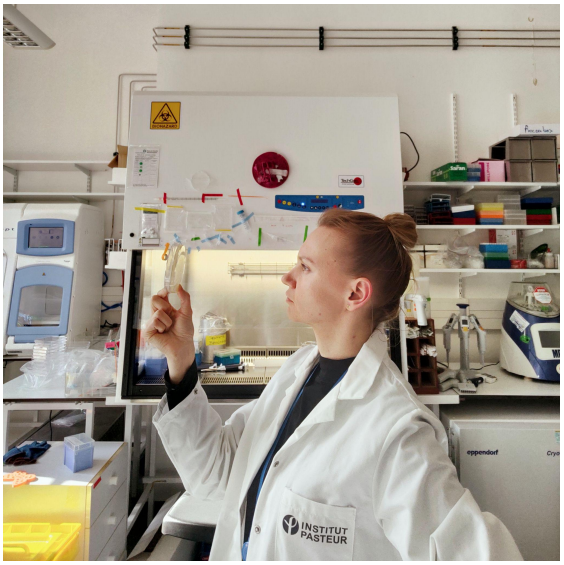


Thanks to the funding programme under the *Agreement for scientific cooperation between Polish Academy of Sciences (PAS) and Centre National de la Recherche Scientifique (CNRS)* I have a pleasure to be a visiting scientist in the Evolutionary Biology of the Microbial Cell Laboratory lead by Prof. Simonetta Gribaldo.

The purpose of my stay at Pasteur Institute is to broaden my scientific qualifications, i.e., improve skills in terms of experimental and bioinformatics approaches utilized in the research of non-model bacteria and archaea.

During my visit to the Pasteur Institute, I will work with an anaerobic bacterium called *Veillonella parvula* - a new experimental model in bacterial cell envelope research. *V. parvula* studies are of great medical significance as the species is an abundant component of the human microbiome. What is more, despite belonging phylogenetically to the Firmicutes, *V. parvula* surprisingly harbours an outer membrane (OM) with lipopolysaccharide (LPS), which is characteristic typical of Gram-negative bacteria, making it an evolutionary enigma and a promising new model to study the diversity and function of bacterial cell envelopes (Figure 1). Gribaldo's group has developed a wide range of genetic tools for *V. parvula* opening the way to experimentally gather insights about the biogenesis and functioning of its outer membrane and the consequences of its loss.

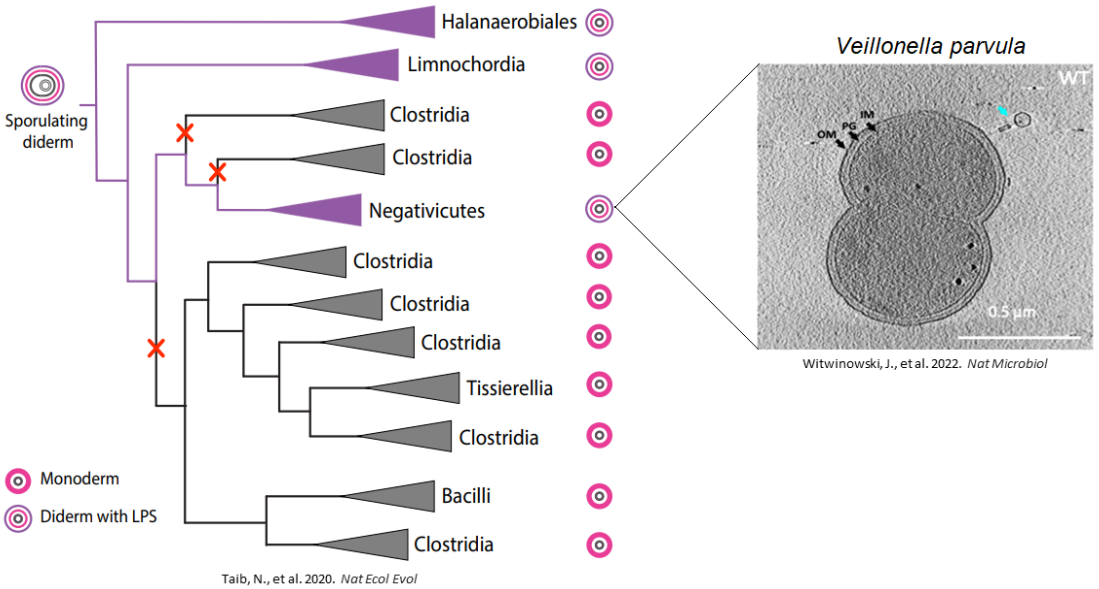
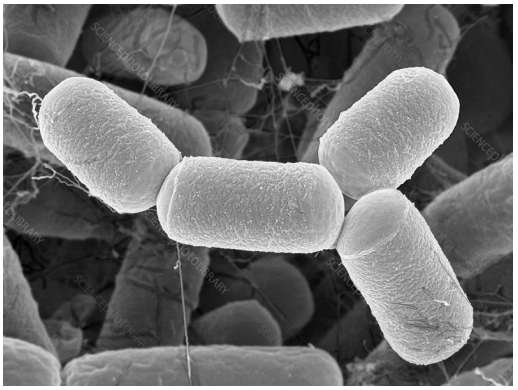


Figure 1. Distribution of monoderm and diderm cell envelopes, and cryo-electron tomography of *V. parvula* WT (OM=outer membrane, PG=peptidoglycan, IM=inner membrane).

One of the aims of my research stay at Gribaldo's group is to develop and optimize the transposon sequencing technique (Tn-Seq) for *V. parvula*. Tn-Seq is a method that combines genome-wide transposon mutagenesis with high-throughput sequencing to estimate the fitness contribution or essentiality of each genetic component in a bacterial genome. A strength of this method is that it allows direct linkage of phenotype to genotype in a high-throughput manner. Ultimately it aims to elucidate the function of each genomic feature and is therefore a critical tool to help interpret genome sequencing data being generated. The idea of the project is to determine which genes are co-essential in *V. parvula* to further study the diderm to monoderm transition. Interesting phenotypes will be characterized and observed by high-resolution microscopy. Finally, I will analyze the results *in silico* by receiving training from skilled bioinformaticians in the Gribaldo's group.

Prof. Gribaldo and her team are also experts in the evolution of Archaea. They have developed *Methanobrevibacter smithii* as a new archaeal model organism from the human microbiome (Figure 2), but genetic tools are unfortunately still lacking. By joining the group, I will therefore be exposed to the subject and learn how to culture them.



Methanobrevibacter smithii

Figure 2. Scanning electron micrograph (SEM) of the human-associated archaeon *Methanobrevibacter smithii*.

The hands-on experience I'm going to acquire during my stay at Institut Pasteur would be a great asset in terms of the research I am conducting at IBB PAS. At present, I am working as a researcher in a Laboratory of Environmental and Evolutionary Systems Biology that focuses on the comparative and evolutionary genomics of microbes. We investigate microbial consortia from natural and experimental environments. Our group analyzes metagenomes and the biodiversity of unique habitats, starting from a gold and arsenic mine, through hydrogen and methane-producing bioreactors, wastewater treatment plants to laboratory sewage tanks.

We are keenly interested in investigating the genomes of selected microorganisms with unusual characteristics, like the ZS207 strain described by our team, belonging to *Acinetobacter lwoffii* species, isolated from the biofilm found in a gold mine in Złoty Stok, Poland. This isolate is interesting because it has developed numerous mechanisms enabling

survival in an environment containing a high level of arsenic and heavy metals compounds which makes it potentially useful in bioremediation.

We plan to examine other, poorly characterized strains (bacterial and archaeal) displaying unique and valuable attributes, isolated from the microbial communities that we explore. The new skills in culturing and manipulating both archaeal and bacterial non-model anaerobic microorganisms will be very important to this line of research.

We are currently investigating the anaerobic microbial community from the sugar factory wastewater treatment plant. Our *in silico* analyses revealed extremely high diversity of bacterial and archaeal isolates present in the examined WWTP. We are also performing research that aims to characterize the microbial community diversity of the sewage sludge that has been collected from a sedimentation tank belonging to IBB PAS. At some point in its use, the flow of the laboratory wastewater was practically stopped. We suspected that these problems may be due to the nature of the unique microbiome. Samples collected during maintenance work were processed for bacterial and archaeal 16S rRNA gene profiling. Preliminary results indicate high biodiversity and significant differences in the proportions of the occurrence of individual phyla of Bacteria and Archaea at the input and output of the sedimentation tank. We plan to continue the isolation and identification of individual species of microorganisms, including archaeal ones. The results of the project will be the first full characterization of microbiological communities selected under the conditions of long-term collection of sewage in biological and chemical laboratories.

Due to our common interest in exploring microbial diversity in the environment, I will have an opportunity to expand skills, both experimental and theoretical (i.e., *in silico* analyses) concerning handling of non-model archaeal and bacterial strains. This will allow us to extend the research that I briefly described above, performed by our team at IBB PAS. Learning the operating protocols and methods of successful cultivation and enrichment of slow-growing, anaerobic microorganisms would be a new competence of remarkable value. The chance to work with Prof. Gribaldo's group would broaden my knowledge in the biology of anaerobes and allow me to learn the most useful techniques used in research on cultivable and non-cultivable microorganisms, which I will then utilize in my work at IBB PAS.