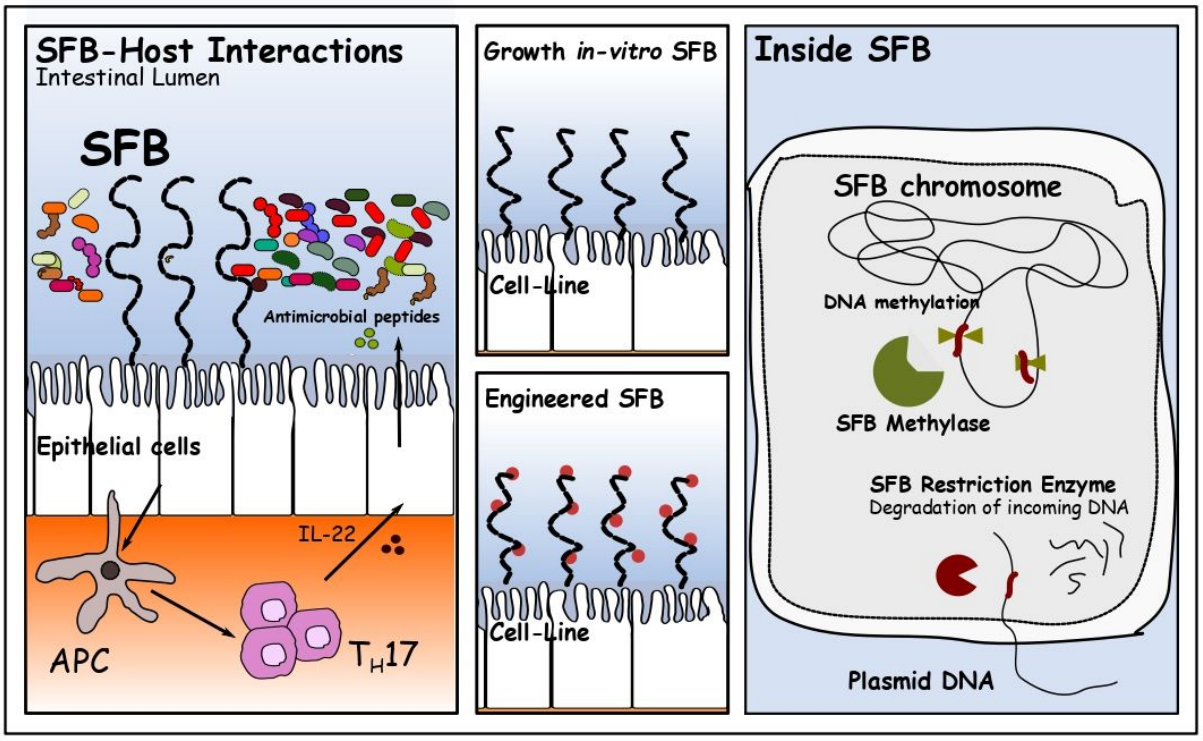
Nom Prenom: Ramachandran, Gayetri
Prix Jeune Chercheur 2019

Characterisation of methyltransferases from gut-inhabiting and immunostimulatory bacteria called Segmented Filamentous Bacteria.

Ramachandran,G[§#], Cerf-Bensussan, N[§] and Schnupf, P[§#].

§ Laboratory of Intestinal Immunity, Institut IMAGINE-INSERM 1163 Université Paris Descartes-Sorbonne Paris Cité 24, boulevard du Montparnasse, 75015 Paris
# Laboratory of Host-Microbiota Interaction, Institut Necker Enfants Malades (INEM)-INSERM 1151 Université Paris Descartes-Sorbonne Paris Cité, 156, rue du Vaugirard, 75015 Paris

---



"What makes us human is, in my opinion, the combination of our own DNA, plus the DNA of our gut microbes."

by Sarkis Mazmanian, renowned microbiologist from Caltech (10/04/2018 BBC Radio 4)

It is quite well known now that a billion bugs reside in our gut. Where they play a vital role in maintaining an immunological, neurological and metabolic equilibrium. Given their functionality, we believe that native or modified microbiota-based therapies can be used to treat diseases.

One such- potentially relevant bacterium is Segmented Filamentous Bacteria (SFB), which has been shown to have an adhesion-specific immunostimulatory response in murine-models. Unlike other commensals, SFB has an intimate interaction with the intestinal epithelium and is able to induce virtually all immune system components. Thus, we aim to develop a methodology that permits manipulation of SFB for various purposes including vaccine production by tapping into its immunostimulatory properties.

Genetic modification of the microbiota is not easy, as SFB and others are difficult to grow *in-vitro* and the highly restrictive nature of the prokaryotic restriction-methylation (RM) systems inhibit the establishment of foreign DNA.

We overcame the first hindrance by devising our *in-house* patented cell-line based culturing system for SFB, which permits easier growth of the bacterium. This groundbreaking method readily helped us to better handle SFB outside the mouse-model.

To solve the RM inhibition problem, we would like to study the poorly characterized SFB DNA methyltransferase system. Methyltransferases are enzymes that catalyze the transfer of methyl group from the methyl donor S-adenosyl-l-methionine (SAM) substrate to specific DNA sequences to identify self DNA. Therefore, we would like to to study the important features of these SFB methyltransferases.

Our aim is to express SFB methyltransferases in *E.coli.* Then, assess their activity using a bioluminescent assay, the **pMTase-Glo™ Methyltransferase Assay** supplied by **Promega**. The robust and replicable utility of pMTase-Glo™ Methyltransferase Assay would help us examine the essentiality of amino acids involved in SAM substrate binding and DNA recognition domains. Finally, it would aid us in better understanding the systems that inhibit the establishment of foreign DNA in SFB.

In the end, we think that this work would be the starting point of innovative treatment and preventive strategies using immunological potent microbes like SFB to fight against life-threatening infectious diseases.