P W6 - Characterization of *Streptococcus thermophilus* lytic bacteriophages from mozzarella cheese plants and evidence of a temperate bacteriophage from the lysogenic host strain *Streptococcus macedonicus* AI4

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Phage attack still represents the main cause of fermentation failure during the manufacture of mozzarella cheese, in which *Streptococcus thermophilus* is employed as starter culture. Thereby, the success of commercial lactic starter cultures is related to the use of strains not susceptible to phage infections. The characterization of lytic phages from *Streptococcus thermophilus* is a leading tool for the selection and use of efficient starter cultures.

In this study 26 bacteriophages isolated from mozzarella cheese whey samples were examined in terms of their DNA restriction profiles, packaging mechanisms and host range. The DNA restriction analysis was carried out by using seven different enzymes (*Eco*RV, *Cla*I, *Eco*RI, *Hind*III, *Hae*III, *Pst*I and *Sal*I) leading to a clustering of the phages. The bacteriophages were classified within the two main groups of *S. thermophilus* phages (*cos*- and *pac*-type) using a multiplex PCR method based on the amplification of conserved regions in the genes coding for the major structural proteins. Most of the phages belong to the *cos*-type group, whereas only few of them gave a PCR fragment distinctive of the *pac*-type group. Twenty presumptive *S. thermophilus* strains, were used to define the host range of the phages, analysed by sequencing of the 16S rDNA and tested for the presence of prophages by mitomycin C induction. Three of them were finally identified as *S. macedonicus* and a temperate phage (PZ1φ was induced from strain *S. macedonicus* AI4. PZ1φ belongs to Bradley's phage group B1, having a hexagonal head and a long, non-contractile tail. The DNA homology between PZ1φ and *S. thermophilus* bacteriophage was even confirmed by Southern analysis. At the best of our knowledge, this is the first evidence for a phage within the *S. macedonicus* species.

Moreover, the amplification of the variable region of the antireceptor gene VR2 and the classification of phages confirmed the correlation between typing profile and host range.

P W7 - Occurrence Of Enteric Viruses In Italian Shellfish And Relation To Climatic-Environmental Factors

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Shellfish are recognized potential vehicles of foodborne diseases, since they can accumulate the pathogenic microorganisms by filter-feeding. Many shellfish-associated outbreaks have been attributed to enteric viruses, particularly noroviruses (NoV), a *Caliciviridae* genus responsible for 70% of human gastroenteritis worldwide and one of the most important causes of gastroenteritis in Europe. In Italy, the real incidence of NoV is yet unknown; however outbreaks of NoV gastroenteritis related to seafood consumption have already been registered. As for NoV, hepatitis A virus (HAV) has been cause of outbreaks in several countries and shellfish consumption is a main risk factor for HAV infection. The aim of this study was to investigate the prevalence of HAV and NoV in shellfish harvested from the Po river delta, focusing on the relation between viral contaminations and environmental factors (water pH, salinity, temperature, dissolved O_2 and hydrometric levels of the Po River).

Two harvesting areas, a lagoon and an open sea point, were selected. Shellfish (mussels and clams) were collected every 15 days for one year, with a total number of 120 samples. The presence of HAV and NoV was detected by RT-nested PCR and Real time PCR and NoV amplicons were sequenced for genomic characterization. Water parameters were measured *in situ* through GSM communication.

Virological analyses showed no contaminations by HAV and 10 NoV-positive samples (8.3%), all collected from the lagoon. Four samples belonged to the Genogroup I (GGI), and 6 samples belonged to GGII. Contamination was detected throughout the year with a higher frequency from February to April. This period was also characterized by some relevant variations in the hydrometric levels of the Po River, probably due to rain events. Water parameters showed no significant differences in the two areas with predictable fluctuations related to seasonal variations.

The absence of HAV-positive samples suggests that HAV in shellfish has distinctive geographical features and Northern Italian production areas are less influenced than Southern endemic areas. The presence of NoV could be ascribed to an increase of the hydrometric level of the tributary river during the rainy periods. The increase in the load of viruses could be due to the higher water flow and the remix of the sea bottom, where the virus can deposit and survive for long periods.

Poster Session X – Stress Response

P X1 – The Expression of *csp*-Genes after Cold-Shock in *Yersinia Enterocolitica* and *Yersinia Pseudotuberculosis*

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Yersinia enterocolitica and *Yersinia pseudotuberculosis* are important psychrotrophic food-borne pathogens, infections of which are usually acquired through ingestion of contaminated refrigerated food. The ability to produce cold-shock proteins rapidly and efficiently enables the bacteria to adapt to low temperatures. However, the cold-shock response has been studied mainly in mesophilic organisms.

The expression of cold-shock genes of *Y. enterocolitica* strain 8081 and *Y. pseudotuberculosis* strain IP32953 was studied by microarray analysis. Cells were grown at 30 °C to logarithmic growth phase after which temperature was reduced to 5 °C. Cells were harvested at different time points following temperature downshift. Total RNA was extracted from the cells, reverse transcripted to cDNA and labeled after which DNA microarray analyses were performed.

The expression of cold-shock protein genes was rapid and substantial both in *Y. enterocolitica* and *Y. pseudotuberculosis*. Expression levels stayed high as late as seven hours after cold-shock. Apparently this highly efficient transcription of cold-shock protein genes contributes to the ability of these bacteria to survive cold storage during the food chain.