

highly thermo-resistant phage of the widespread 936 phage species [2]. Results are presented for detection of pure phage isolates by LAMP compared to a standard PCR-based assay. We further tested LAMP with DNA extracted directly from different whey preparations and compared its sensitivity with that of the standard PCR assay.

References

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LMV05

Rapid quantification of viable *Legionella*

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Introduction: We present a new method for rapid detection and viability assessment of *Legionella pneumophila* in water. Culture based methods require 7 to 10 days for this task and are therefore unsuitable for use in emergency situations. Methods like PCR and lateral flow assay allow a more rapid detection of *Legionella* cells, but do not differentiate between live and dead cells or lack sensitivity for the quantification of low bacterial loads.

Materials and Methods: A transparent macroporous membrane is modified by immobilization of *Legionella* specific antibodies. Bacteria are selectively captured from a water sample by filtration through the membrane. Captured bacteria are stained by incubating the membrane with an anti-*Legionella pneumophila*-Phycocerythrin conjugate solution and an esterase based viability dye. Microscopic counting of single and double stained cells is used for quantitative determination of total and viable *Legionella* load.

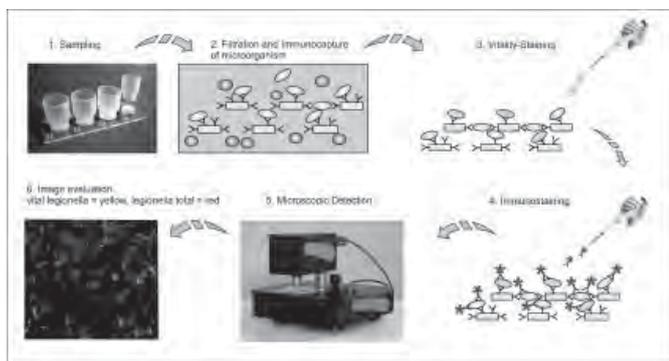
Results: High resolution pictures of individual fluorescent stained cells captured to the membrane were acquired. The limit of detection for esterase positive *Legionella* was 10 cells per membrane corresponding to 100 cells per 100 ml of tap water. The method is robust against particulate contaminants up to 10 µm in size. Total time for one analysis was about 1 hour.

Experiments with other microorganisms and sample matrices e.g. *Salmonella* in meat indicate that the test system can also be used for the quantification of specific bacterial loads in food hygiene and biotechnological applications.

Discussion: The method presented here permits rapid quantification of highly diluted target cells. Living bacteria can be distinguished from dead. The sensitivity for *Legionella pneumophila* is sufficient for detection of contaminations at the federally mandated exposure limit. The short analysis time makes this method useful for finding contamination sources during outbreaks of Legionellosis and for monitoring the success of decontamination efforts. Parallelization of sample and reagent handling and automation of image acquisition appear feasible and would expand the method into high throughput screening. The system requires only minimal, portable equipment and can be used outside laboratory environments.

Figure

1



LMV06

Fast and effective killing of *Bacillus atrophaeus* endospores using a new generation of light-activated vitamin B2 derivatives

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Spore forming bacteria like *Bacillus* or *Clostridium* provoke massive problems in the food and packaging industries as well as in medical and biotechnological processes due to the highly intrinsic resistance of spores against a variety of stress factors. Only strong chemical or physical agents show a satisfactory result in spore decontamination, but these measures exhibit harmful potentials to humans and are often also harmful for the environment. In addition, many chemicals are not allowed to get into contact with food. Alternatively, the photodynamic inactivation (PDI) of microorganisms and spores presents several positive aspects regarding the killing efficacy and the environmental hazard. PDI is based on positively charged and non-toxic dyes (photosensitizers) that attach to the negatively charged spore surface. Upon irradiation the photosensitizers generate reactive oxygen species, especially singlet oxygen, that kill spores via oxidative damage. In a cross-disciplinary approach of physicists, chemists and biologists, we developed new Flavin derivatives (FLASH-01a, FLASH-07a) that are based on naturally occurring Vitamin B2 (Riboflavin). We added one (FLASH-01a) or eight (FLASH-07a) positive charges to Riboflavin that allow the attachment of photosensitizers to the spore surface. The new Flavins convert efficiently visible light energy into singlet oxygen with a quantum yield of 0.75 ± 0.05 and 0.78 ± 0.05 , respectively. The absorption spectrum of FLASH-07a matched closely to the emission spectrum of the non-coherent light source. Incubation of FLASH-01a or FLASH-07a with *Bacillus atrophaeus* endospores for 10 seconds and a following irradiation of 10 seconds with 70 J/cm^2 caused a biologically decrease of spore survival of $\geq 3 \log_{10}$ orders ($\geq 99.9\%$) *in vitro*. Immobilized spores on food related surfaces like polyethylene terephthalate (PET) were efficiently killed with $7.0 \log_{10}$ orders (99.99999%) using the same parameters as for *in vitro* experiments. Thus, PDI with this new photosensitizers offer a great potential for a safe and sustainable use in food industry and environmental technologies as well as in medical applications.

LMV07

Inhibitory effect of nitrite on growth and survival of pathogens - molecular analysis of a preservation method

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The use of sodium nitrite in the curing process is a common food preservation technique for meat products, because of its suggested antimicrobial effect. Indeed, the addition of nitrite to raw sausages results in a better elimination of *Listeria monocytogenes* and *Salmonella* Typhimurium. *In vitro* growth analyses of *L. monocytogenes* and *S. Typhimurium*, taking into account various parameters relevant for sausage ripening like pH-value, NaCl-concentration, temperature and oxygen availability, revealed, that the inhibitory effect of nitrite massively increases in combination with lowering the pH. *L. monocytogenes* is more sensitive to the combined inhibitory effect of nitrite and acidification than *S. Typhimurium*. It is assumed, that during sausage ripening not nitrite itself, but rather reactive derivatives are responsible for the observed antimicrobial activity of the curing agent sodium nitrite. One reactive nitrogen species that has often been mentioned in this context is nitric oxide. Interestingly, both pathogenic bacteria, *L. monocytogenes* and *S. Typhimurium*, are exposed to nitric oxide during the infection process. However, whereas *S. Typhimurium* is well equipped with NO-detoxification systems (HmpA, NorV, NrfA), no such systems are described for *L. monocytogenes*. Global transcriptional analysis with microarray analyses or next generation sequencing revealed that acidified nitrite causes massive transcriptional changes in both organisms. Whereas a global general stress response is induced in *L. monocytogenes* (up-regulation of genes encoding for proteins involved in general stress response, down-regulation of genes encoding for proteins

involved in cell division, translation, metabolism of lipids and others) a more directed response can be observed in *S. Typhimurium*, with *hmpA* being one of the strongest induced genes. The pathogen specific nitrite stress response might influence the ability of these organisms to withstand nitrite stress in the food product.

LMV08

Characterization of *Staphylococcus carnosus* Strains for the Application in Raw Ham Production

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Particular *Staphylococcus carnosus* strains are used as starter cultures in fermented meat. The aim of this study was to investigate if *S. carnosus* strains can be identified that improve the organoleptic properties of fermented raw ham. Therefore, bacterial strains must fulfill certain characteristics, e.g. an active nitrate reductase, which is important for the development of a stable color, and proteolytic activity, which is important for aroma production. Moreover, strains should be regarded as safe according to the qualified presumption of safety (QPS) system.

More than 70 staphylococcal strains, both commercially available strains and isolates from different raw meat products, were tested. 42 out of those 74 strains were identified as *Staphylococcus carnosus* by 16S rRNA gene sequencing and species-specific PCR (Blaiotta *et al.*, 2005). Strains were differentiated by RAPD-PCR with seven different primers. These strains were tested according to the guidelines of the National Committee for Clinical and Laboratory Standards (NCCLS) for resistance against 17 antibiotics commonly used in animal health and in human medicine. Two strains were found to be resistant against ciprofloxacin and one strain showed resistance against sulfamethoxazole-trimethoprim. Another four strains were found to be intermediate resistant against cefotaxime. Furthermore, the potential of the strains to produce the staphylococcal toxins SEA-SEE and TSST was investigated by PCR analysis. None of the tested strains carried any genes for toxin production.

The strains were also tested *in vitro* for their ability to metabolize proteins, tributyrin and nitrate, as those are the characteristics which are important for aroma- and color development in ham. Three strains stand out with a nitrate reductase activity of more than 0.6 mM NaNO₂/10⁷ KBE x mL⁻¹. While most of the strains showed a nitrate activity between 0.3 and 0.5 mM NaNO₂/10⁷ KBE x mL⁻¹, four strains showed no nitrate reduction at all.

At present 13 out of 42 *Staphylococcus carnosus* strains show the potential to be used as a starter culture in raw ham. It would be interesting to investigate their characteristics further in a meat model.

Literature: Blaiotta G, Casaburi A, Villani F. (2005) Identification and differentiation of *Staphylococcus carnosus* and *Staphylococcus simulans* by species-specific PCR assays of *sodA* genes. Systematic and Applied Microbiology. 28: 519-526.

LMV09

Who lives out there? - Molecular monitoring of the smear microbiota composition and diversity of surface-ripened red-smear cheese by high-throughput sequencing

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The surface of red smear cheeses is characterised by the establishment of a multifaceted microbial ecosystem during the ripening period, which is crucial for the development of appropriate sensorial as well as hygienic properties of the final product. However, microbial contaminants or the type of packaging during storage may cause severe smear defects, which often manifest in off-odors and a moist and sticky surface smear [1].

We employed high-throughput sequencing to gain a better insight into the smear microbiota composition and the microbial diversity of different cheese surface smears. The results of the 16S rDNA based metagenome and 16S rRNA based metatranscriptome analysis were in line with culture-dependent analysis and molecular community fingerprinting methods, but offered a much broader interpretation. An unexpected high diversity of

microorganisms was present in the community profiles realized by high-throughput sequencing. Surprisingly, not only typical smear microorganisms were detected, but also various bacteria not previously thought to be involved in cheese ripening. Our data also suggest that differences in the relative abundance of 16S rRNA between various smear samples are due to an altered metabolic activity of smear microorganisms.

In conclusion, total 16S rRNA sequencing proved to be a powerful tool for the study of the complex cheese surface microbiota, also providing insights into the metabolic activity of the ecosystem.

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LMV10

Growth behavior of different lactic acid bacteria in lupin flour and lupin protein isolate

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All over the world, fermented foods play a major role in the human diet. Besides food preservation, a number of advantages are linked with the fermentation process like improved flavor and digestibility or reduction of antinutritives^{1,2}. In contrast to soy, the fermentation of lupin, a high protein and high nutritive legume³, has not been studied extensively so far. Especially research information on the interaction of secondary plant metabolites with microorganisms is scarce. To contribute to this, the objective of this study is to investigate the fermentation performance of different lactic acid bacteria on lupin containing substrates (*L. angustifolius* cv. *boregine*). The metabolism of *Lactobacillus* (*Lb.*) *plantarum*, *Bifidobacterium* (*B.*) *animalis* ssp. *lactis*, *Pediococcus* (*P.*) *pentosaceus* and *Lactococcus* (*L.*) *lactis* ssp. *lactis* was investigated at their respective optimum growth temperature on a 10% lupin flour (LF) and 10% lupin protein isolate (LPI) suspension. Carbohydrate content (10%) and composition (mainly sucrose, raffinose and stachyose) of the protein suspension was thereby adjusted to that of lupin flour. Growth behavior including the competitiveness of the strains tested by statistical MALDI-TOF MS analysis of microbiota, lactate/acetate production and sugar utilization (analyzed by HPAEC) was evaluated. Fermentation experiments revealed that all selected microorganisms were able to grow and metabolize sugars on LF in the same order of magnitude as on LPI. In comparison, the growth rate of *B. animalis* ssp. *lactis* was slightly higher in LPI suspension and greater amounts of organic acids (+51.1% lactate, +51.2% acetate) were produced. On the contrary, *Lb. plantarum* showed a significantly higher metabolism activity on LF and *L. lactis* ssp. *lactis* was only dominant in the flour sample. Moreover, fermentation performance of *P. pentosaceus* was not significant different (p>0.05) on the two substrates.

In conclusion, fermentation behavior of the studied microorganisms was apparently not influenced by the secondary plant metabolites occurring in lupin flour used in this study.

References

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LMV11

Non-thermal atmospheric plasmas for food decontamination

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Introduction: Gentle sanitation of fresh fruits and vegetables is highly demanded since especially produce that is eaten raw increases the risk of food borne illnesses. Currently used disinfection or sanitation methods for fresh fruits and vegetables lack antimicrobial effectiveness, but are high in costs, water consumption or chemicals. Non-thermal atmospheric pressure