Grant Proposal Application

Ministry of Health - Chief Scientist Office

1. Title Page

Research Title: **Characterizing human milk oligosaccharide utilization by infant gut *Bacteroides* species**

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Institute: **The Hebrew University**

Research Authority: The Hebrew University

Date of Application: 02/01/2021

Co-Investigators:

2. Name: _____ Institute: _____
3. Name: _____ Institute: _____
4. Name: _____ Institute: _____
5. Name: _____ Institute: _____
6. Name: _____ Institute: _____

Is the project associated with a commercial company? YES ☐ NO ☒

Is the project associated external parties? YES ☐ NO ☒

If yes, please elaborate:

Signature of the PI: _____

Signature of the Research Authority: _____

2. Abstracts

Characterizing human milk oligosaccharide utilization by infant gut *Bacteroides* species

The infant gut microbiome is very dynamic, and stabilizes to an adult-like state around the age of three. The initial colonization of the infant gut is a complex process, mainly influenced by delivery mode and infant feeding (formula vs. breast milk). Human Milk Oligosaccharides (HMOs) are a family of glycans found in breast milk, and despite being the third-largest component in human milk, infants are unable to digest these sugars, and they serve as food for the infant gut bacteria. The importance of HMOs in shaping the unique infant microbiome has been a subject of avid research in the past decade, and most of the research focused on *Bifidobacterium* species. HMOs promote the growth of *Bifidobacteria*, which are found at higher abundances in breastfed compared to non-breastfed infants.

The strong association between HMO consumption and the abundance of *Bifidobacterium* species is well established. However, the infant gut microbiome is diverse and consists of many different species other than *Bifidobacterium*. Moreover, some breast-fed infants do not have *Bifidobacterium* species in their gut communities, suggesting there are other bacteria that are capable of utilizing the carbon sources in HMOs. When examining the infant microbiome composition, *Bacteroides* species are the obvious first candidates for such utilization, as they are known to have an excellent ability to utilize a vast range of carbohydrates, and they are commonly found in the infant gut.

The overarching goal of this proposal is to expand the HMO-microbiome studies beyond *Bifidobacterium* species and shed light on the mechanistic differences across *Bacteroides* strains in terms of HMO utilization. We will identify *Bacteroides* strains capable of utilizing breast milk sugars, and their relevant enzymatic pathways. We will address this big question by investigating these three specific objectives: (1) Establish a *Bacteroides* strain collection from infant stool samples; (2) Identify HMO-utilizing bacteria by high-throughput measurement of growth dynamics of isolated strains on HMOs; and (3) Identify the HMO-utilization genes based on transcriptional induction across many strains.

Our proposed research will highlight the importance of expanding the HMO-microbiome studies beyond the *Bifidobacterium* species, sheds light on the differences across *Bacteroides* strains in terms of HMO utilization, and digs deeper into the mechanisms that enable HMO utilization by *Bacteroides*. Already in our preliminary data, we show the importance of HMOs in the establishment of *Bacteroides* communities in the infant gut, and we will continue to explore this path even further in the proposed infant isolate collection. A better understanding of the impact of HMOs on the infant microbiome will assist us in developing better infant formula that best-mimics breastmilk, and provides the infant microbiome with all its needed sugars.