**Lavoro scientifico del secondo anno di dottorato in Biotecnologie e Bioscienze**

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During the second year of my PhD in Biotechnologies and Bioscience, conducted at the Probiogenomic Laboratory, under the supervision of Professor Francesca Turroni, I worked on different aspects regarding the biology of bifidobacteria. Bifidobacteria are dominant members of the infant gut microbiota, and they are considered health-promoting bacteria, acting as natural modulators of the host immune system, with a huge impact on the physiology and the metabolism of the human large intestine. Different studies have been demonstrated the existence of a vertical transmission of bacteria from mother to child. In this context, the type of the birth and the type of breastfeeding plays an important role, influencing the composition of the infant gut microbiota. Until few years ago, the intestine was considered sterile before birth, but recent studies demonstrated the possible existence of a pre-birth microbiota and of a placental microbiota. In this context, as bifidobacteria is one of the first colonizer of the gastrointestinal tract, we decided to investigate their transmission from mother to child before the natural delivery by means of animal models (*Rattus norvegicus*). Experiments were performed to evaluate the bifidobacterial inheritance, involving a group of animals treated with a mix of bifidobacterial strains belonging to three different species, i.e., *B. bifidum*, *B. breve* and *B. longum* and a second group with a single *Bifidobacterium* strain. During the gestation period, the presence of *Bifidobacterium* strains in faecal samples was monitored through a qPCR approach based on strain-specific primers. Following caesarian delivery of the pups, rats were sacrificed and their caecum was assayed for the presence of bifidobacteria, revealing the occurrence of bifidobacterial DNA of the strains administered in both mother and newborns. Since *B. bifidum* PRL2010 strain showed the highest occurrence of vertical transmission from mother to newborns, we decided to further examine the maternal inheritance of this specific strain. For this reason, we set up a second experiment, following the same procedure. Also in this case, PRL2010 DNA was identified in mother caecal samples and in newborn caecal samples, but the abundance level was lower respect the previous study where we used a mix of three *Bifidobacterium* strains. In order to investigate the presence of *B. bifidum* PRL2010 in other body sites of rats, we performed qPCR experiment on blood and placenta of mothers. Remarkably, these experiments revealed the presence of DNA belonging to PRL2010 in the placental samples but not in blood, suggesting that the mother’s placenta can be reached of only PRL2010 DNA. In order to further characterize the bifidobacterial composition of caecal samples from mothers and puppies, ITS (Internal Transcribe Spacer) bifidobacterial profiling analyses were performed. Interestingly, mothers and puppies shared bifidobacterial clusters of identical sequences (Operational Taxonomy Units - OTUs), supporting the notion of (bifido)bacterial transmission of DNA and/or cells . The identification of vertically transmitted bifidobacterial strains represents a key example of microbe-host co-evolution.

In addition to vertical transmission of bifidobacteria from mothers to newborns, we decided to investigate the horizontal transmission, also called Horizontal Gene Transfer (HGT), of bifidobacterial genes to other microorganisms. HGT events involve genes that can be transmitted to other microorganisms by different mechanisms, such as insertion sequence (IS) elements, prophages and plasmids. In this context, a big issue affected to the human health, involve the transmission of Antibiotic Resistance (AR) genes to pathogenic bacteria. For these reasons, the resistome of the *Bifidobacterium* genus and the correlation between the bifido-resistome and mobilome of this genus were investigated. The analysis was conducted on 625 different *Bifidobacterium* strains, belonging to 67 (sub)species retrieved from the NCBI database. The number of putative AR genes identified among strains was 13,870, which constituted less than 1% of the total bifidobacterial genes analyzed. According to the predicted mechanism of action, the predicted AR genes identified were classified in seven different classes. The most represented class was the glycopeptide, which included genes that are predicted to counteract glycopeptide antibiotics, such as vancomycin, teicoplanin and telavancin. We cannot state that all predicted AR genes were functional, but they represented the potential arsenal to contrast antibiotics. Furthermore, the 625 bifidobacterial genomes were analyzed in order to investigate the presence of Mobile Genetic Elements (MGEs), such as IS and prophage-like elements, revealing the presence of 16,065 predicted genes encoding for transposases and 598 prophage sequences. In order to evaluate the homology among identified prophage-like elements a genomic based-alignment clustering was performed. Interestingly, this analysis identified four main homology clusters, in which the taxonomic origin of the corresponding *Bifidobacterium* hosts was very heterogeneous. In order to evaluate the presence of predicted AR genes located on the mobile elements as well as close to it, flanked genes of the predicted resistome were investigated. These regions represent Mobile Genetic Hotspots (MGHs) that could result in HGT events, shedding antibiotic resistance to other bacteria. 201 putative MGHs were identified, recognized in 120 *Bifidobacterium* strains analyzed. This data suggested that less than the 1.5 % of the total *Bifidobacterium* resistome could be involved in HGT events. Therefore, almost all putative MGHs identified encompassed transposons that could not be classified as conjugal transposon, reducing the possible HGT events of AR genes.

In conclusion, the reconstructed putative resistome revealed that only a limited number of bifidobacterial genes were putatively involved in AR mechanisms. Nevertheless, most of identified MGHs in the *Bifidobacterium* genus could not be transfer to other microorganisms, due to the transposition mechanism of the identified IS elements flanking putative AR genes. These finding underlined the safety of the *Bifidobacterium* genus.