UDC 663.12:663.15:664.8.039.7
ISSN 1330-9862

review

The Yeast Flora of Maize Silage

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Received: September 10, 1997
Accepted: December 4, 1997

Summary

A literature review of yeast species prevailing in various silages is given. The yeast flora of maize silage is dominated by Candida lambica (Issatchenkia orientalis), C. milleri, Saccharomyces exiguus (Candida holmii) and Sacch. dairensis. Particular attention is paid to the role of these species in the aerobic deterioration of maize silage, and to ways of preventing aerobic spoilage of silage.

Keywords: aerobic spoilage, maize, silage, yeasts

Introduction

Maize (*Zea mays* L.) is widely grown as fodder for livestock. In the late autumn the plants are chopped. In order to prevent deterioration by butyric acid bacteria or aerobic moulds the crop is ensiled by compressing it and by keeping oxygen away from it as much as is possible. A spontaneous lactic acid fermentation follows. Inoculation with lactic acid bacteria generally is not necessary. In most cases the pH drops from 6 to 4 within two days due to the formation of lactic and acetic acids *(1)*. Lactic acid bacteria prevailing in maize silage 2–20 days after ensiling were shown to be the heterofermentative *Lactobacillus brevis* and the homofermentative *L. plantarum* (unpublished results).

Anaerobiosis and low pH together warrant microbiological stability. No spoiling microorganisms are known to grow under these conditions. Undissociated acetic and lactic acids decrease growth rates of many microorganisms. If, however, the silos are opened and the silage exposed to air, deterioration takes place within 5 days. This is caused by oxidation of lactic and acetic acids, resulting in a pH rise, and by oxidation of residual sugars and other fermentation products. It has been known for a long time that yeasts are the most important microbiological agents responsible for the aerobic spoilage of silage *(2,3)*. Since then the yeast flora of many silages has been analyzed.

Yeast flora of silage

In Table 1 literature data on yeast species prevailing in various silages are compiled. In most farm-scale grown crops ascomyceotus yeasts predominate. In Table 1 names of anamorphs are listed if no ascospores had been observed. The names of the teleomorphs are given in brackets, if known. Most of these species are strongly fermentative, but fermentation by *Candida famata* and *Geotrichum candidum* is usually weak or absent. Some crops rich in essential oils or mustard oils, *i.e.* spearmint, turnip foliage and leek, were ensiled in jam jars *(4)*. Non-fermentative ascomycetes, *i.e. Candida famata* and *Stephanoascus ciferrii*, or basidiomycetes, *i.e. Rhodotorula* sp. and *Trichosporon* sp. predominated their yeast flora. Another yet unidentified *Trichosporon* sp. was isolated from the aerobic zone of brewer's grains silage *(5)*.

One may wonder how strictly aerobic yeasts can grow in numbers up to 3.5×10^5 CFU/g, *i.e. Rh. minuta* in spearmint. A simple calculation shows that ensiled crops contain enough oxygen to explain this. Assuming that the yeast cells are spheres with a radius of 3.5 μm and that the dry weight is about 27%, the average mass of a yeast cell will be about 0.048 ng. Spearmint will contain about 16.8 μg dry yeast/g. Assuming that sugars were the only source of carbon and energy and that the yield coefficient was 0.5 g dry yeast per gram of sugar consumed, about 18.5 μg of oxygen/g silage was needed for the observed yeast growth. If the solubility of oxygen in the crop is the same as that in water, about 8 μg of oxygen/g silage will be present under conditions of air saturation. If the compressed crop still contained some air, *e.g.* 5% of its volume, an additional amount of about 10 μg of oxygen/g crop would be available in the gas phase.

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In total this amount of oxygen is just enough to explain the growth of 3.5×10^5 yeast cells in silage. Usually, however, only 10^3–10^4 CFU of non-fermentative yeasts are counted per gram silage. These numbers can easily grow at the expense of the amount of oxygen initially present. This calculation stresses the importance of keeping oxygen away from the silos. The spoilage yeasts, which gain energy from fermentation as well as from respiration, use the available amount of oxygen in a more efficient way than the strictly oxidative species and thus have the potency to grow out to more numerous populations. In farm-scale silos some air ingress cannot be prevented. During storage, some air can enter the silage by leakage and diffusion through the silo wall or the covering plastic sheeting. A slow but continuous loss of nutritive value of the silage is the result. Air ingress into silage is unavoidable after opening the silo for feeding.

Analysis of the yeast flora of different maize silages, at various times after ensiling, revealed predominance of the species listed in Table 2 *(1,6)*. Several species detected in maize silage (Table 1) were not found. In this study *Candida milleri* was distinguished from the physiologically almost similar *C. holmii*, the imperfect state of *Saccharomyces exiguus*, by differences in vitamin requirement. Strains of *C. holmii* required only biotin; strains of *C. milleri* biotin and pantothenate. Attempts to distinguish both *Candida* sp. by an ELISA of the heat-stable extracellular antigens were unsuccessful *(7,8)*. Those of

Table 1. Yeast species prevailing in various silages

Species	Crop	Reference
Candida boidinii	Grass	*(21)* Jonsson and Pahlow 1983
Candida famata	Maize	*(22)* Woolford *et al.* 1978
(Debaryomyces hansenii)	Rocket	*(4)* Middelhoven *et al.* 1990
Candida holmii	Maize	*(6)* Middelhoven and Franzen 1986
(Saccharomyces exiguus)		*(1)* Middelhoven and van Baalen 1988
Candida krusei	Corn cobs	*(23)* Burmeister and Hartman 1966
(Issatchenkia orientalis)	Maize	*(24)* Pelhate 1977
		(25) Hara *et al.* 1979
		(1) Middelhoven and van Baalen 1988
	Grass	*(21)* Jonsson and Pahlow 1983
Candida lambica	Oats	*(26)* Barry *et al.* 1980
(Pichia fermentans)	Grass	*(21)* Jonsson and Pahlow 1983
		(4) Middelhoven *et al.* 1990
	Maize	*(4)* Middelhoven *et al.* 1990
		(6) Middelhoven and Franzen 1986
	Lucerne	*(4)* Middelhoven *et al.* 1990
	Hemp foliage	*(4)* Middelhoven *et al.* 1990
Candida milleri	Maize	*(6)* Middelhoven and Franzen 1986
Candida melinii (Pichia canadensis)	Maize	*(22)* Woolford *et al.* 1978
Candida silvicola (Pichia holstii)	Lucerne, wheat	*(27)* Moon and Ely 1979
Candida tenuis	Lucerne, wheat	*(27)* Moon and Ely 1979
Candida valida	Oats	*(26)* Barry *et al.* 1980
(Pichia membranaefaciens)	Maize	*(22)* Woolford *et al.* 1978
Endomycopsis burtonii	Grass	*(21)* Jonsson and Pahlow 1983
(Hyphopichia burtonii, Pichia burtonii)	Lucerne	*(27)* Moon and Ely 1979
Endomycopsis selenospora (Guilliermodella selenospora)	Wheat	*(27)* Moon and Ely 1979
Geotrichum candidum	Maize	*(1)* Middelhoven and van Baalen 1988
(Galactomyces geotrichum)	Grass	*(4)* Middelhoven *et al.* 1990
Pichia fermentans	Grass	*(28)* di Menna *et al.* 1982
		(21) Jonsson and Pahlow 1983
Pichia anomala	Corn cobs	*(23)* Burmeister and Hartman 1966
	Grass	*(21)* Jonsson and Pahlow 1983
		(4) Middelhoven *et al.* 1990
	Beetroot, hemp	*(4)* Middelhoven *et al.* 1990
Pichia canadensis	Wheat	*(27)* Moon and Ely 1979
Pichia membranaefaciens	Maize	*(25)* Hara *et al.* 1979
	Oats	*(26)* Barry *et al.* 1980
Rhodotorula minuta	Turnip	*(4)* Middelhoven *et al.* 1990
Rhodotorula mucilaginosa	Spearmint	*(4)* Middelhoven *et al.* 1990
Saccharomyces cerevisiae	Grass	*(21)* Jonsson and Pahlow 1983
Saccharomyces dairensis	Maize	*(6)* Middelhoven and Franzen 1986
	Beetroot, witloof	*(4)* Middelhoven *et al.* 1990
Saccharomyces exiguus	Grass	*(28)* di Menna *et al.* 1982
		(21) Jonsson and Pahlow 1983
	Maize	*(25)* Hara *et al.* 1979
		(6) Middelhoven and Franzen 1986
	Turnip, leek	*(4)* Middelhoven *et al.* 1990
Stephanoascus ciferrii	Oats	*(26)* Barry *et al.* 1980
Trichosporon capitatum (Dipodascus capitatus, Geotrichum capitatum)	Brewers's grains	*(5)* Middelhoven *et al.* 1985
Trichosporon sp.	Leek	*(4)* Middelhoven *et al.* 1990

Table 2. Yeast species prevailing in maize silage *(1,6)*

Candida famata (Debaryomyces hansenii)
Candida holmii (Saccharomyces exiguus)
Candida krusei (Issatchenkia orientalis)
Candida lambica (Pichia fermentans)
Candida milleri
Geotrichum candidum (Galactomyces geotrichum)
Pichia anomala
Saccharomyces dairensis
Saccharomyces exiguus

C. milleri CBS 6897 failed to raise antibodies in the rabbit; IgG directed against *Sacch. exiguus* reacted in a competitive ELISA but not in a sandwich ELISA. Moreover it was not specific; cross-reactions were observed with eight other yeast species occurring in foods and fodder, *C. milleri* included.

One of the reasons why the data presented in Table 2 deviate from those of Table 1 might be the difference in temperature during ensiling. For this reason maize samples were ensiled in jam jars at constant temperature. The yeast and fungal flora analyzed after 2 weeks varied with the temperature *(4)*. At 20 °C species mentioned in Table 2 were found to predominate, but at 25 °C and 30 °C the weakly fermentative ascomycete *Arxula adeninivorans*, originally described as *Trichosporon adeninovorans (9)*, was found, accompanied with the non-fermentative ascomycetous black yeast *Exophiala jeanselmei* and the filamentous fungus *Verticillium psalliotae*. Species like *C. melinii* or *Pichia membranaefaciens* (Table 1) were not found among the predominant species in maize silage.

Development of the yeast flora of maize silage

The yeast flora in a laboratory-scale silo at 20 °C was followed in time. After two days the yeast flora of fresh maize foliage *(Cryptococcus laurentii, Rhodotorula ingeniosa, Rh. mucilaginosa, Sporidiobolus salmonicolor* and *Sporobolomyces roseus)* had vanished *(1)*. Due to a lactic acid fermentation by the heterofermentative *Lactobacillus brevis* and the homofermentative *L. plantarum* the pH dropped from 6 to 4. Basidiomycetous non-fermenting yeasts were replaced with ascomycetous fermenting species, of which *C. milleri* predominated during the first two weeks of anaerobiosis. It was accompanied with *C. holmii, C. lambica* and *C. krusei*. In a later stage these species sometimes were accompanied with *Candida famata (Debaryomyces hansenii), Geotrichum candidum* and *Pichia anomala*. After two days of anaerobiosis the total yeast count was already 10^7/g. It remained that high for about 7 days and gradually decreased to 10^4/g after 122 days. Anaerobic silage is a hostile environment, even for spoilage yeasts.

After about 4 months the fodder was subjected to aerobic deterioration. This resulted in a dramatic increase of yeast numbers. After 100 hours the total yeast count was about 10^9/g and lactic and acetic acids, ethanol and fructose had been consumed completely. This was due to growth of *C. milleri, C. holmii* and *C. lambica*. The pH rose to 7.8.

Simulation models

In order to predict successfully the time course of microorganisms growth during aerobic deterioration of silage, simulation models have been developed by several authors. Courtin and Spoelstra *(10)* predicted that the stability of a silage is largely dependent on the initial numbers of yeasts and the concentration of organic acids. Silages with a large yeast population, *e.g.* 10^5 CFU/g, will be spoiled by these organisms upon exposure to air. If the yeast population is small, *e.g.* 10^2 CFU/g, acetic acid bacteria will take over. Muck *et al. (11)* and Pitt *et al. (12)* proposed another model, taking in account only yeasts as spoilage organisms. It predicts that aerobic instability is caused by high yeast and mould populations prior to aerobic exposure, high pH associated with high dry matter content, low buffering capacity and high concentrations of water-soluble carbohydrates which stimulate fungal growth. Aerobic stability is greatest when the pre-ensiling forage is highly buffered, of low dry matter content and contains sufficient water-soluble carbohydrates to allow fermentaiton to the lowest possible pH with no residual water-soluble carbohydrates.

Biochemical activities of silage yeasts

Except for *C. lambica* yeast strains isolated from maize silage did not assimilate lactic and acetic acids *(6)* under conditions prescribed for taxonomic studies *(13)*. Under conditions resembling those in silage, *i.e.* at pH = 4 in the presence of a complex nitrogen source, all strains assimilated both organic acids, but growth of *Saccharomyces dairensis* was very slow *(6)*. All strains, except those of *Sacch. dairensis*, tolerated acetic acid at 5 g/L and grew at pH = 4.0 in a mineral salts medium containing lactic acid (10 g/L), acetic acid (5 g/L), yeast extract (1 g/L) and vitamins.

The less frequently occurring yeast species *C. famata, Geotrichum candidum* and *Pichia anomala* assimilated acetoin and butane-2,3-diol in medium supplied with yeast extract. *G. candidum* assimilated these minor fermentation products also in the absence of yeast extract *(1)*. Diacetyl was not assimilated. The meso-, D- and L-enantiomers of butane-2,3-diol have been detected in silage at total concentrations of up to 0.87% of the dry weight *(14)*. *P. anomala* is notable for assimilation of soluble starch. It is not known to which extent starch in maize silage is dissolved. Ethanol was readily assimilated by all strains studied *(6)*.

Improvement of aerobic stability of silage

Since it became known that yeasts are the most important microbiological agents causing aerobic instability of silage, many investigators have tried to suppress yeast growth by adding inhibiting substances to the forage prior to ensiling. Some of the recent attempts are recorded here. Kitamoto *et al. (15)* demonstrated the activity of killer strains of *Kluyveromyces lactis* on silage yeasts. Spoelstra *et al. (16)* added poultry manure to maize forage. They found an increased conversion of water-soluble carbohydrates into lactic acid, probably due to an increased buffering capacity of the silage.

Aerobic stability increased somewhat. This was not the case if the gas phase of the silage was replaced with carbon dioxide *(17)*. Driehuis *et al. (18)* inoculated maize forage with the heterofermentative *Lactobacillus buchneri*. At the highest inoculum level tested, *i.e.* 10^6 CFU/g, the chemical composition of the silage was switched in favour of acetic acid and propionic acid. This resulted in a tremendous improvement of aerobic stability and a decrease in yeast counts.

An increase in lower volatile fatty acids can also be achieved by adding these compounds to silage. Driehuis and van Wikselaar *(19)*, following the example of many predecessors, studied the effect of formic acid, acetic acid and propionic acid on the microbial flora and the aerobic stability of maize and grass silages. Treatment of maize silage with formic acid considerably improved aerobic stability of maize silage, in spite of high yeast numbers. Acetic and propionic acids decreased yeast numbers but did not improve aerobic stability. The reason for this controversy is that aerobic instability of maize silage can also be caused by *Acetobacter* sp. *(20)*. These bacteria are able to oxidize ethanol and lactic acid to acetic acid, and completely mineralize acetic acid in subsequent oxidation steps. Formic acid killed the acetic acid bacteria in maize silage, but did not reduce yeast numbers. These yeasts do not cause aerobic deterioration and apparently are not the same as those found in maize silage without formic acid. Unfortunatly, no attempts were made to identify the yeast species present in maize silage treated with formic acid. Grass silage responded differently to additions of formic and propionic acids *(19)*. In grass silage treated with propionic acid yeast counts were very low and aerobic stability high. Treatment of grass silage with formic or acetic acids did not reduce yeast numbers; aerobic stability was only slightly better than in the control silage. Contrary to maize silage, grass silage seems not to permit growth of acetic acid bacteria.

Middelhoven and Franzen *(6)* observed that in 4 out of 13 maize silages the yeast flora was dominated by *Sacch. dairensis*. This yeast oxidizes lactic and acetic acids much slower than the other yeast species. Maize silages with *Sacch. dairensis* as the predominating yeast species are expected to be more stable than the other ones. At present, no experimental data are available to support this hypothesis. Unfortunately, the growth factors favouring *Sacch. dairensis* in maize silage are unknown.

Prevention of aerobic spoilage can best be achieved by giving yeasts and acetic acid bacteria no opportunity to oxidize valuable fermentation products. In the Netherlands silos are narrow and after opening the surface of the silage exposed to air is kept as small as possible. Loosened silage removed from the silo is fed to the cattle preferably the same day. Loss of nutritive value is largely prevented in this way.

Acknowledgements

Thanks are due to Dr. Stefanie Oude Elferink and to Dr. Sierk Spoelstra for providing literature data and for critical reading of this manuscript.

References

1. W. J. Middelhoven, A. H. M. van Baalen, *J. Sci. Food Agric. 42* (1988) 199–207.
2. F. Weise, *Landbauforschung Völkenrode, 13* (1963) 111–116.
3. T. Beck, F. Gross, *Das Wirtschaftseigene Futter, 10* (1964) 298–312.
4. W. J. Middelhoven, I. M. de Jong, M. de Winter, *Antonie van Leeuwenhoek, 57* (1990) 1563.
5. W. J. Middelhoven, H. de Kievit, A. L. Biesbroek, *Antonie van Leeuwenhoek, 51* (1985) 289–301.
6. W. J. Middelhoven, M. M. Franzen, *J. Sci. Food Agric. 37* (1986) 855–861.
7. W. J. Middelhoven, S. Notermans, *J. Gen. Appl. Microbiol. 34* (1988) 15–26.
8. W. J. Middelhoven, S. Notermans, *Int. J. Food Microbiol. 19* (1993) 53–62.
9. W. J. Middelhoven, M. C. Hoogkamer-te Niet, N. J. W. Kreger-van Rij, *Antonie van Leeuwenhoek, 50* (1984) 369–378.
10. M. G. Courtin, S. F. Spoelstra, *Grass Forage Sci. 45* (1990) 153–165.
11. R. E. Muck, R. E. Pitt, R. Y. Leibensperger, *Grass Forage Sci. 46* (1991) 283–289.
12. R. E. Pitt, R. E. Muck, N. B. Pickering, *Grass Forage Sci. 46* (1991) 301–312.
13. J. P. van der Walt, D. Yarrow, Methods for the isolation, maintenance, classification and identification of yeasts. In: *The Yeasts, a Taxonomic Study*, N. J. W. Kreger- van Rij (Ed.), Elsevier, Amsterdam (1984) pp. 45–104.
14. M. Fenton, G. W. Matlison, *Can. J. Anim. Sci. 60* (1980) 543–545.
15. H. K. Kitamoto, S. Ohmomo, T. Nakahara, *J. Dairy Sci. 76* (1993) 803–811.
16. S. F. Spoelstra, L. Tjoonk, A. Steg, *Proceedings of the Fifth International Symposium on Agricultural Wastes*, Chicago, U.S.A. (1985) pp. 159–166.
17. R. E. Muck, S. F. Spoelstra, P. G. van Wikselaar, *J. Sci. Food Agric. 59* (1992) 405–412.
18. F. Driehuis, S. F. Spoelstra, S. C. J. Cole, R. Morgan, *Proceedings 11th International Silage Conference*, Aberystwyth, UK (1996) pp. 106–107.
19. F. Driehuis, P. G. van Wikselaar, *Proceedings 11th International Silage Conference*, Aberystwyth, UK (1996) pp. 256–257.
20. S. F. Spoelstra, M. G. Courtin, J. A. C. van Beers, *J. Agric. Sci. Cambridge, 111* (1988) 127–132.
21. A. Jonsson, G. Pahlow, *Landbauforschung Völkenrode, 33* (1983) 72–78.
22. M. K. Woolford, H. Honig, J. S. Fenlon, *Das Wirtschaftseigene Futter, 24* (1978) 125–139.
23. H. R. Burmeister, P. A. Hartman, *Appl. Microbiol. 14* (1966) 35–38.
24. J. Pelhate, *Folia veterinaria latina, 7* (1977) 1–16.
25. S. Hara, M. Itoh, Y. Ohyma, *Jap. J. Zootechn. Sci. 50* (1979) 548–556.
26. T. N. Barry, M. E. di Menna, P. R. Webb, J. N. Parle, *J. Sci. Food Agric. 31* (1980) 133–146.
27. N. J. Moon, L. O. Ely, *Mycopathologia, 69* (1979) 153–156.
28. M. E. di Menna, J. N. Parle, R. J. Lancaster, *J. Sci. Food Agric. 32* (1982) 1151–1156.

Kvasci u silaži kukuruza

Sažetak

Dan je literarni pregled vrste kvasaca koji prevladavaju u različitim silažama. Od kvasaca u silaži kukuruza najčešći su Candida lambica (Issatchenkia orientalis), C. milleri, Saccharomyces exiguus (Candida holmii) i Sacch. dairensis. Osobito je istaknuta uloga tih vrsta pri aerobnom kvarenju silaže kukuruza te način njegova sprječavanja.