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Characteristics of the indigenous microflora from five silage crops in 1987 (1990)

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CHARACTERISTICS OF THE INDIGENOUS MICROFLORA FROM FIVE SILAGE CROPS IN 1987[1]

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Summary

Indigenous lactic acid bacteria (LAB) were isolated from five silage crops in the 1987 growing season: wheat, alfalfa, corn, interseeded grain sorghum and soybeans, and forage sorghum. All crops had post-harvest LAB counts that exceeded 5×10^5 colony-forming units/g. There were no significant correlations between rate of fermentation during the first 7 d post-ensiling and the indigenous LAB counts. However, corn and sorghum, which fermented rapidly, had higher populations of homofermentative LAB, and the isolates showed higher rod to cocci ratios compared to the other three crops. Most of the homofermentative rods isolated were *Lactobacillus plantarum*, and most of those isolates had slow growth rates and narrow growth temperature ranges. A variety of heterofermentative lactobacilli were isolated from all five crops. Two unidentifiable *Streptococcus* species were isolated from wheat and alfalfa.

(Key Words: Microflora, Silage, Lactic Acid Bacteria.)

Introduction

In the absence of supplemental LAB, silage fermentation is controlled primarily by the indigenous (naturally occurring) microflora present when the crop enters the silo. Several researchers have studied the indigenous microflora from individual crops, but to our knowledge, few have reported comprehensive results involving several crops in one geographical region. Our objectives were to isolate and identify indigenous LAB from several Kansas crops during the 1987 growing season. These isolates were then compared to each other and to commercial silage inoculant strains for growth rates, temperature optima, and acid-producing capabilities.

Experimental Procedures

All five silages made were from crops grown under dryland conditions near Manhattan in 1987. A description of the crops and their chemical compositions are presented in Table 39.1. The LAB isolated from the post-harvested, pre-ensiled samples of the five crops were identified and characterized according to the diagram shown in Figure 39.1. The five crops

[1]Partial financial and laboratory assistance and commercial silage inoculant strains were provided by Chr. Hansen's Bio Systems, Milwaukee, Wisconsin.
[2]Project Leader and Research and Development Manager, respectively, Chr. Hansen's Bio Systems.

Table 39.1. Description, Harvest Date, Stage of Maturity, and Chemical Composition of the Five Silage Crops

Item	Arkan wheat	Riley alfalfa	Pioneer 3183 corn	DeKalb 42 sorghum + Pershing soybeans	Pioneer 947 forage sorghum
Harvest date	June 4	July 31	August 19	August 22	September 4
Stage of maturity	Dough	10% bloom	Mid-dent	Late-dough	Late-dough
Dry matter, %	41.0	39.6	37.5	34.4	35.2
Buffer capacity[1]	27.3	54.5	18.3	25.2	27.7
	---------- % of the crop DM ----------				
Crude protein	10.9	20.6	6.0	16.9	7.2
Acid detergent fiber	31.4	31.2	23.9	29.2	27.2
Water soluble carbohydrates	9.2	4.7	8.6	7.2	8.0

[1]Milliequivalents of NaOH per 100 g of crop DM required to raise the pH from 4.0 to 6.0.

105 of this report.

Results and Discussion

Results of the study are presented in Tables 39.2 through 39.5. Wheat and alfalfa underwent much slower fermentations than the other three crops (Table 39.2). All five silages reached sufficiently low pH values for acceptable preservation, and all were lactic acid-dominant silages. Corn and sorghum, crops that typically ferment rapidly, had the highest proportion of homofermentative LAB (Table 39.3). Homofermentative LAB are preferred over heterofermentative LAB because the former ferments sugars almost entirely to lac-

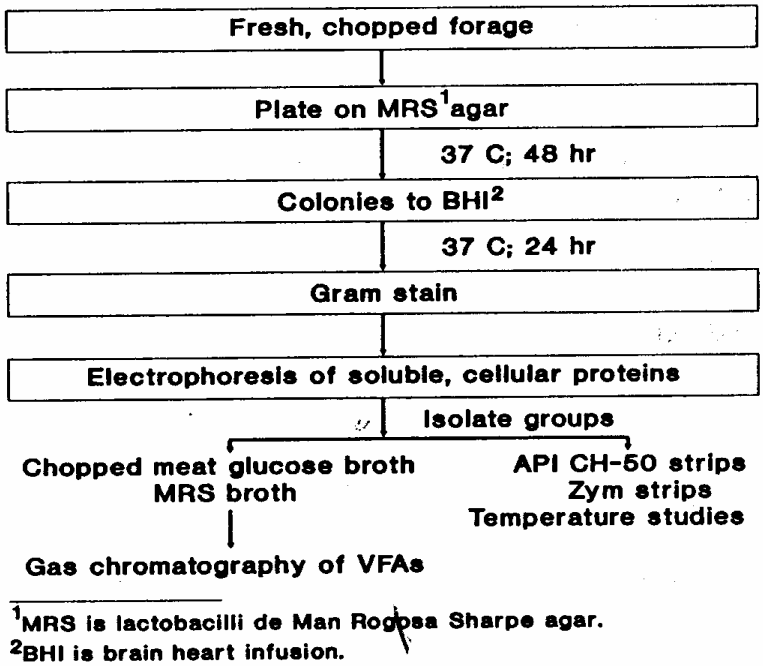

Figure 39.1. Isolation, Identification, and Characterization of the LAB Present on the Five Silage Crops

tic acid, whereas the latter ferments sugars to lactic acid and other products, principally acetic acid, ethanol, and carbon dioxide.

Four of the five crops had LAB populations predominated by *L. plantarum* (Table 39.4). The exception, alfalfa, was dominated by unidentified *Streptococcus*. Growth studies with indigenous isolates of *L. plantarum* and *Pediococcus* species showed that some had growth rates comparable to commercial silage inoculant strains (Table 39.5). However, a greater percentage of isolates was slower growing, had a narrower temperature range for growth, and did not produce as much acid as the commercial strains.

These data support our recommendation that properly selected strains of supplemental (commercial) LAB be added to silage crops to assure a rapid, efficient silage fermentation over a wide range of environmental and silage-making conditions.

Table 39.2. pH and Chemical Composition Over Time for the Five Silages

Time post-filling and item[1,2]		Wheat	Alfalfa	Corn	Sorghum + soybeans	Forage sorghum
Hour 12:	pH	—	5.86	4.72	5.91	4.75
	LA	—	.43	.68	.23	1.04
Hour 24:	pH	5.76	5.66	4.24	4.92	4.65
	LA	.90	.60	1.95	.72	1.87
Hour 48:	pH	5.66	5.47	3.94	4.21	4.41
	LA	1.79	1.34	3.70	3.29	3.67
Day 4:	pH	5.10	5.40	—	4.17	4.22
	LA	2.24	2.13	—	5.12	4.80
Day 90:	pH	4.23	4.98	3.81	4.09	4.10
	LA	5.58	4.47	5.05	6.23	5.60
	AA	1.40	2.69	1.27	3.12	1.42
	ETOH	.17	.24	1.41	.54	.70
	NH_3-N	.17	.30	.07	.13	.07

[1]LA = lactic acid; AA = acetic acid; ETOH = ethanol; and NH_3-N = ammonia-nitrogen.
[2]Acids, ethanol, and NH_3-N are percent of the silage dry matter.

Table 39.3. Numbers and Characteristics of the LAB Isolated from the Five Silage Crops

Item	Wheat	Alfalfa	Corn	Sorghum + soybeans	Forage sorghum
	------ colony-forming units/g of crop -------				
LAB count	5.0×10^6	8.6×10^5	5.0×10^5	4.5×10^6	5.8×10^6
	---------- % of the LAB isolated ----------				
Rods	50.0	64.3	88.2	84.2	75.0
Cocci	50.0	35.7	11.8	15.8	25.0
Homofermentative	60.0	35.7	82.3	78.9	91.7
Heterofermentative	40.0	64.3	17.7	21.1	8.3
Grow at 15 C	10.0	57.0	94.1	100.0	100.0
Grow at 47 C	40.0	0	0	0	0

Table 39.4. Indigenous LAB Identified from the Five Silage Crops[1]

Wheat	Alfalfa
L. plantarum (20)[2]	*Streptococcus* B[3] (29)
L. cellobiosus (20)	*L. plantarum* (21)
Leu. mesenteroides (20)	*L. brevis* (21)
Streptococcus A[3] (20)	*L. cellobiosus* (14)
L. casei (10)	*L. casei* ssp. *pseudoplantarum* (7)
P. pentosaceous (10)	*S. faecium* (7)
Corn	**Sorghum + soybeans**
L. plantarum (76)	*L. plantarum* (42)
L. brevis (6)	*L. curvatus* (16)
L. fermentum (6)	*P. cerevisiae* (10)
P. pentosaceous (6)	*L. brevis* (10)
Leu. dextranicum (6)	*L. buchneri* (5)
Sorghum	*L. fermentum* (5)
L. plantarum (42)	*L. leichmanii* (5)
L. curvatus (24)	*P. acidilactici* (5)
P. pentosaceous (16)	
P. acidilactici (8)	
L. brevis (8)	

[1]L. = Lactobacillus; P. = Pediococcus; S. = Streptococcus; Leu. = Leuconostoc.
[2]Percentage of the isolates identified as that species from this crop.
[3]Strains identified by a capital letter are unrecognized species of that genus. *Streptococcus* A closely resembled *S. faecalis*. *Streptococcus* B closely resembled *S. faecium*.

Table 39.5. Comparison of Indigenous *L. plantarum* and *Pediococcus* Species Present on the Five Silage Crops to Commercial Silage Inoculant (CSI) Strains

Item	Growth rate[1]	pH[2]	Temp. range[3], °C	Optimum temp.[3], °C
L. plantarum				
wheat	.210	3.7	23-43	40
alfalfa	.185	3.9	23-40	37
corn	.220	3.6	20-45	40
sorghum + soybeans	.230	3.7	20-45	37
sorghum	.200	3.7	23-43	37
CSI	.225	3.6	20-43	40
Pediococcus				
wheat	.185	4.3	15-35	30
alfalfa	.160	4.4	15-35	30
corn	.175	4.3	15-30	30
sorghum + soybeans	.190	4.4	20-35	30
sorghum	.180	4.2	20-35	30
CSI	.190	4.2	15-40	32

[1]Absorbance (abs.) recorded at 600 nm. Growth rate = (abs. at 12 hr − abs. at 0 hr)/12.
[2]pH of ENS medium after 24 hr of incubation at optimum temperature. The ENS medium is a minimal bacteriological medium which is similar in composition to grass forages.
[3]Temperature range and optimum temperature studies done in ENS medium.