ROLE AND CONTROL OF STARTER LYSIS IN CHEESE PRODUCTION AND RIPENING

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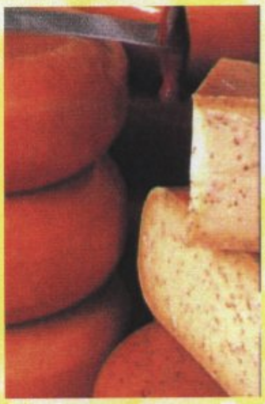
Introduction

Lysis of lactic acid bacteria plays a crucial role in dairy fermentations. Especially in cheese the energy reserves of the starter bacteria are quickly depleted during the maturation. This prevents the translocation of extracellularly produced large peptides into the cell. Therefore it is generally assumed that lysis of the starter bacteria enhances involvement of the intracellular bacterial enzymes in the gradual process of flavour formation by conversion of the milk proteins and their breakdown products. We have studied different aspects related to the phenomenon of lysis:

1. The development of rapid and sensitive methods to analyse and quantify lysis *in situ*, in cheese, by making use of fluorescent probes.
2. The selection of industrial starter strains which have a high lysis efficiency.
3. The effect of bacteriocin-immunity on the cell wall composition and stability (1).



Acknowledgements

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References

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Results

In situ analysis of lysis

To study the activity and integrity of the lactic acid bacteria *in situ*, we are working on a non-destructive method using fluorescent techniques. A *Lactococcus lactis* strain, harboring the lytic genes *lytA* and *lytH* from bacteriophage fUS3 under strict control of the *nisA* promotor (2), was used to obtain controlled lysis of the starter in M17 medium (Figure 1)

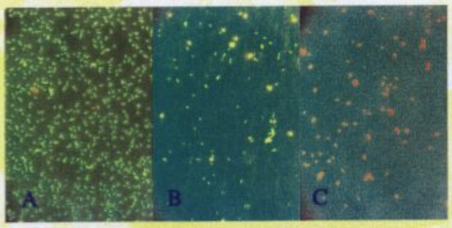

***Figure 1.** Fluorescent labelling of live (green) and dead (red) cells with respectively probe SYTO 9 and PI, in a culture of strain NZ3900, harboring pNZ8038F, before (A), during (B) and after full induction of lysis (C) with different doses of nisin.*

These experiments show that the induced lysis results in 1) large reduction in total cell count due to complete desintegration of the majority of the cells and 2) complete permeability and hence PI staining of the remaining cells. Currently the procedure is being implemented in cheese model systems where lysis is expected to result in mainly permeabilization and not desintegration.

Selection of lysis-sensitive strains

Approximately 200 *Lactococcus lactis* strains were isolated from different sources and compared in cell stability and sensitivity of the cell walls for the lytic enzyme mutanolysin (Figure 2).

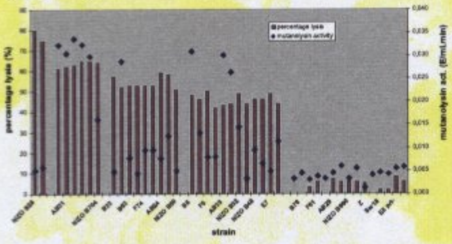

***Figure 2.** Lysis of several L. lactis strains in M17-medium with 0.3% glucose after 21 days incubation at 30°C (bars), as well as the sensitivity of the cells (harvested at OD 0.4 at 600nm) for mutanolysin (20U/ml) at 37°C (◆).*

Cells with high stability always showed a low sensitivity to mutanolysin. The reverse was not always the case, indicating that not only cell-wall composition is involved in lysis behaviour.

Effect of bacteriocin-immunity on the cell stability

Nisin-immune starters were developed by introduction of the nisin-sucrose transposon Tn*5276*-NI containing a deficiency in a structural gene for nisin production (3). Transconjugants of *L. lactis* SK110 showed increased stability in cheese (Figure 3). Cheese manufactured with the transconjugant was more bitter than cheese manufactured with strain SK110 (data not shown). This can be explained by the reduced release of debittering enzymes, due to the higher stability of the transconjugant.

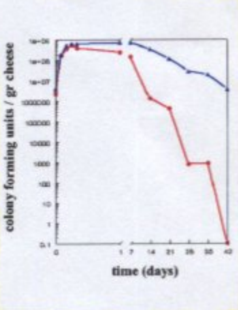

***Figure 3.** Lysis of strain SK110 (●) and its nisin-immune transconjugant SK110: Tn5276-NI (▲) during Gouda cheese production.*

The sensitivity of purified SK110 peptidoglycan for mutanolysin was higher than the cell walls of the transconjugant (Figure 4), which is due to differences in the amount of cross-links in the peptidoglycan structure (data not shown). This suggests that a gene encoded by transposon Tn*5276*-NI affects the peptidoglycan composition.

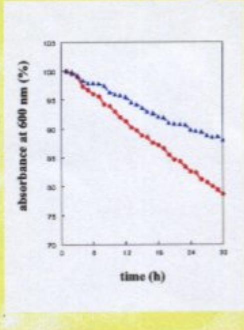

***Figure 4.** Relative changes in absorbance (600 nm) after treating 1.4 mg/ml isolated cell walls of strain SK110 (●) and its transconjugant (▲) with mutanolysin (10U/ml) at 37°C.*

Conclusions

- Fluorescent labeling gives good opportunities to develop a rapid and sensitive method to analyse lysis *in situ*.
- Lysis efficiency is extremely variable between different *L. lactis* strains.
- Introduction of transposon Tn*5276*-NI alters the susceptibility of the host strain to lysis via a changed peptidoglycan composition.

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