

Silage Technology Class: Spring, 2001

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Silage Microbiology



Epiphytic Microorganisms

Lactic acid bacteria
Enterobacteriaceae
Yeasts and molds
Clostridia
Aerobic bacteria
Bacillus



Lactic Acid Bacteria

- **Leuconostoc**
Spherical, pairs or chains, heterofermentative
- **Pediococcus**
Spherical, pairs or tetrads, homofermentative
- **Enterococcus**
Spherical or ovoid, pairs or chains, homofermentative



Lactic Acid Bacteria

Metabolic Characteristics:

- Lactic acid fermentation from simple sugars
- Do not ferment polysaccharides
- Non proteolytic
- Deaminate amino acids
- Ferment organic acids



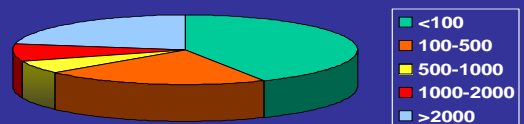
Lactic Acid Bacteria

Quantity and Quality of LAB:

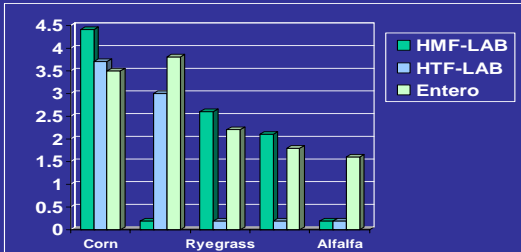
- Very low in numbers
- 4 to 240 cfu/g
- 80% of LAB on standing crops were *Leuconostoc* species
- Enumeration: MRS
(Stirling & Whittenbury, 1963)



Lactobacilli Numbers on Corn Plants (Speckman et al., 1981)



LAB Species on 5 Standing Crops (Andrieu & Gouet, 1990)



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Enterobacteriaceae

- Gram -, non spore forming
- Non pathogenic
- Facultatively anaerobic
- *Erwinia, Enterobacter, Escherichia, Klebsiella, and Rahnella*

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Enterobacteriaceae

Metabolic characteristics:

- Respiratory and fermentative metabolism
- Ferment sugars to acetic acid, ethanol, CO₂ and H₂
- Do not ferment polysaccharides
- Nonproteolytic, but deaminate amino acids

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Enterobacteriaceae

- 10⁴ - 10⁶ cfu/g
- Counts on standing plants:
alfalfa: 10³-10⁶ cfu/g
corn: 10⁵-10⁷ cfu/g
- Enumeration: violet red bile glucose medium

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Yeasts

- Facultatively anaerobic
- Nonfermentative: *Cryptococcus, Rhodotorula, Sporobolomyces and Torulopsis* (10¹ - 10⁷ cfu/g)
- Fermentative: *Candida, Hansenula, Pichia, Saccharomyces, Torulopsis* (below 100 cfu/g)

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Yeasts

Metabolic characteristics:

- Ferment sugars to alcohol and utilize organic acids, including lactic acid
- Limited proteolytic activity

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Molds

- Aerobic
- Fungal spore forming
- *Aspergillus, Penicillium, Mucor*, etc.
- Enumeration: malt agar medium plus antibiotics



Molds

Metabolic characteristics:

- Respiratory metabolism of sugars
- Cellulolytic activity
- Proteolytic activity



Clostridia

- Gram +, spore forming
- Obligately anaerobic
- Habitats: soil and feces
- Not epiphytic on forage plants (contamination)



Clostridia

Metabolic characteristics:

- Ferment sugars and lactic acid to butyric acid
- Optimum pH: 7.0-7.4
- Optimum water activity: forage DM <30%
- Degrade proteins to NH_4 , amides, CO_2 or organic acids



Aerobic Bacteria

- Gram - rods, aerobic
- *Pseudomonas, Flavobacterium* and *Xanthomonas*
- Predominant on the plant
- Strictly respiratory metabolism (convert sugars to CO_2 and H_2O)



Bacillus

- Gram + rods, spore forming
- Facultatively anaerobic
- Low occurrence on forage plant, soil is one of its habitats
- Vegetative cells less tolerance of pH, endospores resist harsh environments



Bacillus

Metabolic characteristics:

- Cellulolytic and proteolytic activities
- Ferment sugars to lactic acid, acetic acid and neutral compounds



Factors Affecting the Quality and Quantities of Epiphytic Microorganisms

- Forage crops
- Maturity and cutting
- Weather or season
- Wilting
- Chopping



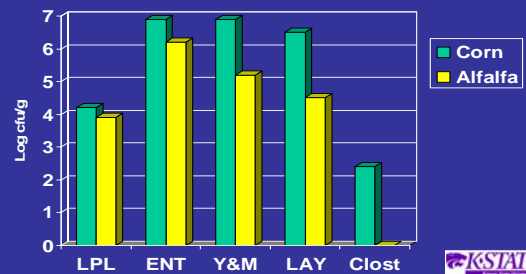
Forage Crops

- LAB 3 times higher on corn plants than grass (Pahlow, 1989)
- Homofermentative LAB percentages different on corn plant (46%) and grass (59%) (Russer, 1989)
- Epiphytic microorganisms higher on corn plant than alfalfa (Lin et al., 1992)



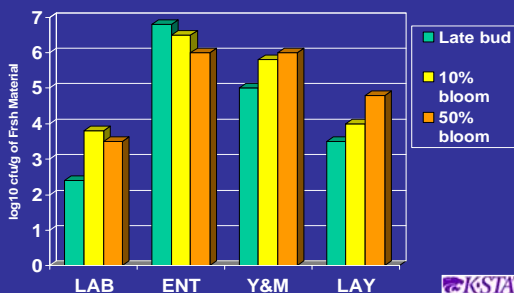
Corn vs. Alfalfa

(Lin et al., 1992)



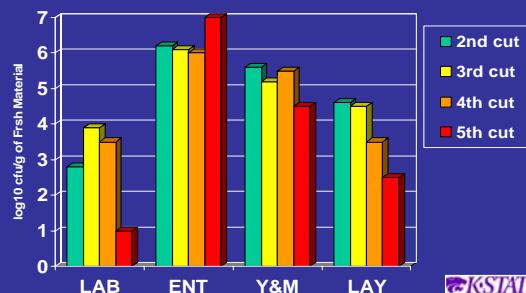
Alfalfa: Stage of Maturity

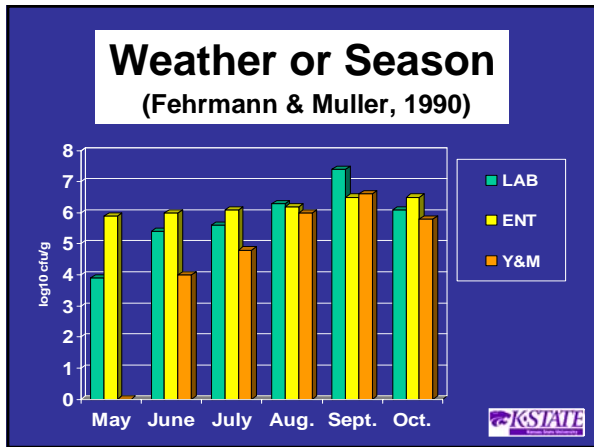
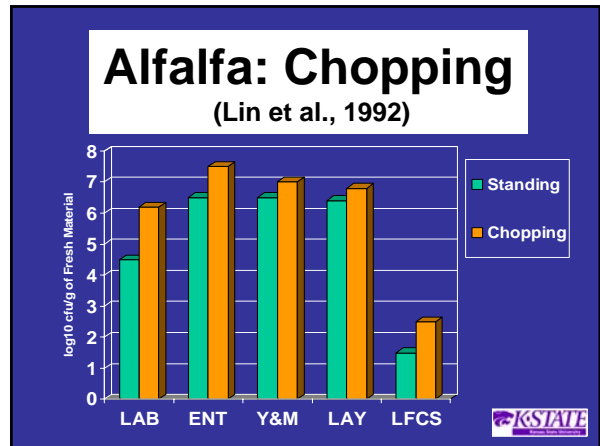
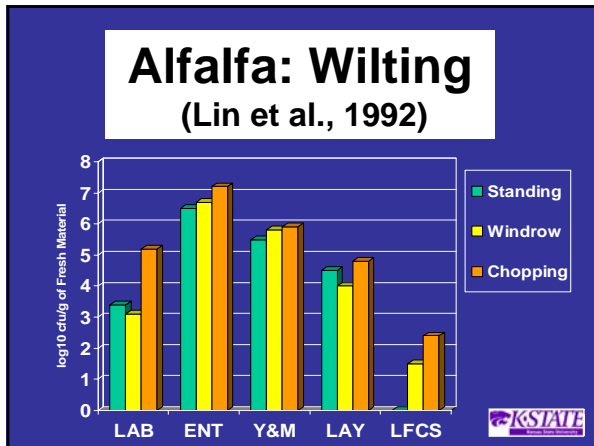
(Lin et al., 1992)



Alfalfa: Cutting

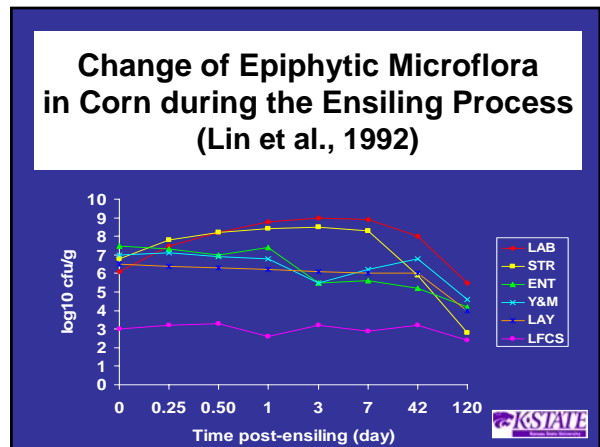
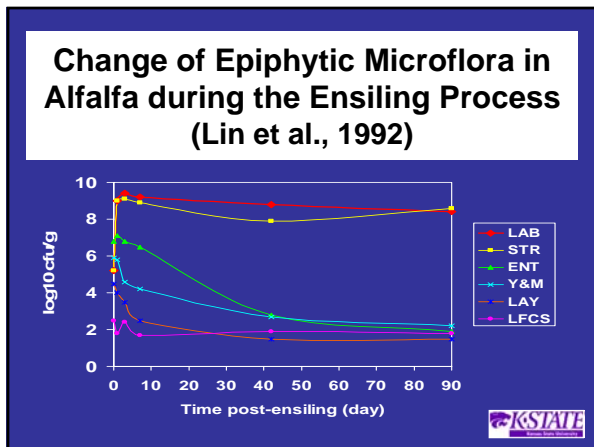
(Lin et al., 1992)



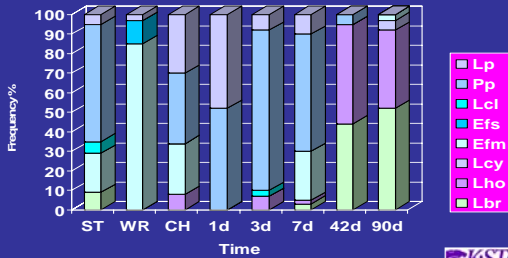


Microbiology of Silage during the Ensiling Process

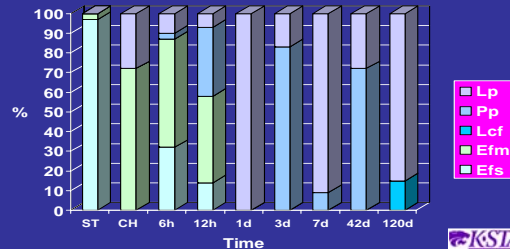
- a. Population changes
- b. LAB succession
- c. Factors affecting microbial changes



LAB Succession during the Pre-ensiling and Ensiling Periods (4th cutting, late-bud alfalfa)



LAB Succession during the Pre-ensiling and Ensiling Periods (Pioneer 3379 whole-plant corn)



Factors affecting Microbial Changes

1. O₂ in silo
2. DM content
3. pH decline

Factors affecting Microbial Changes

O₂ in silo:

- Bacterial and fungal growth
- O₂ is influenced by: forage DM, chopping length, packing density, silo type, and sealing technique

Factors affecting Microbial Changes

DM content:

- Lower DM contents (<30%) increase chance of clostridial growth
- Higher DM contents (>50%) enhance aerobic bacterial and fungal growth

Factors affecting Microbial Changes

pH decline:

- Highly related to lactic acid content & buffering capacity
- Lactic acid production is correlated with homofermentative LAB & WSC content
- Higher pH increases chances of enterobacteria, clostridia & bacilli growth

Microbiology of Silage during Feedout



Silage Aerobic Deterioration

- Temperature rise
- Higher pH value
- Increased CO₂ production
- Increased DM loss



Silage Aerobic Deterioration

- Loss of nutritional components
- Reduced preservation potential
- Accumulation of degradation products causing feed refusals by livestock

(Lindgren et al., 1988; Holzer et al., 1999)



Factors influencing Deterioration

- Oxygen
- Composition of the microbial population
- Substrate type (DM, additives)
- Temperature



Microbial Populations involved in Deterioration

- Yeasts
- Molds
- Bacilli
- Acetic Acid Bacteria



Yeasts

- Grow fast when exposed to air
- Assimilate lactic acid and acetic acid (↑ pH)
- Respiratory metabolism of residue sugars (↑ temperature, and CO₂ production)
- Threshold for aerobic deterioration: 10⁵ cfu/g

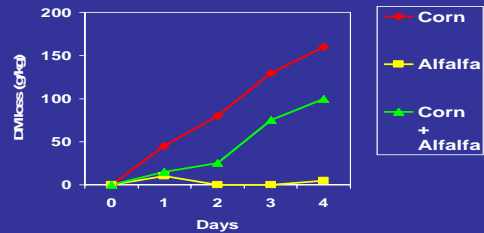


Molds

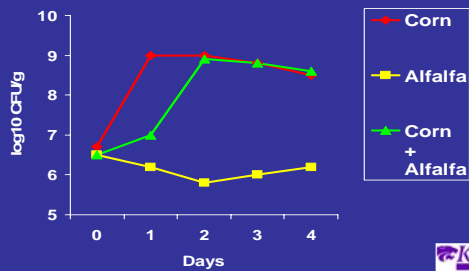
- Grow more slowly than yeasts
- Further deteriorate silage by respiratory metabolism of carbohydrates and proteins
- Mycotoxin production
- *Monascus, Geotrichum, Byssochlamys, Mucor, Aspergillus, Penicillium, Fusarium*



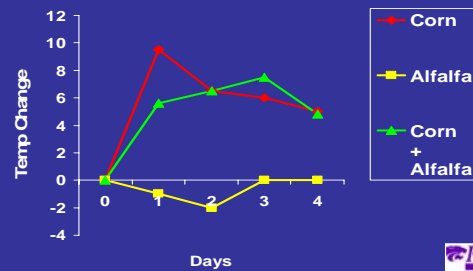
Patterns of Change in DM Loss (O'Kiely & Muck, 1992)



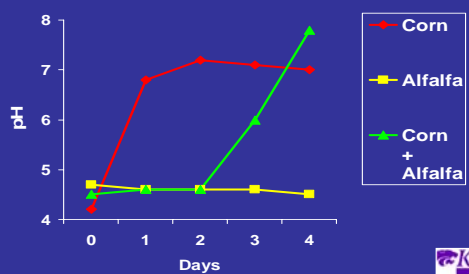
Patterns of Change in Yeast Count (\log_{10} CFU g^{-1}) (O'Kiely & Muck, 1992)



Patterns of Change in Temperature ($^{\circ}C$) (O'Kiely & Muck, 1992)



Patterns of Change in pH (O'Kiely & Muck, 1992)



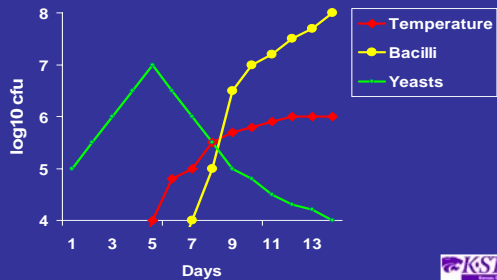
Bacilli

Play a secondary role in aerobic deterioration:

- Low pH intolerant
- Higher temperature tolerant
- Proteolytic bacilli found to initiate aerobic deterioration of corn silage (Woolford et al., 1985)



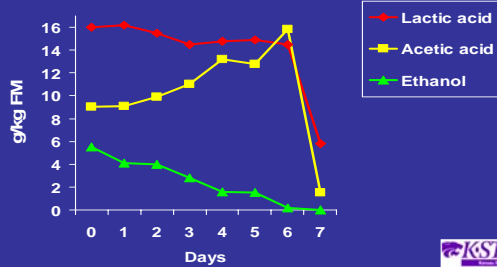
Changes in Temperature, Yeasts, and Bacilli during Aerobic Storage (Lindgren et al., 1985)



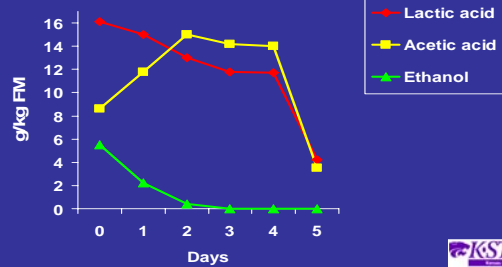
Acetic Acid Bacteria (*Acetobacter*)

- Aerobic
- Grow at low pH
- Metabolize ethanol, lactic acid and acetic acid

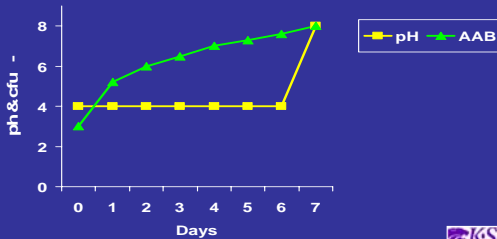
Changes in Chemical Composition of Corn Silage Exposed to Air (Exp. 1) (Spoelstra et al., 1988)



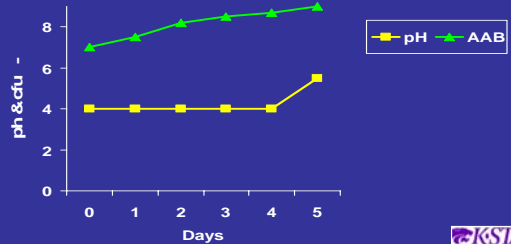
Changes in Chemical Composition of Corn Silage Exposed to Air (inoculated with *Acetobacter*) (Exp. 1) (Spoelstra et al., 1988)



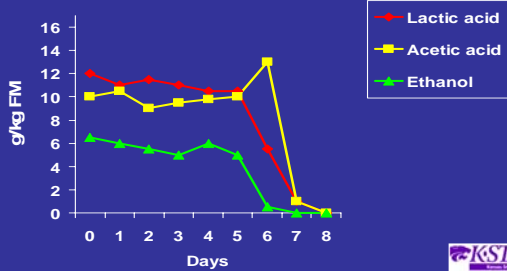
Changes in Microbiological Composition of Corn Silage Exposed to Air (Exp. 1) (Spoelstra et al., 1988)



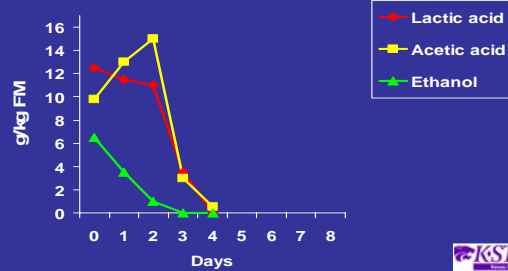
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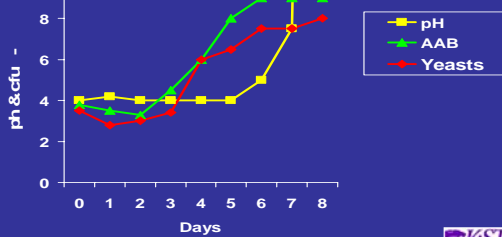
Changes in Chemical Composition of Corn Silage Exposed to Air (Exp. 2) (Spoelstra et al., 1988)



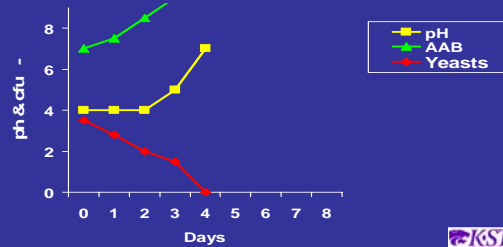
Changes in Chemical Composition of Corn Silage Exposed to Air (inoculated with *Acetobacter*) (Exp. 2) (Spoelstra et al., 1988)



Changes in Microbiological Composition of Corn Silage Exposed to Air (Exp. 2) (Spoelstra et al., 1988)



Changes in Microbiological Composition of Corn Silage Exposed to Air (inoculated with *Acetobacter*) (Exp. 2) (Spoelstra et al., 1988)



Aerobic Stability of Corn Silage inoculated with *Acetobacter* or Yeast

Trial 1	Initial Count	Count	Aerobic Stability
Inoculant	Yeast	AAB	(h)
None	2.5	<2	136
AAB Strain	2.5	6.3	24
Yeast Strain	6.3	<2	44

Aerobic Stability of Corn Silage inoculated with *Acetobacter* or Yeast

Trial 2	Initial Count	Count	Aerobic Stability
Inoculant	Yeast	AAB	(h)
None	3.4	<3	>144
AAB Strain 2	3.4	5.4	36
AAB Strain 3	3.4	5.6	62
AAB Strain 4	3.4	3.6	54
AAB Strain 8	3.4	6.6	56
<i>Acetobacter aceti</i>	3.4	7.8	108