Chapter 5: Concluding remarks

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The establishment of the intestinal microbiota starts at birth and the colonization process is influenced by numerous factors including mode of delivery, gestational age, mode of feeding and environmental factors. The comprehension of all aspects related to the initial bacterial progression is of paramount relevance, particularly considering that this phenomenon may influence health in the adult life. The major objective will be to determine the putative role of all microorganisms including the uncultured ones, among them in particular the hydrogenotrophs. For this reason, the present thesis intended to quantify hydrogen-consuming microorganisms starting from the first days until the second year of life, in order to understand possible correlations with delivery and feeding conditions. Three populations were considered as study objects namely SRB, acetogens and methanogenic archaea and methods applied were qPCR, PCR-DGGE and Illumina sequencing. The insight offered by our work revealed that probably the infant microbiota ecology is more complex than previously proposed. The study of those neglected communities revealed the need of developing more powerful tools for the detection of low abundant populations. The major limitation in the investigation of scarce represented microorganisms is due to the DNA extraction protocol. Our research achieved an improved detection of hydrogenotrophic populations thanks to the mechanical DNA extraction protocol chosen for processing fecal samples. Furthermore, it has to be noted that the limited number of subjects generally involved hampers studies involving infant samples. The limited number of samples reduces the possibility of doing significant statistical analysis and results are affected by inter-individual characteristics. However, firstly, our preliminary data presented a step towards the understanding of hydrogenotropic ecology in infants. Our data reported the presence of SRB, acetogens and methanogens in 16 tested babies. The deeper analysis of the Clostridium XIVa group, which includes many acetogenic species, revealed the ubiquitous presence of Blautia genus and allowed the recovery of H. effluvii in infant fecal specimens. Secondly the longitudinal study of Lachnospiraceae family among the acetogenic population in 25 babies aged 15 days-24 months showed a high prevalence of R. gnavus. Thus extensive investigations on large numbers of babies are necessary to get a deeper insight into the putative ecological role of those microorganisms in the gut. Third, we studied methanogenic population shifts in control and pectin-formula fed piglets groups. The animal model has been used as a surrogate for the infant model for the intervention study. This research detected, as expected, that the Methanobrevibacter genus followed by M. cuniculi are the dominant microorganisms in the pig gut ecosystem. Moreover, to the best of our knowledge it was the first time that M. woesi was

identified in pig feces. This study may represent a relevant step towards a better comprehension of gut archaea composition and its putative impact on host physiology. At the end of my experiments it has been possible to identify a “fil rouge” shared by all presented work. Investigations on low abundant populations, communities that have previously been neglected, is complex. The key to success in studying such microorganisms is mainly linked to the quality of the DNA extraction protocol. It must also be mentioned that infant fecal samples are hard matrixes to manage. In fact, contrary to adult specimens, babies’ samples are variable depending on the infant phase. Typically during the neonatal period feces have a high water content, during weaning they contain more dietary fibers. The amount of fecal matrix for the DNA extraction represents the first crucial point. Equal weight of feces may not result in the same DNA yielded both qualitatively and quantitatively hence the variations have to be estimated. The DNA extraction protocol is another critical point that was overcome by using a mechanical bead beating protocol. Downstream bio-molecular analysis embodies an additional challenge. To date, there is no evidence that previously described primer pairs may correctly detect hydrogenotrophic populations. This thesis underlines the need of further investigations to get a deeper insight on the putative role of the hydrogenotrophic populations and, more extensively, of other low abundant microorganisms. New developed molecular tools could foster research efforts in this field.