



DEFENS
DEPARTMENT OF FOOD, ENVIRONMENTAL
AND NUTRITIONAL SCIENCES

Ready-to-Eat Foods as a Vehicle of Microorganisms in the Microbial Deprivation Hypothesis

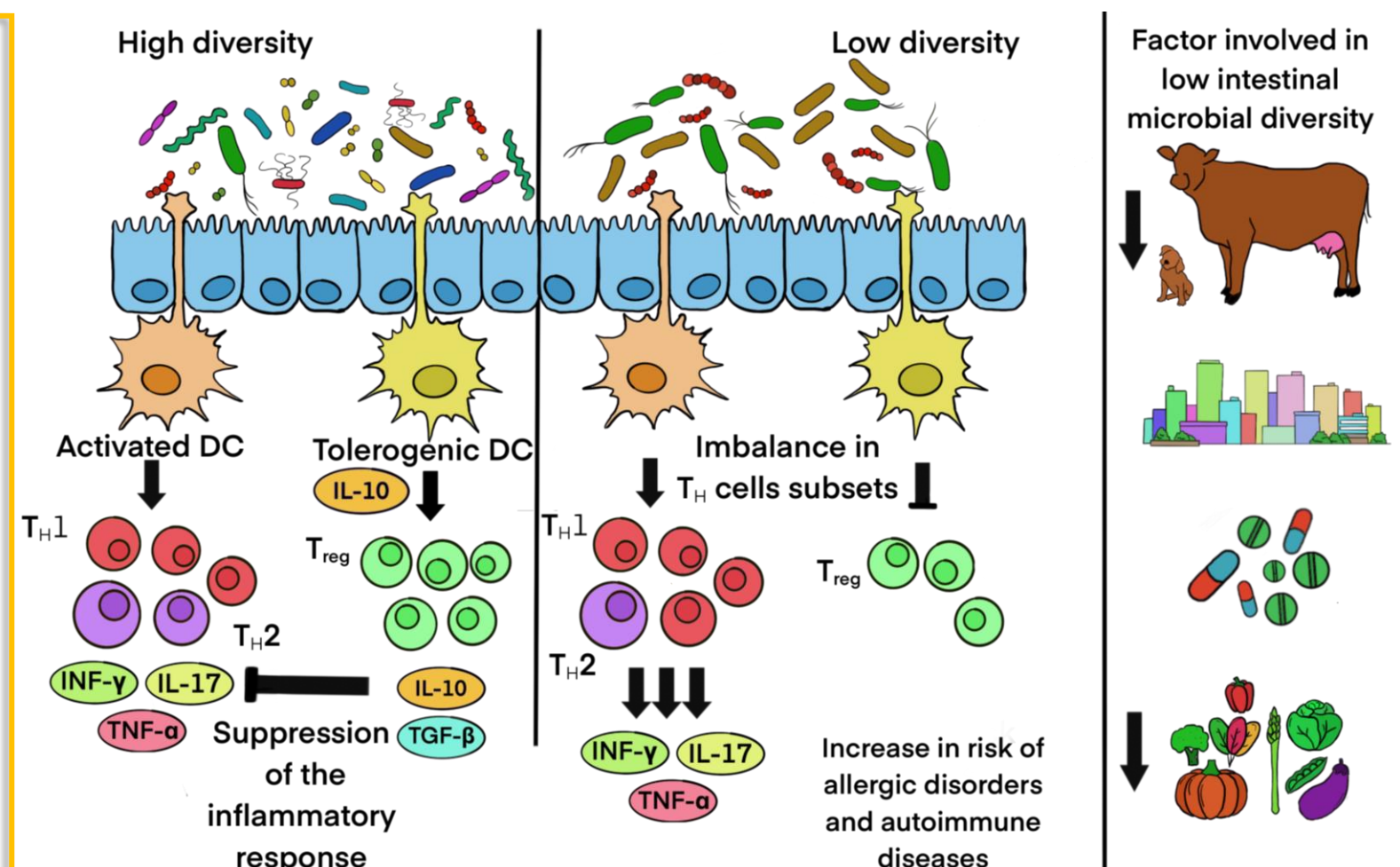


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AGRICOLA
MODERNA

1. State of the art

In 1989, Strachan observed a higher occurrence of allergic rhinitis in first-born children, when compared to their younger siblings. Such observations became the basis for further investigations, which brought about the development of the so-called hygiene hypothesis (Strachan, D. P., 1989). However, evidence from later studies suggested that the increased occurrence of allergic rhinitis in first-born children was not to be associated with and had no dependency from hygienic practices. A new hypothesis was raised, that the immune system impairment might actually depend on the entirety of modern living conditions and life-style (diet, sanitation, antibiotic use, etc.), leading to a reduced contact with non-pathogenic microorganisms, that play a pivotal role in training and properly stimulating our immune system (Scudellari, M., 2017). The food we eat is, indeed, a vector for microorganisms, and can be a source of gut commensals - especially when food is consumed raw or fermented. But cleaning processes and cleaning agents wash away and kill most of the non-pathogenic microorganisms found on food (Tatsika et al., 2019). And this is especially true for ready-to-eat (RTE) foods which, by definition, are a category of products requiring no cooking nor any other action before human consumption (European Commission Regulation, 2005).



2. Project aims

The aim of the project is to provide a thorough insight on the role of industrially processed and cleaned raw foods, and their influence on the intestinal microbial diversity. The study will focus on the microbial ecology of rocket salads (*Eruca sativa*, *Eruca vesicaria*, *Diplotaxis renuifolia*), a product commonly consumed raw and distributed in the form of RTE foods. A reduced bacterial diversity in highly processed rocket salads (i.e. conventional and washed RTE rocket salad) is expected. The entire microbial community associated with the investigated products will be considered, and differences between RTE rocket salads sourced from retailers and rocket salads bought directly from production sites will be identified and detailed. Finally, differences between conventional and organic RTE rocket salads will also be examined, in order to understand the impact of agricultural practices – such as the use of pesticides or herbicides – on microbial populations.

3. Experimental procedure

Microbial Deprivation Hypothesis

- 15 RTE rocket samples
- 3 time-points

A1: Microbial characterization of commercially available rocket salads, and bacterial strain library set-up.

A1.1: Ecological analysis of rocket salad

A1.2: Isolation of Lactic Acid Bacteria (LAB) and library set-up

A2: Antibiotic resistance profiling of metagenomic DNA isolated from rocket salad

A2.1: *E. coli* library set-up

A2.2: minimum inhibitory concentration test on *E. coli* clones

A3: Characterization of selected strains and microbial fortification of RTE rocket salads

A3.1: Functional characterization of LAB isolates

A3.2: RTE rocket salad reinforcement with selected strains

Outcomes

Proving the impact of industrial processes on depletion of the microbial quote associated to food

Development of a microbial reinforced RTE rocket salad with functional properties

Provide an antibiotic resistance profile of differently processed rocket salad as precise as can be

Reinforcing and dissemination of concept of food-associated microbial contribution to a healthy dietary regimen

4. Objective and milestones

Activity\Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
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A3.2) RTE rocket salad reinforcement with selected strains																								
A4.1)Thesis and papar preparation																								

5. References

- Strachan, D. P. (1989) Hay fever, hygiene, and household size. *Br. Med. J.* **299**, 1259–1260 (1989)
Scudellari, M. (2017) Cleaning up the hygiene hypothesis. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 1433–1436 (2017).
European Commission. Regulation (2005) EC No 2073/2005. *Off. J. Eur. Union*
Tatsika, S., Karamanoli, K., Karayanni, H. & Genitsaris, S. (2019) Metagenomic Characterization of Bacterial Communities on Ready-to-Eat Vegetables and effects of Household Washing on their Diversity and Composition. *Pathogens* **8**



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