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Methodological advancements in foodborne pathogen determination: from presence to behavior

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For biological hazards in foods, that is living organisms that interact with the environment and ultimately with the host, the choice of diagnostic tool shapes the type of information obtained and provides diverse opportunities for further management steps. In this context, food microbiology is experiencing a progressive shift from methods that determine the presence/absence or concentration of pathogenic microorganisms in food toward methods that provide information regarding the 'physiological state' of microorganisms. This shift is continuously accelerated as a consequence of technological advancements in molecular biology methods that nowadays reach unprecedented depth of biological information in a single experiment or from a single sample. The focus now is to understand foodborne pathogen behavior throughout the food chain and its impact in disease causing potential.

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Introduction

To assure high level of food safety, there is a continuous need for improved diagnostic tools to be applied both in routine analysis/monitoring but also in epidemiology studies and (quantitative) risk assessment. Despite an observed shift toward diagnostic tools that aim at describing the behavior of pathogens in foods, the regulatory framework still heavily relies on methods of detection and/or quantification that are based on culture assays. Numerous alternative approaches, based on molecular biology assays, essentially PCR and qPCR, have been proposed for most important foodborne pathogens. For *Listeria monocytogenes*, a highly recognized foodborne pathogen, the challenges and needs in terms of detection and enumeration methods in foods have been recently reviewed [1,2].

Moving a step beyond in the effort to define and quantify biological risks, two important questions remain unanswered when culture or molecular assays are employed simply to detect/enumerate pathogens: what is the physiological state of the pathogen at the moment of ingestion and could it be related with its potential to cause disease? Basic *in vitro* studies have addressed aspects that relate with the effect of environmental factors on the physiology of foodborne pathogens [3]. What has been investigated is the impact of the environment on phenotypic characteristics such as growth behavior (lag phase, exponential growth rate), resistance to inactivation treatments (heat or acid inactivation for example), virulence. Two important outcomes have been delineated from previous studies and both of them have implications for food safety. Firstly, microorganisms are able to adapt to adverse environmental conditions and such adaptation could influence their ability to cause disease (their virulence) [4]. Secondly, not all strains within one pathogenic species behave the same way. On the contrary, important strain variability has been observed; it concerns adaptation, virulence and habitat [5]. Molecular mechanisms underlying this observed variability need to be further investigated and considered in microbial risk assessment. In hazard identification, rather than rely on information regarding the microorganism it is reasonable to consider information regarding genes or transcripts, markers of virulence. If mechanistic models become available that explain the observed variability then this information will become integral part of hazard characterization.

From basic *in vitro* studies, it has been shown that regulatory networks that are responsible for microbial stress adaptation partially overlap those associated with virulence [6,7]. Knowledge regarding the cause-and-effect relationship between physiological state and virulence needs to be consolidated. Cutting edge high throughput approaches provide data that move food microbiology science in this direction.

In this review we highlight advancements that aim at describing the molecular mechanisms underlying adaptation to food and virulence. The information gathered could be integrated in mechanistic models in order to predict the behavior of foodborne pathogens. We decided to focus on *L. monocytogenes*, since it has become one of the first model organisms to study virulence and stress response gene expression in food [8].

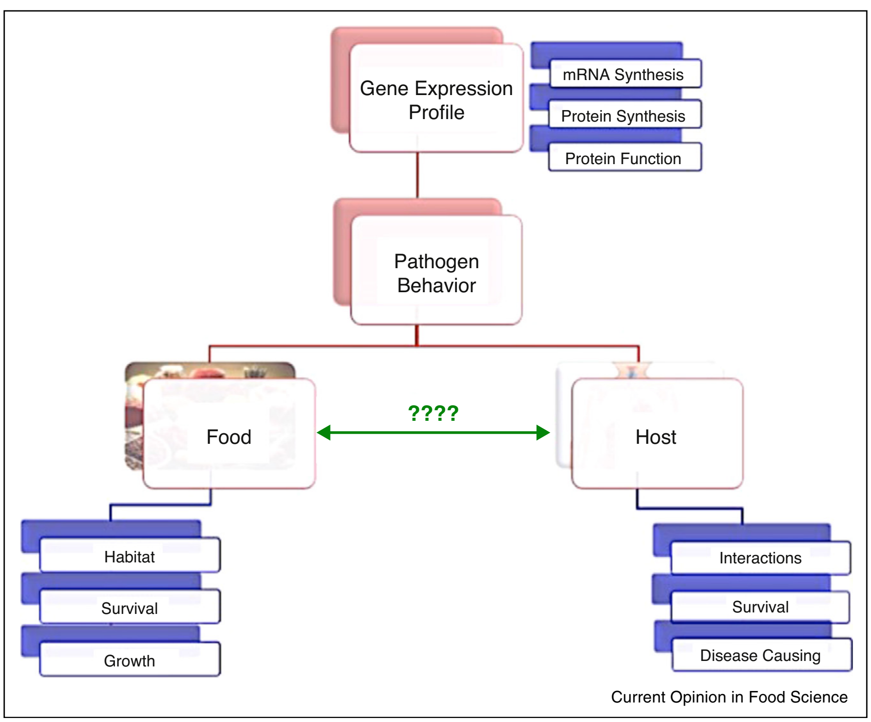
Foodborne pathogens in food and in human infections: a different perspective

Scientific articles studying foodborne pathogens behavior could be mainly categorized into two big areas: food science and medical. The main differences between them regard the overall aim (avoid food contamination/understand virulence in the human host) but also the source of isolation of the strains (food/human tissue) and the serotype considered. Food microbiologists mainly focus on the effect of food on microbial physiology and on the development of different, innovative and reliable methods for the detection of pathogens in foods. On the other hand, medical microbiologists appear more concentrated on the molecular mechanisms of virulence, trying to clarify how disease develops and how pathogens interact with the human host. We believe an integrated view on the subject, from the contamination of the food matrix to the development of a disease, should be achieved to get a complete picture of the foodborne pathogen's behavior (Figure 1). It is essential, although methodologically demanding, to be able to understand and quantify the impact of the food matrix/environment on the characteristics (survival, stress adaptation, virulence) of the pathogen on the subsequent step of host colonization, an aspect still unclear (Figure 1).

Foodborne pathogen behavior in food: different approaches

The behavior of foodborne pathogens in a food sample can be studied by two different approaches, commonly referred to as *in vitro* or *in situ*. *In vitro* studies aim at simulating the food matrix in the laboratory, using synthetic media, under conditions that may influence pathogen behavior. On the other hand, *in situ* studies describe the expression of microbial activities directly in a real food matrix and under common conditions of storage and consumption (Table 1). *In situ* studies are a relatively new field of research for food microbiologists and provide information regarding adaptive responses and virulence potential of foodborne pathogens [9]. Notwithstanding the evidences on the different behavior a pathogen has *in vitro* compared to a real *in situ* situation, the majority of published works have been conducted *in vitro*. This is mainly due to the technical difficulties encountered *in situ* in some laboratory steps, primarily the extraction of good-quality RNA molecules or proteins. In fact, within a food matrix, several inhibitors can be encountered, which may be avoided when the analysis is performed *in vitro*. However, mimicking as closely as possible the food environment is crucial in studying microorganism behavior.

Figure 1



Schematic representation of events involved in the study of pathogen behavior in food science. Pathogen gene expression profile can be studied at three different levels: (i) mRNA synthesis; (ii) protein synthesis; (iii) protein function. The expression profile has a direct impact on pathogen behavior both at the level of food matrix and on the interactions with the human host. In a food matrix, different levels of investigations may be achieved, regarding (i) the type of habitat contaminated; (ii) its survival; and (iii) its growth. On the other hand, inside the human host, the pathogen (i) interacts with different tissues and organs; (ii) survives inside the body; (iii) triggers the host immune system to cause the disease. The interactions between pathogen behavior in the food and in the host remain to be investigated.