5 cfu/g. Studies with other LAB inoculants gave mixed results [33,34].

Recent studies of effect of LAB inoculants on the nutritional value of silages other than grass are summarized in Table 3 [35–42]. The experiments described used different inoculants applied at various rates under various ensiling conditions, and therefore, comparisons are also not possible. The effects of LAB inoculants in the drier silages on animal performance were variable. Stokes [36] found reduced DM intake of inoculated silages due to their poor aerobic stability. Kung et al. [38] who used two different inoculants in corn silage observed tendencies for higher fat corrected milk yields and higher DM intake only with MTD1, which agrees with the results obtained with low DM grass silages.

2.3. Shortcomings of LAB inoculants

Inoculants developed under the Whittenbury approach have not always been successful in improving silage quality. Lack of success can be divided into two general areas: failure to dominate fermentation and failure to inhibit adverse microbial activity.

Failure to dominate the fermentation has already been partially addressed. Five factors could lead to an apparent failure of an inoculant to dominate the fermentation.

1. Certainly the most important aspect is the epiphytic LAB population. Both Pahlow [16] and Satter et al. [20] have found that consistency of inoculant performance is related to IF. However, IF should be viewed only as an approximate guide because the epiphytic LAB population is quite variable in growth rate, substrates utilized and products formed [43].
2. The inoculant may have dominated the fermentation in the treated silages, but the control silages were of similar good quality. Therefore, no beneficial effects of the inoculants were detected. Such an explanation cannot be documented but certainly can be suspected in corn silages, for example, which often naturally have a high lactate:acetate ratio. Lin et al. [44] who studied the epiphytic LAB in corn and lucerne silages concluded that "knowledge of the numbers and species of LAB did not predict the outcome of the silage fermentation, and hence, characterization of the epiphytic LAB strains and chemical composition of the ensiled crop, particularly their WSC profiles and buffering capacity, are necessary".
3. The inoculants were infected by phage. While bacteriophages have been a major concern in the dairy-product industry, their presence in silage has been largely overlooked. Tanaka et al. [45] studied the presence of bacteriophages infecting silage-making lactobacilli and their relationship to fermentation quality in ryegrass silages. Twenty five percent of the silage samples contained phages infectious mainly to strains of *L. plantarum* but also to *L. casei*. Phages were detected mainly in high moisture silages, and their presence was associated with poor quality silages. These results may partially explain failures in silage making or of inoculants.
4. The inoculant's strains did not grow well on the target crop. Hill [46] isolated strains of *L. plantarum* from corn, lucerne and sorghum. When these three strains were co-inoculated on these crops, the strain originally isolated from a specific crop could be detected in the silage of that crop in higher numbers than the other two. This suggests that the best isolates for a specific crop would come from that crop. We also have circumstantial evidence on wheat silages that this is true. In several trials commercial forage inoculants failed to improve rate of pH decline even with IF > 10 (e.g. [18]).
5. Technical problems such as the LAB not being viable at the time of application (because of too long storage, heat damage, etc.), uneven mixing of the inoculant and the forage, low ambient temperatures and slow filling of the silo.

Many of the concerns regarding the failure of

Table 3
The effect of LAB inoculants on the nutritional properties of silage and animal performance

Source	Silage	%DM	Inoculant composition	Effect on fermentation [a]	Effect on digestibility [b]	Effect on animal performance [c]
[35]	HMC [d]	72	*L. plantarum, Ent. faecium, P. acidilactici* (2×10^6) [e]	+, AS−	0	DMI+, LWG 0 (steers)
[36]	grass:legume	30–35	*L. plantarum, L. brevis, P. acidilactici, Lact. cremoris, S. diacetylactis*, ± enzymes (2×10^5)	+ **, AS−	0	DMI− **, MY− **, LWG− (cows)
[37]	corn	32	*L. plantarum, Ent. faecium* (1.1×10^5)	+, AS−	0	
	forage, sorghum	29	*L. plantarum, Ent. faecium* (1.1×10^5)	+, AS−	0	
[38]	corn	33	*L. plantarum, Ent. faecium* (10^5)	0		0 (cows)
			L. plantarum MTD1 (10^5)	+ *		FCM+ (cows)
	corn	40	*L. plantarum* MTD1 (10^5)	0		FCM+, DMI+ ** (cows)
[39]	HMEC	64–73	combinations of *L. plantarum* MTD1 and *Serratia rubidaea, Bacillus subtilis, S. thermophilus* (3×10^5)	+, AS+ **	−	DMI 0, LWG 0 (steers)
[40]	wheat	21, 28, 39	*L. plantarum, Ent. faecium* (10^4–10^5)	+ **	0	
[41]	lucerne	40, 55	*L. plantarum* (0.4×10^6)	0	0 (sheep)	DMI 0 (steers, sheep)
[42]	corn		*L. plantarum, Ent. faecium* (10^5)		+ (wethers)	DMI+, LWG+ ** (growing bulls)

[a] Fermentation effect = lower final pH, faster fermentation rate, and/or higher lactate:acetate ratio.
[b] Digestibility effect = higher DM, organic matter, crude protein and/or fiber digestibilities.
[c] DMI = dry matter intake, LWG = liveweight gain, MY = milk yield, FCM = fat-corrected milk.
[d] HMC = high moisture corn, HMEC = high moisture ear corn, AS = aerobic stability.
[e] Number in parenthesis gives inoculation rate in cfu/g forage.
[f] +, 0, − designates improvement, no effect and decrease relative to the control, respectively.
*, **, *** = significant at $P < 0.1$, 0.05 and 0.001, respectively.

inoculants to dominate the fermentation have been addressed and minimized by the level of LAB in these products. A more serious concern is the issue of these products failing to prevent adverse microbial activity. Two types of microbial activity can be problems: clostridial growth and aerobic microorganisms. In very moist silages, such as those frequently made in northern Europe, LAB inoculants may not consistently lower pH enough to inhibit clostridial activity [47,48]. The wetter the silage, the higher the water activity of the crop and thus the lower the pH required to inhibit clostridial growth [49]. Consequently in low DM crops, low sugar contents and/or high buffering capacities may prevent even the efficient fermentation of inoculant LAB from reaching a sufficiently low pH.

Regarding aerobic stability (AS) or resistance to aerobic spoilage, studies have shown that commonly used LAB inoculants comprising homofermentative species sometimes impair the AS of silages (e.g. Kennedy [50], Honig et al. [51], Wyss [52,53], Weissbach et al. [54], Sanderson [37]). Weinberg et al. [55] found that homofermentative LAB inoculants enhanced the aerobic deterioration of mature cereal silages whereas legume silages remained stable. They hypothesized that "high levels of residual WSC, combined with high LA concentrations and lack of protective volatile fatty acids (VFAs, such as acetic, propionic and butyric) in the inoculated silages were associated with aerobic spoilage. This is because both WSC and LA are substrates for moulds and yeasts and VFAs inhibit these organisms." From a theoretical view, this would be expected because at a given pH lactic acid is less inhibitory than acetic acid to yeast growth. Because yeasts are affected only by the unionized form of these acids, the concentration of either acid becomes more inhibitory as pH is lowered [12]. Consequently, if an inoculant lowers pH sufficiently to overcome the negative effects of the shift from acetic to lactic acid production caused by the inoculant LAB, AS may not be affected or may be slightly improved. However, cereal or corn silages normally have sufficient sugars so that fermentation is stopped by low pH with or without an inoculant. In these cases, differences in pH most likely are insufficient to counter the negative effects of the inoculant on AS caused by the shift in fermentation products.

3. New trends in the development of inoculants for silage

Because homofermentative LAB inoculants do not always prevent or reduce undesirable microbial activity, a variety of new approaches are being tried. These include the selection of strains that better match the target crops and consider specific problems such as secondary fermentation in moist crops and AS. They are summarized in Table 4 and can be described as follows.

3.1. Targeting of novel homofermentative LAB strains to specific crops

Albrecht et al. [56] used combinations of strains of LAB species isolated from grass silages that are usually not included in commercial LAB inoculants. Among the species tried were *L. delbrueckii*, *L. casei*, *L. rhamnosus* in addition to standard inoculant species such as *L. plantarum*, *P. acidilactici* and *E. faecium*. A strain of *L. casei* produced the highest levels of lactic acid and lowest pH. Some combinations, including the combination of *L. delbrueckii*, *L. casei*, *L. rhamnosus* and *E. faecium*, gave good results with regard to conservation of grass silage. Results by Idler et al. [57] indicated that inoculants comprising *L. casei* plus *L. rhamnosus* also improved digestibilities of crude protein and crude fiber. These results suggest that other homofermentative species may have value as inoculant strains. However, potential effects on aerobic stability and animal performance were not investigated.

Another group [58] screened *Pediococcus* strains for potential use as inoculants in grass silage. One strain (*P. acidilactici* G24) was most effective and stimulated the epiphytic *L. plantarum* population in ensiled grass which contained 24 g/kg WSC, but not in grass with only 8 g/kg WSC.

Grant et al. [59] isolated a strain of *L. plantarum* from pickle fermentation which was efficient in grass-legume silage but not in corn silage. The lack of effect on corn appeared primarily due to high epiphytic LAB populations on the corn. In one trial, the new strain was tested against two commercial inoculants, providing results better than one of the commercial products.

L. pentosus was isolated recently from Israeli

Table 4
Summary of recent approaches and development in silage inoculants

Source	Crop	Inoculant	Novelty
D. Spangler (personal communication)	wheat	*L. pentosus* isolated from wheat in Israel	Wheat is rich in hemicellulose which yields pentose sugars during ensiling. *L. pentosus* ferments pentoses to LA and acetic acids and therefore, might improve AS
[56,57]	grass	combination of 16 various LAB strains comprising *L. plantarum, L.casei, P. acidilactici, Ent. faecium, L. rhamnosus, L. delbrueckii*	*L. casei* was most efficient in decreasing pH, build-up of LA and lower acetic acid. A combination of *L. casei* + *L. rhamnosus* + *Ent. faecium* + *L. delbrueckii* was also efficient in terms of silage conservation
[58]	grass	*P. acidilactici* G24 isolated from grass	Efficient in terms of silage fermentation in crops with sufficient fermentable sugars
[59]	grass-legume, corn	*L. plantatum* isolated from pickle fermentation	Strain was efficient in grass-legume silage but not in corn silage
R. Muck	corn	*L. buchneri* isolated from lucerne	Should be active at late stages of ensiling and produce lactic and acetic acids. The latter inhibits yeasts and moulds upon aerobic exposure
[63–66]	grass, high moisture corn, whole plant corn, millet, sorghum and wheat	propionic acid bacteria (PAB)	PAB produced propionic acid and improved AS only when the decrease in pH was slow and moderate
[67]	grass, wheat	*L. plantarum* + *P. acidilactici* + *Serratia rubidaea* + *Bacillus subtilis*	*S. rubidaea* and *B. subtilis* produce substances that inhibit yeasts and moulds. Aerobic stability of grass silage was improved, but in experiment with wheat control silage was also stable
[68]	grass (aerated)	*P. acidilactici* JBL 1096	Bacteriocin producing strain. Controlled *L. monocytogenes* during first 14 days of ensiling but not afterwards
[69]	grass	*L. plantatum*, genetically engineered to possess amylase activity cloned from *L. amylovarus*	Can use starch when WSC are scarce
[70]		cloning of genes for cellulase and xylanase activity in *L. plantarum*	Enzymes to release fermentable structural carbohydrates

LA = lactic acid, AS = aerobic stability, WSC = water-soluble carbohydrates.

wheat (D. Spangler, 1995, personal communication). Wheat is relatively rich in hemicellulose which is partially hydrolyzed during ensiling to yield pentose sugars. *L. pentosus* is capable of fermenting these sugars to lactic and acetic acids. The enhanced acetic acid content should help protect the silage upon aerobic exposure. The advantage of this method of producing acetic acid is that no loss of dry matter occurs in this fermentation. This organism is currently being tested in wheat silages.

3.2. Inclusion of heterofermentative LAB in an inoculant

L. buchneri has been tested in corn silage and has improved AS by apparently converting lactic acid to acetic acid when oxygen becomes present (Muck, unpublished data). With such a mechanism, one-third of the lactic acid DM consumed will be lost as CO_2. However, a small loss of 1% or perhaps up to 2% DM may easily offset much larger losses by aerobic microorganisms.

Another potential concern with such species would be effects on animal performance. The effects of lactate on intake are mixed whereas there is stronger evidence that acetic acid depresses intake [60,61]. However, work by Phillip et al. [62] with sheep being intraruminally infused with either corn or corn silage extract suggests that the effects of silage acids on intake may be caused by their raising of rumen osmolality. If this is true, then at least intake should not be affected by the conversion of lactic acid to acetic. Similarly, *L. pentosus* would not be expected to adversely affect intake. Nevertheless, the absence of any potential negative effects on animal performance will need to be confirmed before either strain would be commercially viable.

3.3. Use of organisms other than LAB for silage inoculants

An example is the application of propionic acid bacteria (PAB). It was hoped that such an inoculant would produce propionic acid in the silage and inhibit yeasts and moulds upon aerobic exposure. Wyss et al. [63] used an inoculant comprising lactate and propionate producing organisms in wilted grass silage (DM = 39%) which was exposed to air at different stages of fermentation. The delayed sealing of the silage apparently slowed LA production and pH decline, and under such conditions their inoculant improved AS. Dawson et al. [64] showed that a PAB inoculant used in ensiled high-moisture corn grain produced significant amounts of propionic acid and reduced yeast and mould counts. In this type of silage (DM > 70%), the decline in pH is slow so that the PAB could perhaps be more easily established. Weinberg et al. [65,66] tried *Propionibacterium shermanii* in millet, corn, sorghum and wheat silages. The PAB produced propionic acid only in a wheat silage in which the pH decline was delayed, and in that case AS was improved. In all other silages, the pH decline was rapid, the PAB could not proliferate, and no improvement in AS was detected. Consequently it appears that the success of current PAB strains depends on maintenance of a high pH so that PAB can grow to significant levels.

Another product [67] contains selected strains of *Serratia rubidaea* and *Bacillus subtilis* along with *L. plantarum*. The former strains were included in the inoculant because they inhibit yeasts and moulds by secretion of bacteriocin-like substances which are not fully characterized. When used in big bale grass silages, the numbers of moulds decreased significantly. An inoculant containing combinations of these species was used in high moisture ear corn and resulted in improved AS [39]. A similar product was developed for wheat silages which also contained, along with the above mentioned microorganisms, *P. pentosaceus*. The *Pediococcus* species is capable of fermenting pentose sugars which result from hemicellulose hydrolysis in wheat silages. In a single trial with wheat silage, no improvement in AS was observed because the control silages were also stable. However, this inoculant did not impair aerobic stability (Weinberg et al., 1994, internal report).

3.4. Selection of LAB strains for their ability to control pathogens in silage

Fenlon et al. [68] challenged a bacteriocin-producing *P. acidilactici* (strain JBL 1096) with *Listeria monocytogenes* in aerated grass silages. The pediococci inhibited the listeria in the initial 14 days of ensiling but not afterwards.

3.5. Genetic manipulation

Several groups are working at developing LAB strains with the ability to degrade polysaccharides. Fitzsimons et al. [69] transferred a gene coding for amylase activity from *L. amylovarus* to strains of *L. plantarum* by either integration to the chromosome or to an autoreplicative plasmid. The recombinant strains exhibited stable amylase activity and grew well in pure cultures in media containing starch. The authors hypothesized that such bacteria may have a potential as silage inoculants for crops low in WSC and high in starch.

Cloning and expression of genes for cellulase and xylanase activity in *L. plantarum* has been demonstrated in studies in culture media [70]. Such enzymes were inactivated when the pH decreased below 4.5. If the transformed strains were to be applied as silage inoculants in crops low in WSC, the cellulase and xylanase activity would release fermentable sugars from structural carbohydrates, promoting the ensiling fermentation. The pH inactivation would prevent the overdigestion of crop fiber.

This latter feature would be advantageous. Yeasts and moulds grow more rapidly on sugars than fermentation products [12] so that producing excess sugar may have a detrimental effect on AS. A review of cell-degrading enzyme additives for silage [19] found that enzyme products could worsen as well as improve AS, depending on the case. It was hypothesized that increasing fermentation products and lowering pH would have a positive effect on AS whereas excess production of sugars could possibly negate such benefits.

4. Discussion

The ensiling process is complex and comprises interactions of different chemical and microbiological processes. Also, different silages and different methods of ensiling present a variety of needs. Therefore, the development of inoculants for silage is not as simple as was perceived a generation ago. When Whittenbury [14] formulated his criteria for silage inoculants, the main objective was to improve the fermentation stage. This was enhanced by using homofermentative LAB that ferment WSC efficiently, resulting in a rapid decrease in pH, a fast build-up of LA and stabilization of the silage with minimal losses of nutrients. However, research over the years has shown that such inoculants do not meet the whole range of requirements presented by different types of silages.

Therefore, new criteria for silage inoculants should be formulated that address specific needs. New approaches in the development of silage inoculants should consider both fermentation efficiency, aerobic stability and animal performance. Because ensiling problems vary, the criteria for new silage inoculants may include crop specificity, DM content of the target silage and silo type.

Crop specificity may be important for two primary reasons: firstly as shown by Hill [46], inoculant strains were most competitive when used on the plant species from which they were isolated. Different strains even of the same species do not have identical properties and vary in their fermentation characteristics ([3] p. 288). Secondly, crops vary in potential ensiling problems. For example, corn and small grain cereal silages tend to have poor aerobic stability. In contrast, legume silages are stable upon aerobic exposure because they might contain a protective factor not yet defined [71,72]. In legumes, protecting protein, improving fiber digestibility and/or inhibiting clostridial fermentation may be of greater concern.

In addition to crop type, some silages are more susceptible to aerobic deterioration than others because of silo type or ensiling conditions. For example, some silo types are more susceptible to air penetration than others, such as bale silages in which surface area to volume ratios are greater. Also crops ensiled too dry for the type of silo or silages removed at too slow a rate will be prone to aerobic spoilage. For these cases as well as for susceptible crops, inoculants should be developed that are also beneficial during aerobic exposure and have the ability to inhibit yeasts, moulds and/or acetic acid bacteria. As shown in the current review, various species are being tested that might not be as efficient in the fermentation stage of ensiling but may be advantageous during aerobic exposure. A combination of homofermentative LAB and such organisms could perhaps improve overall inoculant performance under conditions where aerobic losses are a particular problem.

For moist crops (DM < 25%), the problem of clostridial fermentation should be addressed. Are there vigorous LAB strains that rapidly decrease the pH below 4.0 by using polysaccharides or other carbon sources in the plant that current strains do not use? Some of the genetic engineering approaches previously mentioned (Table 4) may yield LAB that could be successful is this manner. Is it possible to find strains that specifically inhibit clostridial bacteria like the approach taken by Fenlon et al. [68] for finding activity against listeria?

Considerable effort should be devoted to understanding how inoculants affect animal performance. This is not just for the sake of scientific curiosity, but because improvements in animal performance are in many cases the principal economic justification for inoculant use. Earlier reviews ([3] pp. 285–289, [13,19]) have noted that inoculants by virtue of shifting LAB fermentation to homofermentative pathways should improve rumen fermentation in the animal and possibly have some positive effect on palatability. Such effects, however, would not appear adequate to explain some of the observed increases in animal performance. An earlier review [19] and this one have noted that some studies have found improvements in DM and fiber digestibility. This may help explain enhanced animal performance in some cases. However, from Tables 1 and 2, there are clearly cases where increases animal performance in the inoculated treatment cannot be explained by typical means because neither fermentation nor digestibility were affected by the inoculant. This suggests that a probiotic effect may be an important mechanism in some inoculants.

A probiotic is defined as a culture of microorganisms, which when applied to man or animal, beneficially affects the host by improving the properties of the indigenous microflora. It is speculated that they have the potential to inhibit pathogens and to detoxify carcinogens [73]. It is also well known that LAB produce a variety of antimicrobial substances such as bacteriocins [74,75]. Thus they might inhibit detrimental microorganisms both in silage and the rumen. Wallace and Newbold [76] summarized experiments in which lactobacilli or enterococci were used as probiotics in calves and lambs. When live bacteria were used, counts of coliforms in the rumen decreased, incidents of scouring in calves decreased, and improved intake and liveweight gain were observed. Perhaps the probiotic effect could explain animal performance improvements in silages where silage quality by normal measures appeared to be unaffected by inoculation. Hypothetical modes of action of this effect might be either at the ensiling level or in the rumen, where specific LAB strains produce beneficial substances which promote specific rumen microbial populations, resulting in enhanced animal performance. Clearly more research in this area is warranted and could lead to inoculants with enhanced animal performance benefits.

Genetic engineering could play an important role not only in developing new inoculant strains but in determining how inoculants function. Novel techniques of molecular biology could enable investigators to trace inoculant strains in the silage or in the rumen to study their effect on rumen microbial populations. An important question in this context is whether the inoculant's LAB strain(s) survive rumen conditions. Such studies could employ plasmid-mediated marking of strains with resistance to antibiotics [77,78] and/or the use of strain-specific probes of plasmid DNA or ribosomal RNA [79,80]. These techniques could enable investigators to distinguish between the indigenous microbial population and the added strain. The latter technique would permit identification and quantification of specific strains without the need to culture them and has been used in studying rumen microbial ecology [81,82].

In summary, research studies have indicated that the many commercial products that are available currently as silage inoculants generally improve fermentation but do not always satisfy the diversity of needs mentioned here. A variety of approaches are presented that are either under testing or are proposed that could serve as guidelines for the silage inoculant industry. The efficacy of the new products will need to be proven in both laboratory and field experiments, and such testing must also consider effects on animal performance.

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