

## Master Thesis

**Subject: Active new drugs as an alternative therapy to antibiotics. Screening of probiotic bacteria from Portuguese table-olives. Ability for gut cell adhesion and antimicrobial activity.**

**Student:**

**Host Institution:** ITQB; Food Microbial Technology Laboratory

**Supervisor in ITQB:** Dulce Brito (Senior Researcher)/ Cidália Peres (Team Leader)

### The problem and progress to date

Olives may be a source of an immobilization potentially probiotic strains while containing fibres and many beneficial nutrients for humans as anti-oxidizers constituting a basis of functional food development. It is a relevant aspect for the valorization of the Mediterranean diet, and it opens the way for the classification of fermented table-olives as functional foods.

Research suggests that a vegetable food matrix, such as olives, might be an efficient vehicle for administering probiotics in a tasty, nonrefrigerated functional product. In probiotic applications, selected LAB strains are used as food supplements that may favourably influence the intestinal flora of human and animal hosts, e.g. by competitive exclusion of gastrointestinal pathogens. The bacteriocins from olive lactics may constitute an alternative or a complement to common antibiotics, since they can kill bacteria that carry multiple resistances to antibiotics (Dulce Brito, Cristina Serrano, Alexandra Pereira, Amélia Delgado, Mónica Oleastro, Lurdes Monteiro and Cidália Peres. 2007. Evaluation of the inhibitory activity of *Lactobacillus* sp. from table-olives against *Helicobacter pylori* Abstrat no: PO91. *Helicobacter*. 12:438). So, probiotic bacteria are a safe source of microbial metabolites, which may constitute a future alternative to antibiotic therapy, as acquired resistances are spreading among bacteria from a growing number of genera and habitats. Intake of probiotics stimulates the growth of beneficial microorganisms.

A desired characteristic for a probiotic is the capacity of tack to the intestinal tract, allowing to its permanence and performance in the digestive ecosystem. This characteristic depends in part on the properties physiciest-chemistries on the bacterial cellular wall. So, to exert their beneficial effect after human consumption, probiotic bacteria need to survive first the manufacturing process of the carrier food and then the gastrointestinal ecosystem. Those aspects are already tested in 'in vitro' assays using some of the potential probiotic strains. All of them showed to withstand 'in vitro' tests for the simulation of the digestion. It was confirmed the maintenance of a strain's ability to tolerate acidic conditions, survive and grow in the presence of bile, and metabolize selective substrates.

To complete information in our battery of strains it is necessary to study some other characteristics fundamental for to screen probiotic bacteria. One of the most important selection criteria for probiotics is the ability to adhere to the intestinal mucosa, and is considered to be a prerequisite for colonization (Paola Lavermicocca, Francesca Valerio, Stella Lisa Lonigro, Maria De Angelis, Lorenzo Morelli, Maria Luisa Callegari, Carlo G. Rizzello, and Angelo Visconti. 2005. Study of Adhesion and Survival of *Lactobacilli* and *Bifidobacteria* on Table Olives with the Aim of Formulating a New Probiotic Food. *Appl Environ Microbiol*. 71(8): 4233–4240).

While the capacity to colonize the human gastrointestinal tract is not a pre-requisite for the active function of a probiotic strain in the digestive tract, it is a desirable characteristic. If the probiotic strain can adhere to the gastrointestinal tract epithelium, it can colonise, i.e. establish and grow within the tract, and continually produce metabolites that may mediate the beneficial effect.

**The objective of this study is to evaluate the ability of strains for the intestinal cell adhesion and to determine the effect of the microbial cells stability on adhesion and antimicrobial property.**

### Intestinal epithelial adhesion properties and capability of adhesion

- a) Adhesion properties will be monitored by studding the adhesion to intestinal cells (eg, Caco-2).
- b) Stability of freeze-dried powder of selected strain: effect on cell viability by analyzing the colony forming units per gram (CFU/g) of dried material, capability for posterior adhesion, and maintenance of production of antimicrobial compounds.
- c) Ability to inhibit known gut pathogens, spoilage organisms, or both. Survival studies of *Listeria monocytogenes*, *Salmonella* sp. and *Escherichia coli* O157:H7 in the presence of probiotic olive strains.

**(to complete for the candidate, after bibliography revising)**