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Ecology of silage microorganisms

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Introduction

The biochemistry and microbiology of ensiling have been investigated in depth in several studies (McDonald et al., 1991; Rooke and Hatfield, 2003; Pahlow et al., 2003). It is, however, difficult to combine these results to address as to why the catabolic diversity in silage ecosystem is large and what are the driving forces for such complexity. As an example, certain lactic acid bacteria under the presence of air reduce O₂, by which, reactive oxygen species (e.g. superoxide, hydrogen peroxide) which are essentially detrimental to them are formed (Pahlow, 1991). In this work, we aim at dissecting factors of importance to the silage ecosystem and provide an overview of some of the catabolic pathways employed by silage microorganisms.

Respiration versus fermentation

Understanding similarities and differences between respiration and fermentation is essential for understanding causes and effects in the silage ecosystem. In both processes, chemical energy of substrates is released by means of oxidation-reduction (redox) reactions. Electrons are transferred from substrates to compounds with higher reduction potentials, *i.e.* the electron acceptors, by electron carrier molecules (e.g. nicotinamide adenine dinucleotide). A larger difference between reduction potential of electron donors and acceptors means a greater magnitude of the $\Delta G^{0'}$. In biological systems, O₂ has the highest reduction potential (Madigan et al., 2012). The half-reactions, *i.e.* oxidation of the substrate and reduction of the electron acceptor, always take place together so that the electron carrier molecules become again available and the process of energy release continues, a phenomenon referred as a balanced reaction (Madigan et al., 2012). It is therefore appeared that this process is heavily dependent on the presence of electron acceptor compounds in the system.

In fermentative pathways, the redox balance is reached by reduction of intermediates (e.g. pyruvate) that are formed from the up-taken substrates. This results in an incomplete oxidation and excretion of semi-catabolized substrates, *i.e.* fermentation end-products. In respiratory pathways, external electron acceptors (EEAs), e.g. O₂, or nitrate, are exploited instead. A further distinction between the two is that in fermentation, the released energy is stored via substrate level phosphorylation but in respiration, oxidative phosphorylation is also employed. This in turn gives substantial superiority to respiration regarding energy conservation efficiency.

Silage ecosystem

A low pH of silage causes stress to acid tolerant microorganisms while being detrimental to the others. This effect is the result of passive entry of short chain fatty acids (e.g. acetic and propionic acids) into the cell before dissociation and decreasing the intracellular pH to

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detrimental levels. Silage microorganisms employ different strategies to withstand a low pH. Active removal of H^+ is the most important defensive response (De Angelis and Gobbetti, 2004). It seems, therefore, under low pH conditions, silage microorganisms are in a greater demand of energy.

When air penetrates into a silo, growth of yeasts is enhanced (Jonsson and Pahlow, 1984). This increased population growth has been attributed to the lactate assimilating ability of certain species, collectively known as lactate assimilating yeasts. Firstly, such classification of silage yeasts (*i.e.* lactate vs. non-lactate assimilating) is an approximation because the response of different yeast species to lactate depends heavily on environmental conditions (*e.g.* N source and pH) and adaptation time (Middelhoven and Franzen, 1986). Secondly, other fermentation end-products, such as ethanol, can also be assimilated by yeasts when air is present. Therefore, this classification is incomplete and could be misleading. The increased growth is, however, explained by the fact that respiratory species (*e.g.* *Wickerhamomyces anomalus*) can switch from fermentation to respiration (Madigan et al., 2012), by which, the efficiency of energy conservation is improved.

A similar strategy of increasing energy gain can also be found in prokaryotic species. For instance, *Escherichia coli* is able to anaerobically respire when nitrate is available (Madigan et al., 2012). *Leuconostoc* spp. and *Lactobacillus plantarum* will ferment glucose to acetic acid instead of lactic acid under the presence of O_2 and nitrate, respectively (Rooke and Hatfield, 2003). Through this externally balanced fermentative pathway, two extra moles of ATP are gained. Lactic acid bacteria generally lack catalase for detoxification of reactive oxygen species (Pahlow, 1991; Sanders et al., 1999) but as discussed in the Introduction, they still opt reducing O_2 . This phenomenon might be explained by the theory that silage microorganisms prioritize enhancement of energy gain due to an inflated energy demand. On standing forages, this inflation is owing to starvation, irradiation and climatic conditions and during ensiling, it is caused by the low pH.

Anaerobic condition together with the closed system of ensiling favor lactic acid bacteria as these conditions allow accumulation of lactic acid and, thereby, out-competition of pH sensitive microorganisms. The ability of silage microorganisms to produce anti-microbial compounds that directly target competitors, however, should not be ruled out. For instance, it is known that *L. plantarum*, *L. buchneri* and *W. Anomalus* have the ability to produce certain anti-microbial compounds (Gollop et al., 2005; Passoth et al., 2006; Olstorpe et al., 2011).

Different strategies used by silage microorganisms for increasing energy conservation efficiency can be summarized as shown in Figure 1.

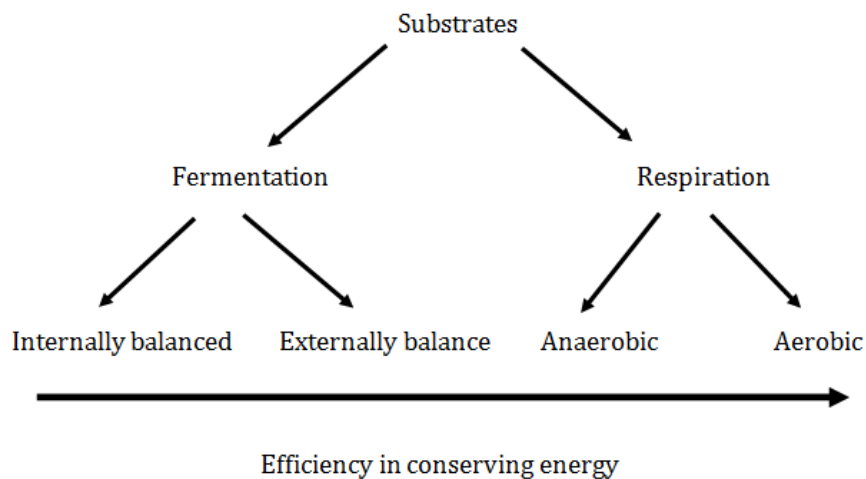


Figure 1 A summary over catabolic pathways used by silage microorganisms.

The metabolic ability of microorganism (*e.g.* yeasts cannot practice anaerobic respiration) and redox balance status (*i.e.* availability of EEAs) are two factors that determine pathway. Substrate availability and silage pH can also play important roles (McDonald et al., 1991). The complexity is even further increased by the fact that some microorganisms can utilize certain end-products during starvation. It was, for instance, found that under the presence of citrate, *L. plantarum* can ferment lactic acid, using citrate as the EEA (Lindgren et al., 1990).

Implications

Modeling silage quality from pre-ensiled forage composition could be of great benefit to farmers. Advice on the correct kind of additives, wilting strategies, etc. could eventually be improved. Such models can also play important parts in silage research and additive evaluations. However, most of the attempts in this regard (Wilkinson et al., 1983; Pitt et al., 1985; Mogodiniyai Kasmaei et al., 2013) have been largely unsuccessful. The majority of these modeling attempts, including both dynamic and static models, have not taken the role of microbial interactions into account, something which may have contributed to their failures. This has mainly been due to scarcity of the relevant data and/or underestimating the effect of this factor on ensiling results. For constructing more powerful silage models, data on microbial interaction seem to be absolutely necessary.

Improving the aerobic stability of silage upon opening has recently become of great interest. Inoculation with *L. buchneri*, a heterofermentative species, has been suggested (Kleinschmit and Kung, 2006). However, this strategy could lead to an increased dry matter loss, which is explained by a slow rate of pH decline that allows continuation of microbial catabolic activities and formation of CO₂ by heterolactic fermentation (Wilkinson and Davies, 2012). Application of chemical additives can be an alternative strategy to improve both aerobic stability and DM losses (Knicky and Spörndly, 2011). However, the cost and unsustainability are unfortunately disadvantages of this technique (Wilkinson and Davies, 2012). An increased knowledge of

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microbial ecology could improve silage research and result in a new generation of additives or even new storage strategies.

Conclusions

Silage microorganisms strive to enhance energy conservation efficiency. The strategy used is a constant adjustment of catabolic pathways to environmental conditions including substrate availability, redox balance and pH. A better understanding of microbial ecology is needed to improve silage research and technology.

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