

between the predicted proteasome and the content of biometal co-factors. Only bacteria, which harbors manganese depend superoxide dismutase and members of NRAMP-family uptake system, contain a high amount of manganese (6). Additionally, the transition metal ratios change dramatically from bacteria to yeast and to phototrophic organism.

FTP059

Dissemination of dairy bacteriophages in whey powder samples

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Bacteriophages (phages) are a frequent cause for fermentation problems in dairies. When these bacterial viruses infect lactic acid bacteria, which are important lactic acid and flavour producers, serious delays or even complete failures of fermentation batches may occur resulting in significant financial losses. We have previously shown that phages of *Lactococcus lactis* starter strains may reveal remarkably high thermal stability [1]. Hence, these phages are not inactivated properly by heat treatment conditions used for pasteurization of raw milk and may propagate in the dairy resulting in high phage numbers in cheese whey (i.e., up to 10⁹ plaque-forming units [pfu] per ml). Since large quantities of whey are processed into whey powder by spray-drying, we wanted to assess the dissemination of surviving dairy phages in whey powder samples.

Whey powder samples obtained from 11 whey powder producing plants were tested with a representative set of 59 starter strains encompassing mesophilic (*L. lactis*) and thermophilic (*Streptococcus thermophilus*) acid-producing and mesophilic flavour-producing bacterial isolates (*Leuconostoc pseudomesenteroides*, *Leuconostoc mesenteroides*). Notably, lytic lactococcal phages were present in samples from all 11 dairies, while *S. thermophilus* phages and *Ln. pseudomesenteroides* and *Ln. mesenteroides* phages were detected in samples obtained from 8 and 6 dairies, respectively. Maximal phage titers were 6x10⁷ (*L. lactis*), 1x10⁹ (*Ln. pseudomesenteroides*) and 1x10⁴ (*S. thermophilus*) pfu per gram of whey powder. Many of these phage populations revealed extended host ranges, illustrating the potential risk of re-cycling phage-contaminated whey components in dairy fermentation processes. Nearly all of the lactococcal phages isolated from whey powder belonged to the wide-spread 936 phage species known to include also the most heat-resistant phages.

[1] Atamer, Neve, Heller, Hinrichs. 2012. Thermal resistance of bacteriophages in the dairy industry. In: Bacteriophages in dairy processing, pp. 195-214, Nova Publishers, Hauppauge

FTP060

Expression of subunit ND5 of the respiratory complex I (NADH:quinone oxidoreductase) from *Yarrowia lipolytica* in *Saccharomyces cerevisiae* leads to an increased salt sensitivity

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Complex I in the inner mitochondrial membrane is the first enzyme of the electron transport chain. Electrons from the oxidation of NADH are transferred to ubiquinone. This might induce conformational changes coupled to the transport of protons or other cations across the inner membrane. Our special interest is the ND5 subunit of the mammalian complex I, which is known to play a role in neurodegenerative diseases. The membrane bound ND5 subunit is considered to be involved in the proton pumping process and there is increasing evidence that ND5, also a homologue of bacterial H⁺/Na⁺ antiporters, is also able to translocate sodium ions (Gemperli, Schaffitzel et al. 2007). In addition human ND5 expressed in *S. cerevisiae* leads to an increased salt sensitivity (Steffen, Gemperli et al. 2010). In this study the optical density and the number of colony forming units (CFU) of *S. cerevisiae*, containing wild type and the F123L and E144G variants of ND5 from *Y. lipolytica* in membranes of the ER, were determined after incubation in a medium with or without added salt for 24, 48 and 72 hours. Cells containing wildtype ND5 grown without added salt exhibited the highest cell densities up to OD₆₀₀ = 20, corresponding to 1 x 10⁸ colony forming units (CFU). Growth of *S. cerevisiae* containing wildtype ND5 was heavily impaired when 800 mM NaCl or KCl was present in the medium. This inhibitory effect was diminished with *S. cerevisiae* producing ND5-E144G. We observed a 20-fold increase in CFU at 800 mM NaCl or KCl compared to cells producing wildtype ND5. The results are in accord with a presumed cation transport activity which affects

ion homeostasis in *S. cerevisiae*. Support for this notion comes from Na⁺ transport studies with ND5 in vesicles derived from endomembranes of *S. cerevisiae*. Wild type ND5 promoted Na⁺ uptake (50 nmol after 90 sec) whereas vesicles containing the ND5-E144G variant exhibited diminished Na⁺ transport activity (10 nmol after 90 sec). Thus, we conclude that the individual ND5 subunit of complex I from *Y. lipolytica* exhibits cation transport activity.

Gemperli, A. C., C. Schaffitzel, et al. (2007). "Transport of Na⁺ and K⁺ by an antiporter-related subunit from the *Escherichia coli* NADH dehydrogenase I produced in *Saccharomyces cerevisiae*." Arch Microbiol 188(5): 509-521.

Steffen, W., A. C. Gemperli, et al. (2010). "Organelle-specific expression of subunit ND5 of human complex I (NADH dehydrogenase) alters cation homeostasis in *Saccharomyces cerevisiae*." FEMS Yeast Res 10(6): 648-659.

FTP061

Structural characterization of lantibiotic immunity proteins

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Lantibiotics are peptide-derived antibiotics that inhibit the growth of Gram-positive bacteria mainly via interactions with lipid II and lipid II-dependent pore formation in the bacterial membrane. Due to their general mode of action the Gram-positive producer strains need to express immunity proteins (LanI proteins) for protection against their own lantibiotics. Subtilin producing *Bacillus subtilis* and nisin producing *Lactococcus lactis* strains possess four immunity genes which code for the lipoproteins SpaI and NisI and the ABC-transporter SpaFEG and NisFEG, respectively. Little is known about the immunity mechanism protecting the producer strain against its own lantibiotic on the molecular level.

The expression of SpaI or NisI alone is sufficient to confer subtilin immunity to *B. subtilis* or nisin immunity to *L. lactis*. Interestingly there is no cross-immunity between SpaI and NisI, despite the high sequence and structural similarity of subtilin and nisin but is in agreement with the limited sequence similarity between SpaI and NisI.

In order to elucidate this highly specific immunity mechanism we solved the structure of a 15 kDa biologically active fragment of SpaI by NMR which is the first structure of any LanI protein. NMR investigations of a full length construct of SpaI lacking the diacylglycerol anchor suggest that the 30 N-terminal amino acids are unfolded in the absence of a membrane. However, this N-terminal stretch interacts with liposomes in NMR titration experiments. When mutating this stretch *in vivo* the SpaI mediated immunity of *B. subtilis* against subtilin is not affected.¹

The structure of the 25 kDa NisI protein in comparison to the 17 kDa SpaI will give insights to the highly specific immunity mechanism of LanI proteins. We were able to purify the full length NisI to high purity and stability for the structure determination by NMR. The ¹⁵N-HSQC spectrum of NisI shows well dispersed peaks with some overlap in the center indicating a well folded protein with possible unfolded termini.

Our results are the first step on the way to understand the immunity mechanism of subtilin and nisin producing strains on a structural level at atomic resolution.

Reference

¹Christ N.A., Bochmann S., Gottstein D., Duchardt-Ferner E., Hellmich U.A. Düsterhus S., Kötter P., Entian K.D. and Wöhnert J. (2012) *JBC* **287**, 35286-98.

FTP062

Application of encapsulated baker's yeast as attractant for soil living insect pests

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As several soil living important pest insects like western corn rootworm and wireworms use CO₂ for host (plant roots) location the use of attractants based on CO₂ is a promising approach for attract(-and-kill) strategies in pest control. Laboratory experiments have shown that different artificial CO₂