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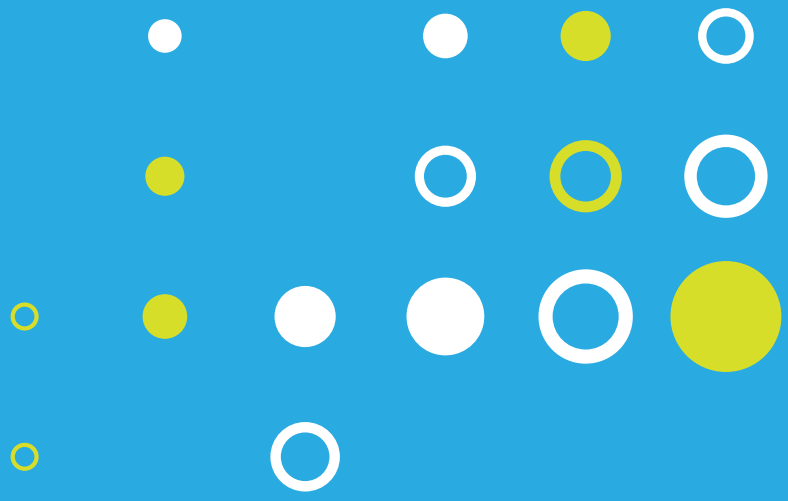

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MI-C09.

IDENTIFICATION AND CHARACTERIZATION OF PHOSPHATIDIC ACID PHOSPHATASE ENZYMES IN *Streptomyces coelicolor*

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Phosphatidic acid phosphatase (PAP) catalyzes the dephosphorylation of phosphatidate yielding diacylglycerol (DAG), the lipid precursor for triacylglycerol (TAG) biosynthesis. Considering the increasing interest of bacterial TAG as a potential source of raw material for biofuel production, we have focused our studies on the identification and physiological characterization of the putative PAP enzymes present in the TAG producing bacterium *Streptomyces coelicolor*. We have identified two *S. coelicolor* genes, named *lppa* and *lppß*, encoding for functional PAP proteins. Heterologous expression of *lppa* and *lppß* genes in *E. coli* resulted in higher levels of DAG in this bacterium. In addition, the expression of these genes in yeast complemented the temperature-sensitive growth phenotype of the PAP deficient strain GHY58 (*dpp1lpp1pah1*). In *S. coelicolor*, the simultaneous mutation of both genes provoked a drastic reduction in *de novo* TAG biosynthesis as well as in total TAG content. Consistently, overexpression of Lppa and Lppß in the wild type strain of *S. coelicolor*, led to a significant increase in TAG production. The present study describes for the first time the identification of PAP enzymes in bacteria, completing the whole set of enzymes required for *de novo* TAG biosynthesis and providing further insights on the genetic basis for prokaryotic oiliness.

MI-C10.

ANTIGENICITY AND PROTECTIVE CAPACITY OF NOVEL VACCINE CANDIDATES FOR *Trypanosoma cruzi*

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We have previously identified a group of 22 novel vaccine candidates for Chagas' disease by screening an epimastigote-subtracted trypomastigote cDNA expression library. Of these, we selected 3 genes for further studies: G2, a hypothetical protein (TcCLB.507003.70), A12, a putative lysosomal membrane glycoprotein (TcCLB.510825.30), and A11, a TcTASV-C surface antigen (TcCLB.511675.3). In this work we analyzed the antigenicity of these proteins in the course of experimental and natural infection with *T. cruzi* and evaluated the protective capacity of TcTASV-C. Sera obtained from humans and infected animals reacted against the recombinant proteins, suggesting that these genes are expressed in the parasite stages that infect the definitive host and validate them as vaccine candidates. To test the protective capacity of TcTASV-C, we employed a prime and boost vaccination schedule (2 doses of DNA + GM-CSF/mouse and 2 doses of protein + alum/ mouse; TcTASV-C: n=6; controls: n=6). Fifteen days after the last dose, animals were challenged with the highly virulent *T. cruzi* RA strain (lineage VI). Vaccinated animals presented lower levels of both circulating parasites and mortality (50% vs 100% at 30 d.p.i.) than controls, suggesting that TcTASV-C induces a partially protective response.

MI-C11.

BtaE: A POLAR ADHESIN INVOLVED IN BINDING OF *Brucella suis* TO HOST CELLS AND VIRULENCE IN MOUSE

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Increasing evidence indicates that adhesion of *Brucella* spp. to host cells is an important step to establish infection. We have previously shown that an unipolar type I monomeric autotransporter (BmaC) mediates the binding of *Brucella suis* to host cells through cell-associated fibronectin. Genome analysis shows that the *B .suis* genome encodes several additional potential adhesins. In this work we show that a protein from the Trimeric Autotransporter Family named as BtaE is involved in the binding of *B. suis* to hyaluronic acid and fibronectin. The *B. suis btaE* mutant was: impaired in the ability to bind to host cells, outcompeted by the wild type strain in co-infections experiments, and showed an attenuated phenotype in the mouse model. Similar to BmaC, the BtaE adhesin was only observed at one of the cell poles. In *Brucella* and other α-proteobacteria, the two daughter cells generated by asymmetric division are differentiated bacteria displaying different pole markers. Using pole markers we observed that BtaE is associated with the new pole. Furthermore, BmaC was also found to be localized to the same (new) pole, suggesting that *Brucella* adhesins are located at the new pole of the bacteria at a particular stage of the cell cycle.

MI-C12.

BB3576 DIGUANILATE CYCLASE PROTEIN REGULATES MOTILITY AND BIOFILM IN *Bordetella bronchiseptica*

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Cyclic diguanylate (c-di-GMP) is a ubiquitous second messenger that regulates diverse cellular functions, including motility, biofilm formation, and virulence in bacteria. Components of this regulatory network include GGDEF domain-containing proteins that synthesis c-di-GMP. In a previous work we demonstrated that heterologous expression of a *Pseudomonas aeruginosa* GGDEF protein modifies biofilm formation capacity and motility in *B. bronchiseptica* (Bb), a pathogenic bacterium that causes respiratory infections in a wide variety of hosts.

In this work, we analyzed the expression of BB3576, a putative GGDEF protein of *B. bronchiseptica*. To this end, bb3576 gene from Bb was amplified and cloned in the replicative plasmid pBBMCR5 under a constitutive promoter and transformed in Bb. Biofilm formation and motility were evaluated in the recombinant bacteria to detect possible changes in c-di-GMP concentrations. As found in other organisms that contain high levels of c-di-GMP, we observed that Bb was able to form biofilm and reduce its motility only in the case bacteria express GGDEF domain-containing protein. Interestingly, high *bb3576* mRNA expression levels were detected by real time PCR in avirulent phase, when motility is present. These results demonstrate the presence of a functional GGDEF protein in Bb and suggest a putative motility regulation function for Bb3576.