



*LVII SAIB Meeting - XVI SAMIGE Meeting*

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*Chlamydia trachomatis* (CT) is the most frequent causative agent of bacterial sexually transmitted infections worldwide. CT is an obligate intracellular pathogen presenting a biphasic life cycle that involves the transition between infectious elementary bodies (EBs) and replicative but not infectious reticulate bodies (RBs). The cyclic transitions between EBs and RBs occur inside a CT-induced intracellular vacuole or “inclusion”. In the presence of stressors such as beta-lactams or interferon-gamma (IFN $\gamma$ ), CT enters into a poorly studied viable but non-cultivable state termed “chlamydial persistence”, which is reversible upon removal of the stressors and considered critical for pathogenesis. Polymorphic membrane proteins (PMPs) are a family of *Chlamydia*-specific autotransporter proteins secreted via a type V secretion system. The genome of CT encodes 9 PMPs (PMPA-I), which have been proposed to play a role in antigenic variation and adherence, however, PMPs functions remain ill-defined due to *Chlamydia* being historically refractory to traditional genetic manipulation. In a previous screen with a collection of ~1000 genome sequenced CT chemical mutants, we identified a PMPC nonsense mutant (pmpC-ns) with a defective phenotype in chlamydial persistence. In order to confirm the role of PMPC in chlamydial persistence, a PMPC-null mutant was obtained via insertional gene inactivation with a group II intron (pmpC::GII). We observed that in control conditions, both wild type (WT) and pmpC::GII CT were able to complete their life cycle and generate similar amounts of infectious EBs. However, upon penicillin- or IFN $\gamma$ -induced persistence, pmpC::GII presented a defective phenotype, consistently showing a decreased production of EBs after removal of the persistence inducers. To further investigate PMPC functions in CT, adherence and invasion assays were carried out in epithelial HeLa cells using fluorescently-labeled WT, pmpC::GII and pmpC-ns CT. We found no statistically significant differences in adherence to HeLa cells between either strain. Nevertheless, pmpC::GII and pmpC-ns CT invasion rates were more than 10 fold lower than that observed for WT CT. Curiously, both pmpC-ns and pmpC::GII displayed an altered phenotype inside the inclusion, characterized by a non-homogeneous distribution of the bacteria, which were instead observed forming “aggregates”. By performing live-cell microscopy of HeLa cells infected with fluorescently labeled WT, pmpC::GII or pmpC-ns CT, we confirmed that lack of PMPC was associated with “auto-aggregation” inside the inclusion, which was not rescued by co-infecting with the WT strain, thus suggesting that homotypic PMPC interactions might prevent this aggregation phenomenon. In conclusion, these results support that PMPC participates in penicillin- and IFN $\gamma$ -induced persistence and CT invasion but not adherence, and also in preventing auto-aggregation of the bacteria inside the inclusion.

## MI-P070-41

### DETERMINATION OF TRIGLYCERIDES IN *Caenorhabditis elegans* FED LACTOBACILLI

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*Caenorhabditis elegans* is regarded as a simple model to assess the *in vivo* effects of probiotics, especially concerning the study of fat metabolism due to its ability to store lipids in intestinal and skin-like hypodermal cells. The main constituents in fat droplets stored in this nematode are triglycerides (TG). The objective of this work was to evaluate TG levels in *C. elegans* feeding with lactobacilli alone or combined in different proportions. The strains used in this study are listed as follow: *Lactocaseibacillus rhamnosus* CRL1425, *Lactiplantibacillus plantarum* CRL1427, CRL1428, CRL1449, CRL1472, *Lactocaseibacillus casei* CRL1430, *Limosilactobacillus fermentum* CRL1446 y *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL1447. The strains of the different mixes were selected based on previously studied functional properties and were combined as follows: mixture 1 (Mix 1) was formed by CRL1446, CRL1449, and CRL1472; Mixture 2 (Mix 2) by CRL1446 and CRL1449, Mixture 3 (Mix 3) by CRL1446 and CRL1472, and Mixture 4 (Mix 4) by CRL1449 and CRL1472. Synchronized nematodes were fed *Escherichia (E.) coli* OP50 (control nematodes) and OP50:Lactobacilli in a ratio of 0:100; 25:75; and 50:50 (treated nematodes) at 18 °C until they reached the L4/adult stage. Then, a 5% solution of Triton X-100 was added and the suspension was sonicated. The lipids were solubilized at 90 °C for 5 min, and the lysate was removed by centrifugation. TG was determined in the supernatant by enzymatic methods. At least 3 biological replicas were used for each or mixtures of strains. The results showed that nematode development was slower in the 0:100 OP50:Lactobacilli ratio, while the 50:50 OP50:Lactobacilli ratio was similar to the control. In a 25:75 ratio, all strains, except CRL1427 and CRL1428, showed a significant reduction in TG levels. The CRL1425, CRL1446, and CRL1447 strains had the highest percentage of TG reduction (75, 70, and 75%, respectively). When the nematodes were fed with Mix 1, Mix 2 and Mix 3 presented a significantly lower TG content than the control, with a reduction percentage of 56, 49, 42%, respectively. However, no significant differences were observed between these mixes. Mix 4 did not induce any change compared with nematode control. In conclusion, *C. elegans* can be used as a screening method for strains with the ability to reduce TG content, which reports an anti-obesity effect of these strains.

## MI-P071-82

### BIOCONTROLLING CAPACITY OF EXTRACELULAR VESICLES FROM PLANT BENEFICIAL BACTERIA

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