Own Preparatory Work

The preliminary aim of the work was to investigate the role of yeasts in aerobic deterioration of grass silage. In the literature there are numerous reports that yeasts are the initiators of aerobic instability in grass and other silages (see LITERATURE REVIEW), especially yeasts with the ability of assimilating lactate (JONSSON and PAHLOW, 1984; JONSSON, 1989).

JONSSON and PAHLOW, 1984, stated that a population of > 5 log cfu/g silage FM at the time of opening causes fast aerobic deterioration if the yeasts can utilise lactic acid aerobically.

In order to investigate whether those observations can be generalised, first own observations were made in small scale laboratory silages in 2002/2003: if ensiled with defined air infusion (2*24 h over a 49 d storage period) (comparable to practical farm conditions) all unstable silages contained at least 5 log cfu/g silage FM. On the other hand, if ensiled strictly anaerobic low dry matter grass silages (~ 25 % DM) were unstable with yeast numbers between 3-4 log cfu/g FM on malt extract agar and similarly on lactate agar which was introduced by JONSSON and PAHLOW, 1984, whereas in unstable high DM silages (35-40 % DM) yeast numbers varied widely from below the detection limit up to 5 log cfu/g FM (Figure 4 in the LITERATURE REVIEW; MARTENS and PAHLOW, 2003).

The above mentioned lactate agar for the enumeration of lactate assimilating yeasts offering lactate as sole carbon source has a pH of 3.4-3.8.

The question arose whether this medium would allow the development of all lactate assimilating yeasts grown under different conditions in silages. The two main differences between low and high dry matter grass silages were seen in pH and osmotic pressure, which are both higher in high DM silages. The pH of the latter can vary between 4.0 and 5.5 or even higher.

MIDDELHOVEN and FRANZEN, 1986, investigated the ability of 6 yeast species (15 type strains) to assimilate lactate by growing them at pH 5.8 and pH 4.0 in liquid cultures. They stated that most of the strains were able to grow with lactate as carbon source at the lower pH even when they did not grow at all at pH 5.8.

Thus, to answer the above mentioned question, in the own work, the pH of the lactate agar was varied between 3.8 to 6.0 in 5 steps. In a second treatment the osmotic pressure was raised by 8.3 % KCl in the different pH levels respectively.

Mixed silage flora was plated on the original lactate agar and on another one with a pH adjusted to the original pH of the silage.

Yeast isolates from grass silages were plated on 5 pH levels and the medium supplemented with KCl.

No distinct effect could be observed except an inhibition by KCl.

As even a yeast identified as *Saccharomyces cereviseae* whose abilities of lactate assimilation are inconsistent for taxonomical purposes grew on all modifications of the lactate medium it was doubted that the only offered carbon source was used.

Growth tests on only Yeast Nitrogen Base agar and on pure agar without external C-source were positive. That led to the assumption that some yeasts can either a) utilise nutrients from agar or b) carry over nutrients in their cells from the former growth medium. Additionally in a mixed yeast flora even an exchange of nutrients on the agar plate is possible, so that yeasts which cannot grow on the plates by their own metabolism can survive by others (HOFFMANN, 2004). DAVENPORT, 1980, also emphasizes that a medium is only part of an environmental system which includes interactions and carry-over. LOUREIRO and MALFEITO-FERREIRA, 2003, describe the general obstacles associated with selective media.

The conclusion was that the plate count method was not specific enough to study the role of yeasts in high DM grass silage deterioration and the factors influencing their growth and activity.