Quantification of bacterial peptidase activity in milk by Bioluminescence-Resonance Energy Transfer (BRET) analysis

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Introduction

Refrigerated storage of raw milk is a prerequisite in the dairy industry. However, psychrotrophic bacteria can proliferate and contribute to spoilage of ultrahigh temperature (UHT) treated milk due to their ability to produce extracellular, heat resistant peptidases. The residual activity of peptidases after UHT treatment may lead to technological problems (off flavors, physico-chemical instability) during the shelf life of milk and dairy products. Early detection of peptidase activity, ideally in raw milk, is therefore urgently needed; methodologies for quantification, however, are still time consuming and lack sensitivity.

Aims & Methods

The aim of the present study was to examine the potential of the BRET-technology as a rapid technique for quantifying peptidase activity. Major tasks were the determination of assay sensitivity and specificity using peptidases present in culture supernatants of four different *Pseudomonas* strains. The azocasein assay and the quantification of free amino groups using fluorescamine after defined storage of samples were used as reference methods.

Results

Culture supernatants were decimally diluted and measured using BRET. Results showed very similar reactions with the biosensor and sensitivity of the assay was comparable for all strains. Accordingly, the specificity for cleavage of the biosensor peptide bridge was equal for all four strains, as well. The identical supernatants were inoculated into UHT milk, stored for 14 days and then analysed for the amount of released amino groups using fluorescamine. Comparison with BRET results showed a similar sensitivity and provides evidence that the BRET assay has a similar detection limit as can be achieved using fluorescamine.

Conclusion

The BRET technology shows potential to serve as a more rapid alternative for detection of peptidase activity compared with determination of released peptide groups. Upcoming analyses will include the testing of more species to confirm specificity for other *Pseudomonas* species, as well.