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Dynamic Stresses of Lactic Acid Bacteria Associated to Fermentation Processes

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1. Introduction

Despite their negligible mass the microbial agents, starters and non starters, play a profound role in the characterization of the fermented foods in terms of chemical and sensorial properties. In fact, fermented foods may be defined as foods processed through the activity of microorganisms. Fermentation processes take a special place in the evolution of human cuisine, by altering the taste experience of food products, as well as extending the storage period. In particular, foods fermented with lactic acid bacteria (LAB) have constituted an important part of human diet and of fermentation processes (involving various foods, including milk, meat, vegetables and fruits) [1] since ancient times. They have played an essential role in the preservation of agricultural resources and in the improvement of nutritional and organoleptic properties of human foods and animal feed. Moreover, these organisms nowadays are increasingly used as health promoting probiotics, enzyme and metabolite factories and vaccine delivery vehicles [2].

It is interesting to outline how the changes of food characteristics during the fermentation process can be described as dynamic fluctuations of the food environment itself and, at the same time, stress source for the microorganisms involved [3, 4], such as LAB. In fact, whenever autochthonous bacteria are adapted and competitive in their respective environment, the environment can be described as stressful for LAB [5, 4]. The fermentation parameters, including temperature, water activity (Aw), oxygen, pH, as well as the concentration of starter cultures, affect the regulatory mechanism and the response mechanisms of LAB, as well as their effects on the final products properties [4].

When LAB are added to food formulations, several factors that may influence the ability of those microorganisms to survive, growth and become active in the new matrix have to be considered [6]. These factors include: 1) the physiological state of the LAB used as starters (whether the cells are from the logarithmic or the stationary growth phase); 2) the physical

conditions of product ripening and storage (eg. temperature); 3) the chemical composition of the matrix (eg. acidity, available carbohydrates content, nitrogen source, mineral content, water activity and oxygen concentration); 4) possible interactions of the starter cultures with probiotics and other microorganisms naturally occurring or added to the system [6].

In figure 1 the main factors affecting the viability and the responses of LAB from production to storage are described [7].
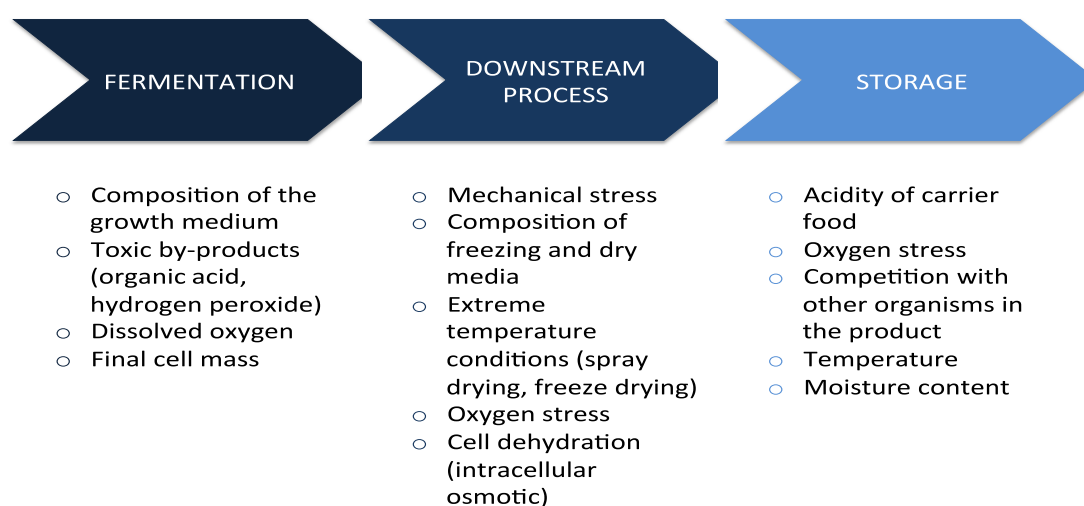
FERMENTATION

- Composition of the growth medium
- Toxic by-products (organic acid, hydrogen peroxide)
- Dissolved oxygen
- Final cell mass

DOWNSTREAM PROCESS

- Mechanical stress
- Composition of freezing and dry media
- Extreme temperature conditions (spray drying, freeze drying)
- Oxygen stress
- Cell dehydration (intracellular osmotic)

STORAGE

- Acidity of carrier food
- Oxygen stress
- Competition with other organisms in the product
- Temperature
- Moisture content

Figure 1. Factors affecting the viability and the responses of LAB to the various fermented foods production steps.

To better elucidate what happens to LAB during fermentation processes, we decided to use a model (defined "virtual food") that mimics various steps occurring during processing and that can affect LAB performances or viability.

2. Lactic acid bacteria and stress: Basic concepts

"Stress results from interactions between subjects and their environment that are perceived as straining or exceeding their adaptive capacities and threatening their well-being. The element of perception indicates that human stress responses reflect differences in personality, as well as differences in physical strength or general health" [8].

Stress has driven evolutionary changes (the development and natural selection of species over time). Thus, the species that adapted best to the causes of stress (stressors) have survived and evolved into the plant and animal kingdoms we now observe. The same evolutionary process regarded microorganisms. In fact, bacteria, irrespective of natural habitat, are exposed to constant fluctuations in their growth conditions. Consequently they

have developed sophisticated responses, modulated by the re-modelling of protein complexes and by phosphorylation dependent signal transduction systems, to adapt and to survive to a variety of insults. To ensure survival to environmental adversities, bacteria may adapt to changes in their immediate vicinity by responding to the imposed stress. These responses are different and vast and depend on the microorganism nature and on the environmental stress and are accomplished by changes in the patterns of gene expression for those genes whose products are required to combat the deleterious [3]. In particular, cellular metabolic pathways are closely related to stress responses and the flux of particular metabolites to understand the hypothetically shifts and implications in the food systems has been studied in LAB [9-13, 4, 14, 15].

LAB are a functionally related group of organisms known primarily for their bioprocessing roles in food and beverages [16]. LAB play a crucial role in the development of the organoleptic and hygienic quality of fermented products. These microorganisms are used as starter cultures in many fermented products (i.e. beer, milk, dough, sausages and wine). Therefore, the reliability of starter cultures in terms of quality and functional properties (important for the development of aroma and texture), but also in terms of growth performance and robustness, has become essential for successful fermentations [17]. There have been some reports describing the physiological stress responses in LAB, particularly *Lactobacillus* species, which have a broad biodiversity [17-21, 13, 22, 4, 14, 15].

LAB evolved specific mechanisms to respond and to survive to environmental stresses and changes (stress-sensing system and defences). In fact, microorganisms could have specific regulators tailored to each of their regulated genes and adapt their expression according to environment. Stress defences are good examples of such integrated regulation systems. Bacterial stress responses rely on the coordinated expression of genes that alter different cellular processes (cell division, DNA metabolism, housekeeping, membrane composition, transport, etc.) and act in concert to improve the bacterial stress tolerance. The integration of these stress responses is accomplished by networks of regulators that allow the cells to react to various and complex environmental shifts. LAB respond to stress in a very specific way dependent on the species, on the strains and on the type of stress. The best-studied stresses are acid, heat, oxidative and cold stresses, although for the latter most of the studies focused on a specific family of proteins instead of analyzing the whole response [4].

Despite the extensive use of LAB, there is a paucity of information concerning the stress-induced mechanisms studied *in vivo* for improving the survival of these organisms during real food processing. A better knowledge of the adaptive responses of LAB is important because the fermentation processes often expose these microorganisms to adverse environmental conditions. LAB should resist to adverse conditions encountered in industrial processes, for example during starter handling and storage (freeze drying, freezing or spray-drying) and during the fermentation environment dynamic changes. These phenomena reinforce the need for robust LAB since they may have to survive and grow in different unfavorable conditions expressing specific functions (for example during stationary phase or storage) [17].

3. Principal responses to the most common stresses

Heat shock response: The effect of heat shock and the induction of a stress response in *Lactobacillus* spp. have been studied for *Lactobacillus delbrueckii* subsp. *bulgaricus* [23] and *Lactobacillus paracasei* [24, 25], *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus helveticus* [26], *Lactobacillus collinoides* [27] , *Lactobacillus sakei* [28], *Lactobacillus johnsonii* [29], *Lactobacillus rhamnosus* [30], *Lactobacillus plantarum* [31-33] and *Lactobacillus salivarius* [34]. The heat resistance of LAB is a complex process involving proteins with different roles in cell physiology, including chaperone activity, ribosome stability, stringent response mediation, temperature sensing and control of ribosomal functions [31]. The time taken to initiate the stress response is different for different treatments and different strains. The major problem encountered by cells at high temperature is the denaturation of proteins and their subsequent aggregation. In addition Earnshaw et al. [35], , Texeira et al. [36] and Hansen et al. [37] described also as response to heat stress the destabilization of macromolecules as ribosomes and RNA as well as alterations of membrane fluidity.

Heat stress response is characterized by the transient induction of general and specific proteins and by physiological changes. In every strain tested the involvement of Heat Shock Proteins (HSPs such as DnaK, GroEL and GroES during the heat stress was clear) [23-38]. The role of these stress proteins is complex; in fact, the bind substrate proteins in a transient non-covalent manner prevent premature folding and promote the attainment to the correct state *in vivo*. The resistance to heat stress is higher when the cells were previously exposed and adapted to this type of stress in the stationary phase, otherwise, when pre-adapted in exponential phase, the cells are more sensitive. In particular, the storage stability of the culture that was heat shocked after stationary phase was superior to that of culture heat shocked after log phase [34, 23, 30].

Cold shock response: It is very important to improve knowledge about LAB behavior in cold environment. In fact, during industrial processes, like in cheese ripening and refrigerated storage of fermented products, these microorganisms are subjected to different temperatures far below the optimal growth temperature. When LAB living cells are exposed to these cold environments, important physiological changes occur, such as decrease in membrane fluidity and stabilization of secondary structures of RNA and DNA, resulting in a reduced efficiency of translation, transcription and DNA replication. The response of microorganisms to these effects is termed cold-shock response during which a number of Cold Induced Proteins (CIPs) are synthesized. The roles of these proteins are at the levels of membrane fluidity, DNA supercoiling and transcription and translation. Few papers have described cold shock proteins and mechanisms in LAB, in particular they have focused on *Lactococcus lactis* and *L. plantarum* [39-42]. Kim et al. [39, 40] tested different LAB to evaluate cold shock effects on cryotolerance. Improved understanding of cold-shock-induced cryotolerance may contribute to the development of environmental conditions that allow improved viability/activity of frozen or freeze-dried commercial LAB starter cultures. The results showed that, as with heat stress, there is also an improvement of the viability of the tested strains as concerning the cryotolerance after a cold shock. The process of freezing

appeared to have different effects on different LAB as well as different effects on strains within the same genus. Moreover, the freezing response of the strains depends on the time of the cold shock process and the induction of cryotolerance appears to be dependent on the growth phase in which the cold shock took place [43-47].

Another interesting study regarding LAB response to sub-lethal cold stress was developed by Montanari et al.[14]. These Authors separated and quantified the cell cyclopropane fatty acids lactobacillic (C19cyc11) and dehydrosterculic (C19cyc9) to study the adaptive response to sub-lethal acid and cold stresses in *L. helveticus* and *Lactobacillus sanfranciscensis*. These microorganisms showed different fatty acids composition and environmental adaptation to short term cold and acidic stresses. In *L. helveticus* C19cyc11 dramatically increased after 2 h at 10°C and with the pH decrease, particularly in micro-aerobic conditions, in the presence of tween 80, and in anaerobic conditions. The increase of lactobacillic acid in *L. helveticus* is necessary to maintain the cell membrane in a suitable state of fluidity. Moreover, cyclopropane fatty acids confer resistance to ozonolysis, singlet oxygen and mild oxidative treatments [48, 49], suggesting a cross protection and response of LAB cell membrane to physicochemical stresses. A combined analysis of the genome-wide transcriptome and metabolism was performed with a dairy *Lactococcus lactis* subsp. *lactis* under dynamic conditions similar to the conditions encountered during the cheese-making process. Specific responses to acid and cold stresses were identified, but also the induction of unexpected pathways was determined. In particular, the induction of purine biosynthesis and prophage [50].

Oxidative stress response: LAB are facultative anaerobic microorganisms that have in common the reduction of part of pyruvate produced to lactate production in order to regenerate NAD+ from NADH formed during glycolysis. They do not require oxygen for growth and, in fact, a negative effect of oxygen on the development of these bacteria has often been observed. It was generally believed that these bacteria could under no condition use oxygen as the terminal electron acceptor [17]. However, many LAB have NADH oxidase and some can even express a functionally active respiratory chain in the presence of heme [51-57]. Respiration-competent LAB differ from the features of *Escherichia coli* and *Bacillus subtilis*, since they carry limited equipment for respiration. All respiring LAB carry genes encoding electron donor (NADH dehydrogenase) and a single electron acceptor (cytochrome bd oxidase) [58]. Addition of heme to the system activates respiration chain NADH oxidase activity, but none of the tested LAB synthesize heme [01].

When for some reasons the generation of free radicals is higher than the rate of their detoxification the cells are exposed to a constraint called "oxidative stress" [59]. For the food-associated LAB a still fragmented picture of the resistance mechanisms present emerges. Representatives of the different mechanisms have been described in different LAB [60-64]. Apart from the toxic effects of oxygen, aeration can induce important changes in the sugar metabolism of LAB. In fact, the presence of oxygen is a factor that greatly affects the outcome of a fermentation process. In general, LAB tolerate oxygen but grow better under nearly anaerobic conditions. However, in the presence of heme and oxygen LAB start respiration metabolism, by which the cell metabolism is reprogrammed so that pH, oxygen status, growth capacity and survival are markedly altered [56]. In the presence of oxygen

and during the fermentation metabolism, H_2O_2 is formed. Numerous species of LAB contain peroxidase and/or catalase to prevent and eliminate these deleterious effects [17]. Concerning the prevention of reactive oxygen species (ROS) formation, the scope of the reactions is the eliminations of free oxygen. In a study on *L. helveticus* the fatty acids composition in the cell membrane changed in response to oxidative stress. In fact, the activity of oxygen consuming desaturase system increased to reduce the free radical damage to the cell [19]. Generally, the response to oxidative stress of LAB is similar, but also depends on the species, on the strains and, with regard to catalase action, on the bacterial density [4]. In *L. lactis* several genes have been identified and the respective encoded proteins have been shown to contribute to oxidative stress resistance. Moreover, the induction of these genes is growth phase-dependent (exponential or stationary) and their products confer multi-stress resistance [52]. General stress resistance mechanisms may also confer resistance to oxidative stress. In fact, in a model system several acid resistant mutants of *L. lactis* that appeared also more resistant to oxidative stress were isolated [64].

Acid stress response: Understanding the acid resistance mechanism used by LAB to survive to by-products of their own metabolism (i.e. homofermentative *L. lactis* converts 90% of metabolized sugar to lactic acid) and the response available in low-pH foods is of great importance. In LAB one of the most effective mechanisms for resistance in acid stress environment is the glutamate decarboxylase (GAD). In fact, few years ago, it was proposed that amino acid decarboxylase functions to control the pH of the bacterial environment by consuming hydrogen ions as part of carboxylation reaction [65]. LAB are also capable of inducing an Acid Tolerance Response (ATR) in response to mild acid treatments. The system induced includes pH homeostatis, protection and repair mechanisms. Genes and proteins, involved in pH homeostasis and cell protection or repair, play a role in acid adaptation, but this role can also extend to more general acid tolerance mechanisms. A more specific study was developed on the effects of lactic acid stress on *L. plantarum* by transcription profiling [66]. The difference, in terms of stress response, into the dissociated or undissociated forms of lactic acid has been highlighted. The toxicity of organic acids depends on their degree of dissociation and thus on the pH. For LAB end product inhibition by lactic acid could result in a disturbance of the regeneration of cofactor NAD+, especially under anaerobic conditions, in which the cell does not have the possibility of NAD+ regeneration by NADH oxidase. The response at membrane fatty acids level to acid stress was studied in *L. helveticus* and *L. sanfranciscensis* [14]. The relevant proportion of dodecanoic acid in the latter species under acid stress suggests that carbon chain shortening is the principal strategy of *L. sanfranciscensis* to modulate fluidity or chemico-physical properties of the membranes in the presence of acid stress. Moreover, a specific shift in leucine catabolic pathway at pH 3.6 was identified in *L. sanfranciscensis* [15]. In fact, the acid stress induced a metabolic shift toward overproduction of 3-methylbutanoic and 2-methylbutanoic acids, accompanied by sugar reduced consumption and primary carbohydrate metabolite production. The metabolites coming from branched chain amino acids (BCAAs) catabolism increased up to seven times under acid stress. While the overproduction of 3-methylbutanoic acid under acid stress can be attributed to the need to

maintain redox balance, the rationale for the production of 2-methylbutanoic acid from leucine can be found in a newly proposed biosynthetic pathway leading to 2-methylbutanoic acid and 3 mol of ATP per mol of leucine. Leucine catabolism to 3-methylbutanoic and 2-methylbutanoic acids suggests that the switch from sugar to amino acid catabolism supports growth of *L. sanfranciscensis* in restricted environments such as sourdough, characterized by acid stress and recurrent carbon starvation.

Osmotic stress response: In the various applications in food and feed industry LAB can be exposed to osmotic stress when important amounts of salts or sugars are added to the product [17]. In fact, in most of the food habitats where lactobacilli live, they are confronted with salt [67] and sugar stress [68]. Study on the differences between salt and sugar osmotic stress revealed that the hyperosmotic conditions imposed by sugar stress are much less detrimental and only transient (transient osmotic stress), because the cells are able to balance the extra and the intracellular concentrations of lactose and sucrose [17]. Bacteria need to adapt to this change in their environment in order to survive [69], and they can do it by accumulating (by uptake or synthesis) compatible solutes, generally of organic origin, under hyperosmotic conditions [17]. The compatible solutes are defined as osmoprotectants. The main strategy to adapt to high osmolarity of non-halophilic bacteria is associated with the enhancement of the osmotolerance [68]. Moreover, the osmoprotectants can also stabilize enzymes and provide protection not only against osmotic stress but also against other type of stresses (high temperature, freezing and drying). The intracellular accumulation of compatible solutes prevents the loss of water caused by high external osmolarity and allows the maintenance of turgor [68]. The accumulation of carnitin, betain and proline was determined in LAB grown in MRS and complex diluted MRS medium (DMRS medium) [70]. Moreover, a specific response mechanism to osmotic stress was identified in a sourdough model system [13]. In particular, the growth of *L. sanfranciscensis* under osmotic stress resulted in a relevant accumulation of 3-methylbutanoic acid. Its synthesis is associated with the BCCAs., is NAD+ dependent and produces NADH during the reaction [71]. The accumulation of 3-methylbutanoic acid as predominant metabolite has been also observed in model systems simulating sourdough as a consequence of osmotic, acid or oxidative stress [12, 15].

High pressure stress response: High-pressure processing (HPP) or high pressure homogenization (HPH) are non-thermal processes capable of inactivating and eliminating pathogenic and food spoilage microorganisms in specific foods [11, 72], and it represents an exceptional stimulus for most mesophilic bacteria. Several proteins are induced after high pressure treatment and some of these have also been involved in the response to other various stresses [8]. The responses to HHP stress have been studied in particular on *L. sakei* and *L. sanfranciscensis* [73, 18]. These Authors suggested the presence of *de novo* protein synthesis as a consequence of HHP stress [73]. As concerning HPH several interesting studies on the responses on *Lactobacillus* spp., at the level of proteolytic and metabolic activities point of view have been conducted [11, 21, 22, 74]. HPH treatment positively affects the proteolytic activity of some of *Lactobacillus* strains, but the activation and the quantitative and qualitative changes of the metabolic activity appear to be the most promising results. The pre-treatment at different pressure was able to induce relevant

changes in term of fermentation dynamics and metabolism with respect to the untreated cells [11]. The same approach was applied on *L. acidophilus* and *L. paracasei* to improve the technological performances of probiotic strains [21, 22, 74]. The sub-lethal treatment with HPH enhanced the capacity of some *in vitro* probiotic features (i.e. hydrophobicity and tolerance to simulated gastric acidity) in a strain dependant way. *L. paracasei* A13 enhanced cellular hydrophobicity and auto-aggregation capacity after HPH treatment at 50 MPa. On the contrary, the HPH treatment decreased these features in the other strains considered. Highest values of hydrophobicity were found for *L. acidophilus* DRU and its bile-resistant derivative *L. acidophilus* DRU+, while lower values were obtained for *L. paracasei* strain [74]. Moreover, the stress responses enable survival under more severe conditions, enhancing resistance to subsequent processing conditions [75]. HPH treatment at 50 MPa can favour the maintenance of cell viability during a refrigerated storage in buttermilk, a suitable medium to maintain the cell viability during refrigeration [76]. The increased viability can be attributed to the increased precocious availability of low molecular weight peptides and free fatty acids such as oleic acid [21, 22].

Competition and communication: Food fermentations are typically carried out by mixed cultures consisting of multiple strains or species [77]. Mixed-culture food fermentations are of primary economic importance. The performance of these cultures, consisting of LAB, yeasts, and/or filamentous fungi, is not the simple result of "adding up" the individual single-strain functionalities, but is largely determined by interactions at the level of substrates, exchange of metabolites and growth factors or inhibiting compounds [77].

General microbial interference is an effective non-specific control mechanism common to all populations and environments including foods. It represents the inhibition of the growth of certain microorganisms by other members of the habitat.

The mechanisms involved are common to all genera and include [78]:

1.   Nutrient competition,
2.   Generation of unfavorable environment,
3.   Competition for attachment/adhesion sites.

Most substrates for food fermentations have a highly heterogeneous physicochemical composition, which offers the possibility for the simultaneous occupation of multiple niches by "specialized" strains, for instance, through the utilization of different carbon sources. In these substrates, coexisting strains often interact through trophic or nutritional relations via multiple mechanisms [77].

Carbon sources are often present at high concentrations in food substrates, and therefore competition concerns the rapid uptake of nutrients and conversion into biomass. In dairy fermentations nitrogen is limiting, and initially organisms compete for the free amino acids and small peptides available. While in the later stages of fermentation, they compete for the peptides released by the actions of proteolytic enzymes [77].

In a cell-density-dependent quorum-sensing system, bacteria produce extracellular signaling molecules such as peptides or post-translationally modified peptides that act as

inducers for gene expression when concentrations of these molecules exceed a certain threshold value [79]. These changes might eventually lead to competitive advantages for the population, more effective adaptation and responses to changing environmental conditions, or the co-ordination of interactions between bacteria and their abiotic and biotic environments [7]. In fact, microorganisms produce diffusible chemicals for the purpose of communication and it has been reported that the stress caused by the exposure of microbial cells to their own cell free conditioned media, containing metabolites and bioactive compounds including ''quorum sensing'' molecules, including 2(5H)-furanones, promotes cell differentiation, autolysis and overproduction of specific metabolites [12, 80, 9, 10]. In this way the microbial cultures used in food fermentations can also contribute (by "secondary" reactions and relations) to the formation of flavor and texture [81].

4. General steps regarding a virtual fermented food process

In the figure 2, the steps that mainly interest food fermentation are reported. A model virtual fermented food was identified to resume the common denominator of the fermented foods dynamics, particularly focused on the reciprocal influences between environmental fluctuation and LAB fermentation.

Whatever kind of food we want to produce, fermented or not, the first step of the process is the formulation: in this phase the main raw materials (meat, milk, fruit and vegetables or their derivatives) are mixed with other ingredients, that have different roles: salts or sugars to improve taste, spices to give specific sensorial quality and as antimicrobials, additives or other substances able to affect physical and structural properties, preservatives to improve microbial stability and shelf life. The addition of those ingredients can be perceived as stress. In fermented products, proper microorganisms, mainly yeasts and LAB, are also added as starter cultures, in order to start and lead the fermentation and to obtain a stable and standard final product. As a consequence, the microorganisms, naturally occurring or added as starter cultures, have to cope with a completely different system: in particular, naturally occurring microflora have to face the changes induced by the ingredients, while the starter cultures, deriving from growth media or added as lyophilized cultures, have to adapt to a real food system, where different sources of stresses are often present.

In particular, the first sub-lethal stress, which LAB face, regards the difference between the growth medium composition and the real food. Generally, LAB lyophilized cultures can be added to the ingredients after a reactivation and subsequently added to the product. This procedure identify the presence of a stress for the LAB cells. Starter cultures are added to the raw materials in large numbers and incubated under optimal conditions, but the adaptation to substrate or raw material is always necessary [82]. It is very important to consider the physiological state of the LAB before the inoculum. This state strongly depends on the time of harvesting of the culture (whether during the logarithmic or stationary phase of growth), on the conditions leading to transition to the stationary phase, on the treatment of the culture during and after harvesting and on the chemical composition of the environment. Therefore it is important during formulation and technological processes to consider also these factors, mainly for those products where microorganisms are added as starter cultures.
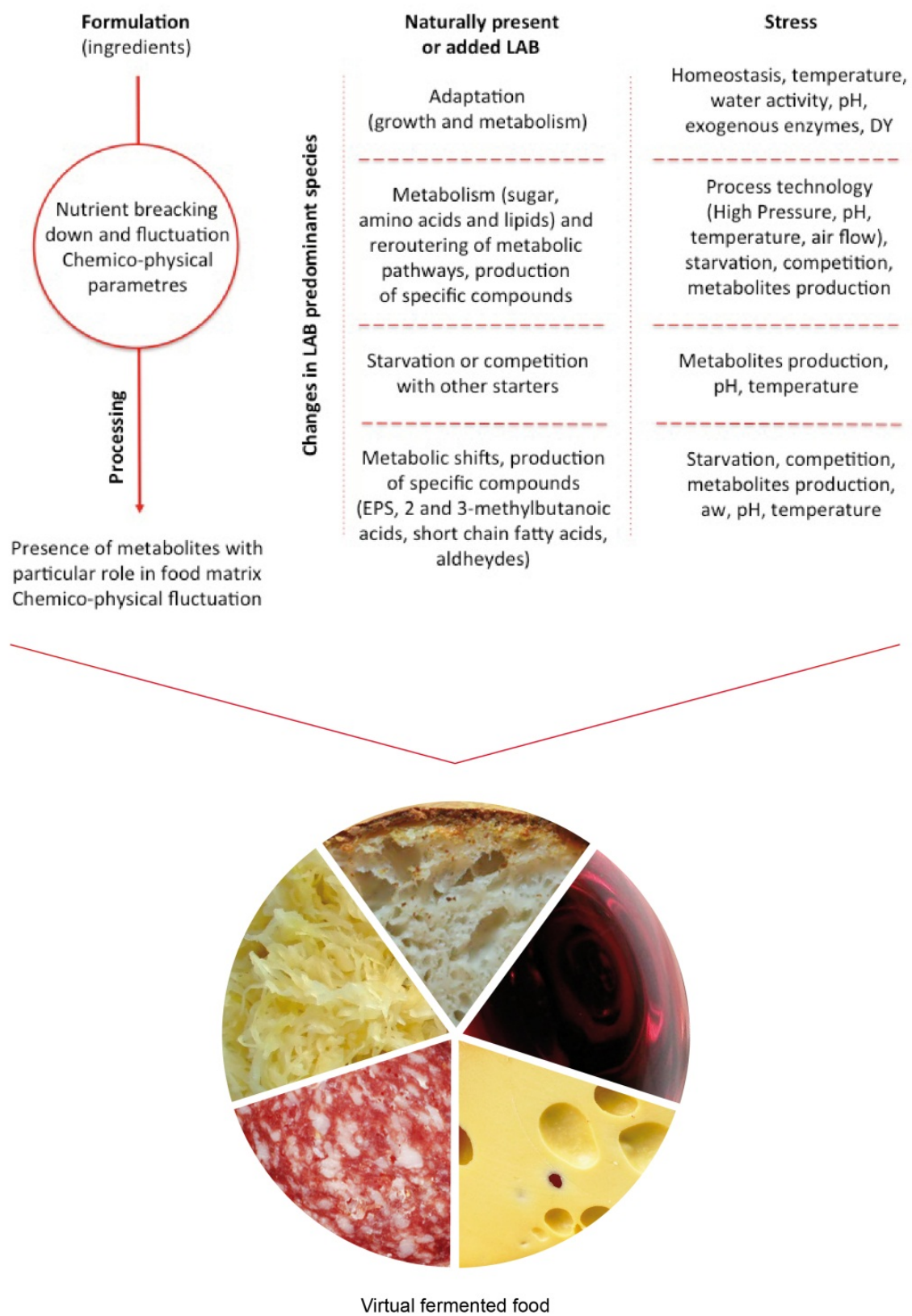
Formulation
(ingredients)

**Naturally present
or added LAB**

Stress

Nutrient breacking
down and fluctuation
Chemico-physical
parametres

Adaptation
(growth and metabolism)

Homeostasis, temperature,
water activity, pH,
exogenous enzymes, DY

Metabolism (sugar,
amino acids and lipids) and
reroutering of metabolic
pathways, production
of specific compounds

Process technology
(High Pressure, pH,
temperature, air flow),
starvation, competition,
metabolites production

Starvation or competition
with other starters

Metabolites production,
pH, temperature

Metabolic shifts, production
of specific compounds
(EPS, 2 and 3-methylbutanoic
acids, short chain fatty acids,
aldheydes)

Starvation, competition,
metabolites production,
aw, pH, temperature

Changes in LAB predominant species

Processing

Presence of metabolites with
particular role in food matrix
Chemico-physical fluctuation

Virtual fermented food

Figure 2. Fermented food model: reciprocal influences between environmental fluctuation and lactic acid bacteria fermentation.

The interaction between the starters and the ingredients and between the starters and the naturally present microbial population can trigger few important mechanisms that will influence the quality and the characteristics of the fermented product. Analogously, many food processes and formulations have been tested for safety by challenge test inoculating pathogen bacterial cells at different growth phases, and the results proved that cells grown to the stationary phase or adapted to various stresses have greater resistance than exponential cells [83].

Other ingredients usually added to obtain safe and stable products are food preservatives, including:

a.   Antioxidants,
b.   Anti-browning agents
c.   Antimicrobials.

These latter are arbitrarily classified into two groups: traditional or "regulatory approved" and naturally occurring [84]. The former includes acidifiers such as acetic acid, lactic acid and citric acid and antimicrobials such as benzoic acid and benzoates, propionate, nitrites and nitrates, sorbic acid and sorbates and sulfites. The latter includes compounds from microbial, plant and animal sources that are, for the most part, only proposed for use in foods as antimicrobials (e.g. lactoferrin, lysozyme, nisin). Throughout the ages, food antimicrobials have been used primarily to prolong shelf-life and preserve quality of foods through inhibition of spoilage microorganisms, while only few are used exclusively to control the growth of specific foodborne pathogens (e.g. nitrite, used for hundreds of years to inhibit growth and toxin production of *Clostridium botulinum* in cured meats). In food formulation antimicrobials are part of a multiple intervention system that involves the chemical along with environmental (extrinsic) and food related (intrinsic) stresses and processing steps. Some of these substances (for example lactic acid and citric acid) provoke a direct acidification of a food or food ingredient, and therefore challenge the microflora inducing and increase of acid resistance of the microflora itself. In fermented food the situation can be somewhat different, because the pH is gradually lowered by LAB creating a pH gradient, more likely than a sharp alteration in the pH due to direct acidification.

A good model describing the shock related to the inoculum of LAB in the raw complex material has been described during the production of fermented sausages [85]. The relatively high pH of raw meat rapidly decreases during the initial fermentation phase because organic acids, mainly lactate, are formed by LAB and the water activity is reduced during ripening, because of the addition of salt as well as drying. Furthermore, adjuvants, such as potassium or sodium nitrite and/or nitrate, are mostly added to optimize the fermentation process.

Generally strains used as starter cultures must tolerate these kinds of stresses and exhibit a high ecologic performance in the stressful food environment. Genes related to stress response are induced when *L. sakei* is inoculated in the raw meat system [86]. In fact, ctsR, a gene that coded for a class III heat shock proteins repressor associated with the environmental stress response of Gram positive bacteria, increased its expression when *L.*

sakei starts to adapt to the raw environment. This mechanism demonstrated that the sudden changes in the environment conditions are perceived as stress by *Lactobacillus* species. In particular, in the case of *L. sakei*, added to raw meat and spices, the principal stress response regarded high osmolarity and temperature shifts. Moreover, the presence of curing salt is regarded as one of the major hurdles in the initial phase of sausages fermentation. Because nitrite was found to be the effective for growth inhibition of pathogens, nitrite was also hypothesized as a stressor for *L. sakei* [85] and the exposure of this strain to stresses can induce changes in metabolic activities in a food environment [4]. The metabolic changes in *L. sakei* resulted in enhanced exploitation of available nutrients or increased activity of glycolytic enzymes, leading to the accelerated production of lactic acid by stress-treated *L. sakei* cells [85]. However, the exposition of *L. sakei* to low temperature and high osmolarity gives rise to the repression of phosphofructokinase and consequently to a decreased flux through the glycolytic pathway [87].

Moreover, it is important to consider that some ingredients can be also antimicrobials because of their own characteristics: in fact, if the recipe includes herbs and spices (aromatic plants, pepper), garlic and onions, an effect on microorganisms can be exerted by specific compounds characterizing these products, such as essential oils, terpenes and sulfur compounds [88].

Another essential aspect affecting the performances and metabolism of LAB are the intrinsic characteristics of raw materials that sometimes act in a synergic way with other ingredients. Considering for example fermented vegetables, the microflora of the starting fresh vegetables is typically dominated by Gram negative aerobic bacteria and yeasts, while LAB make up a minor portion of the initial population [89] and therefore they would not be able to start and lead a fermentation process. However, if anaerobic conditions are settled and salts are added, LAB can have a competitive advantage and induce spontaneous lactic acid fermentation. The growth of specific LAB is dependent on the chemical (substrate, salt concentration, pH) and physical (vegetable type, temperature) environments. As the environments change during fermentation, so can the dominant organisms, often leading to a specific and reproducible succession of bacteria.

In sauerkraut [89, 90] the presence of 1.8-2.2% of NaCl and a temperature of 18°C inhibits many strains of LAB, with the exception of *Leuconostoc mesenteroides* that initiates the fermentation; however this species is sensitive to acid conditions, so after a few days, when the concentration of lactic acid increases, *L. mesenteroides* is replaced by more acid resistant LAB such as *Lactobacillus brevis* and *L. plantarum*, able to further lower the pH up to 3-3.5, stabilizing the final product.

Considering olives fermentation is possible to outline the characteristics of the product affecting LAB: while the brine provides a good environment for LAB growth, with glucose, fructose and mannitol as the main source of fermentable sugars, the presence of high levels phenols (such as oleuropein) exert an antimicrobial activity, inhibiting some strains and selecting the types of organisms that predominate during the fermentation [91-93]. These LAB have to be resistant not only to phenols, but also to lye treatments and water washes,

that can be performed during the processing and increase the initial pH, reducing also the nutrients content on the olive surface. The species able to face these kind of stresses usually belong to the genera *Pediococcus*, *Leuconostoc* and *Lactococcus*; after the first stage of fermentation, when the pH reaches 6, *L. plantarum* rapidly grows and dominate the fermentation, that goes on until the fermentable sugars are depleted. The viability and vigor of *L. plantarum* can be encouraged also by yeasts that are still present in this stage of fermentation and can produce vitamins [94].

Moreover, the presence of some gases can modify the growth performances of LAB. That is also influenced by the mixing step of the ingredients in some food processes (e.g. dough mixing). In fact, in bread making process, the continuous agitation of the dough can increase the microbes exposure to oxygen, and this can be a source of oxidative stress, mainly for LAB that are usually anaerobic or facultative anaerobic. Also in these cases the bacteria can react in different ways, activating metabolic and transcriptional responses in order to detoxify ROS, as previously described.

For the fermented vegetables ,above reported, the rapid consumption of oxygen due to the presence of yeasts and aerobic bacteria in the first stage of fermentation has a positive effect on LAB. In fact, they are exposed only for a short time to oxidative stress and, due to their competitive advantage, they rapidly and intensively grow in the food system.

After formulation, the technological processes involving LAB include a fermentation process.

It is reported that various beneficial phenotypic traits of LAB in food fermentations such as rapid acidification, selective proteolysis, tolerance of osmotic and stresses, resistance to ROS, and ability to thrive in nutrient poor conditions and at low temperatures are influenced by stress responses in various species of LAB [95, 96]. The knowledge of these mechanisms, and mainly of the stress responses activated by the fermentation process parameters can be useful in order to develop strains with optimal fermentation characteristics [83].

The first metabolic reaction regards the oxidation of carbohydrates (this reaction depends on the hetero-fermentative or homo-fermentative species involved) that give rise to acids, alcohols and CO_2. These metabolites are directly involved in flavor, aroma and texture of the product and in a second time can influence the production and the availability of other metabolites such as vitamins and antioxidant compounds [78]. Moreover, the LAB interactions with the ingredients increase also the digestibility and decrease the glycemic index, enhancing the healthy features of the fermented foods [97].

At the same time with carbohydrates oxidation, other metabolic mechanisms interest LAB cells such as proteolysis and lipolysis. The first reaction produces polypeptides with interesting characteristics as antimicrobial compounds, salt substitutes (the oligopeptides are able to increase the palatability of the system), and amino acids deriving aromatic compounds. On the other hand lipolysis produces medium chain fatty acids, with important antimicrobial properties. All these reactions (carbohydrates oxidation, lipolysis and proteolysis) generate precursors for other mechanisms in the cells and in the food matrix

that give rise to the dynamic environment characteristics of fermented foods. It is important to outline that the compounds produced by the cells, metabolizing the substrate, can modify the system, producing also compounds that can stimulate the growth of symbiotic species or inhibit the growth of antagonistic microorganisms.

The conversion of carbohydrates to metabolites as acetic acid, lactic acid or CO_2 implies the acidification of the system. The contemporary pH decrease and the presence of sugar (osmotic stress) stimulate the exopolysaccharides (EPSs) production. In fact, in sourdough EPSs can be involved in acid tolerance of sourdough LAB [98]. EPSs are long-chain polysaccharides consisting of branched, repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose and rhamnose, in different ratios [99]. The presence of EPSs in the system can create a novel stress to the cells. The inclusion of cells within biofilm can increase their resistance to unfavorable environmental factors such as extreme temperature, low pH and osmolarity, the changes in the texture can induce in LAB also specific stress responses.

For example in yogurt production, the acidification by LAB implies proteins coagulation and thereby changes in the viscosity of the milk. In *L. bulgaricus*, during the acid adaptation present in the fermentation milk to obtain yogurt, some cellular changes were observed: the chaperones GroES, GroEL, HrcA, GrpE, DnaK, DnaJ, ClpE, ClpP and ClpL were induced and ClpC was repressed [100]. Some genes involved in the biosynthesis of fatty acids were induced (*fabH*, *accC*, *fabI*), while the genes involved in the mevalonate pathway of isoprenoid synthesis (*mvaC*, *mvaS*) were repressed [101, 102]. The changes in Aw value are depending not only on EPSs production by LAB after the exposition to acidic and osmotic stress, but also on the ingredients composition and on the step of fermentation.

Considering cheese, the Aw decreases during manufacture and ripening as a result of dehydration, salting, and production of water-soluble solutes from glycolysis, proteolysis, and lipolysis; the cheese Aw values range from 0.70 for extra hard cheeses to 0.99 for fresh, soft cheeses, such as cottage cheese, while semi-hard cheeses have Aw values of around 0.90. The cheese pH also decreases during manufacture and ripening [103]. The effects of different Aw and pH on *L. lactis* simulating cheese ripening have been analyzed [103]. The results evidenced that at low Aw, particularly at low pH, the growth and lactose utilization rates decreased and lactose fermentation to L-(1)-lactate switched to a pathway involving nontraditional saccharide products rather than the traditional lactococcal heterofermentative products.

In *L. plantarum* WCFS1 the addition of 300 mM and 800 mM of NaCl induced mild osmotic stress and osmotic stress respectively. In the presence of 800 mM of NaCl several genes showed an increased expression with respect to the control culture. In particular, those genes were associated with various stress responses in prokariotes, i.e. genes encoding Clp protease, an excinuclease, catalase (peroxide stress) and Dpr-like protein (peroxide stress). These differences in the gene expression were also identified in the presence of acid stress. These results suggest that lactic acid stress in *L. plantarum* WCFS1 also induces a more general stress response (as above described for different *Lactobacillus* species). An overlap between the stimulus for lactic acid and those for peroxide and UV radiation has also been

reported for *L. lactis* [104, 66]. The response of *L. sanfranciscensis* to osmotic stress (saccarose 40%) gives rise to the overproduction of 3-methylbutanoic acid and gamma-decalactones when *L. sanfranciscensis* was co-inoculated with yeasts, simulating a sourdough environment. The production of lactones can be indicated as unfavourable environment for microbial growth and metabolism. In fact, these compounds have both particular aromatic and antimicrobial features [13].

The ability of the target strains to dominate the fermentation is related not only to the ingredients (as above described), but also to the fermentation conditions, mainly temperature and atmosphere. If the fermentation is not performed at the optimal growth temperature for the microorganisms, they could be unable to compete with naturally occurring microflora, and consequently the whole process could be compromised. On the contrary, some microbial species have developed specific thermal resistance mechanisms, and they can easily adapt to these unfavorable conditions without implications for the fermentation processes. Moreover, the adaptation to thermal stresses often leads to tolerance to other stresses, in a mechanism usually define "cross protection", as reported for *L. lactis* [105]. The ability of commercial *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* to withstand freezing at –60°C for 24 h was significantly improved by a prior 25 min heat shock at ~40°C or by a 2 h cold shock at 10°C, opening interesting perspectives for the production on resistant starter cultures, both frozen or lyophilized [105].

Other Authors with regard to different stresses reported the "cross protection" mechanism: for example the mechanisms of multiple adaptations to hops of two different strains of *L. brevis* have been characterized [106]. Hop resistance of lactobacilli requires multiple resistance mechanisms. This is consistent with the stress conditions acting on bacteria in beer, which mainly consist of acid stress and the antimicrobial effect of the hop compounds, in addition to ethanol stress and starvation. The effect of interaction of acid stress and presence/absence of oxygen in the system on *L. helveticus* and *L. sanfranciscensis*, in particular on their cell membrane composition, has been reported [14]. Upon acid stress the level of cyclopropane fatty acids increased at the expense of the level of long-chain unsaturated fatty acids. *L. helveticus* and *L. sanfranciscensis*, exposed to acid sub lethal stress demonstrated the same increase in cyclopropane fatty acids. In particular, *L. helveticus* presented higher concentration of C19cyc11 at pH 4 and pH 3, while *L. sanfranciscensis* presented more C19cyc9 at pH 3 in microaerophilic condition without tween 80, at pH 3.6 in anaerobiosis with tween 80, and at pH 4 in anaerobiosis without tween 80. These results demonstrated the same behavior in front of multiple stresses by LAB membrane [106, 14]

Consider the atmosphere, i.e. the presence or not of oxygen, as another important variable during fermentation, it is known that oxygen can inhibit the growth of LAB, especially in the first stages. However, the food system is usually a consortium of different microorganisms: for example in bakery products and in fermented sausages the fermentation is carried out both by yeasts and LAB; the formers can therefore consume the amount of oxygen present in the mix, allowing the growth of LAB. The same thing happens for fermented vegetables, where naturally occurring Gram negative bacteria and yeast rapidly remove the oxygen, promoting the rapid predominance of Lactobacilli.

Some secondary metabolites such as bacteriocins can play a role in LAB performances and metabolism, affecting also the total population and ecology of fermented foods [107, 108]. Bacteriocins are antimicrobial peptides or proteins produced by bacteria that can be active on different microorganisms, depending on their structure. LAB belonging to the genera *Lactococcus, Pediococcus, Lactobacillus, Leuconostoc, Carnobacterium, Propionibacterium* are known to produce bacteriocins with both narrow and broad inhibitory spectra [109]. The use of functional LAB starter cultures (eg. bacteriocinogenic starter cultures), well adapted to the environment and the process conditions applied, may contribute to the development of better controllable and more efficient production processes [110]. An example can be nisin, a peptide produced by *L. lactis* ssp. *lactis*, that has a narrow spectrum affecting primarily only Gram-positive bacteria and their spores, including lactic acid bacteria, *Bacillus, Clostridium, Listeria,* and *Streptococcus*. However some LAB such as *Streptococcus thermophilus* and *L. plantarum* are able to produce the enzyme nisinase, which neutralizes the antimicrobial activity of the peptide [111]. Therefore these LAB could be suitable for a co-fermentation with *L. lactis*.

Another interesting case of bacteriocin production, as a consequence of oxidative stress and carbon dioxide exposure, has been reported [110]: oxidative stress and carbon dioxide are involved in the production of a specific bacteriocin, amylovorin L, by *Lactobacillus amylovorus*, able to inhibit other LAB species. During traditional sourdough fermentation, a decrease in redox potential of the rather firm mixture occurs. The oxygen initially present is consumed by *Candida* spp. or converted into hydrogen peroxide or water, thereby creating microaerophilic or anaerobic environment in which the growth of the desired LAB is favored. While in a large-scale sourdough type II fermentation currently the use of dough mixture with high dough yield is exploited. This sourdough has to be stirred to liberate part of the carbon dioxide produced to prevent running over. During mixing, oxygen is incorporated into the dough. Also, the development of yeast and hence the production of carbon dioxide is favored in continuously stirred sough mixtures with high water content. Elevation of the airflow rates leading to oxidative stress conditions resulted in an enhanced specific amylovorin L production. Growth in the presence of carbon dioxide also increased the specific bacteriocin production. Mild aeration or a controlled supply of oxygen as well as growth in an environment containing high amounts of carbon dioxide might thus contribute to the competitiveness of *L. amylovorus* DCE471 in a sourdough ecosystem [110]. The production of plantaricin A by *L. plantarum* was also demonstrated in relation to a quorum sensing mechanism [79].

Another example of the influence of the process on LAB metabolism has been widely described [112]. These Authors monitored the evolution of the gene expression of *L. plantarum* IMDO 130201 during a sourdough process. In particular, the genes and the metabolites related to acidic stress were analyzed. It is interesting to highlight that during the pH decrease (production of lactic acid by *L. plantarum*) the genes coding for plantaricin production had higher levels of expression at low pH values, indicating that the bacteriocin production was activated under acid stress conditions by *L. plantarum* IMDO 130201 strain. The presence of the pheromone plantaricin A (PlnA) in a system inoculated with *L. plantarum* DC400 was also reported [79]. Biosynthesis of PlnA was variously stimulated

depending on the microbial partner. In fact, *L. sanfranciscensis* DPPMA174 induced the highest synthesis of PlnA, which, in turn, determined lethal conditions for it. The proteome of *L. sanfranciscensis* DPPMA174 responded to the presence of PlnA. The up-regulation of 31 proteins related to stress response, amino acid metabolism, energy metabolism, membrane transport, nucleotide metabolism, regulation of transcription and cell redox homeostasis was found. At the same time, other proteins such as cell division protein (FtsZ), glutathione reductase (LRH_11212) and response regulator (rrp11) were down-regulated. These results demonstrated a hypothetically and interesting waterfall of events all related with stresses response and with the typical fermentation products dynamics (Figure 3). At the same time, the low pH values implied a poor expression of the genes involved in carbohydrate degradation in *L. plantarum* IMDO 130201. The bacterium was directed toward survival at low pH by amino acid conversions rather than by relying on growth [112]. The same behavior was identified in *L. sanfranciscensis* LSCE1 response to pH 3.6 [15]. Under the adopted experimental conditions, which did not produce any decrease in viability of *L. sanfranciscensis* LSCE1, the acid stress, within 2 h, was accompanied by a reduction of the carbohydrate metabolism, as shown by the decrease of ethanol, acetate, and lactate. This mechanism suggests the existence of a switch from sugar to amino acid catabolism that supports survival and growth also in specific and restricted environments, such as sourdoughs, characterized by acid stress and recurrent carbon starvation. Under the acid conditions (pH 3.6) and in the presence of specific nutrients 3-methylbutanoic acid was the predominant metabolite among those detected by solid phase micro-extraction gas chromatographic analysis and mass spectrometry (GC-MS-SPME), released after 2 h of acid stress exposure [15]. The acid stress implied less carbohydrate utilization and ethanol, lactate, and acetate production, but high amino acids catabolism that confers a different and characteristic metabolites pattern. Stress resistance assume great importance as one of the adaptation factors to gastrointestinal tract of probiotic strains as reported in a detailed review [113].

5. Stress resistance of probiotic LAB

There are two main categories of factors that contribute to the optimal functioning of probiotic lactobacilli: factors that allow optimal adaptation to the new niches that they temporarily encounter in the host (adaptation factors) and factors that directly contribute to the health-promoting effects (probiotic factors) [113].

Adaptation factors include stress resistance, active metabolism adapted to the host environment, and adherence to the intestinal mucosa and mucus.

In fact, probiotic lactobacilli encounter various environmental conditions upon ingestion by the host and during transit in the gastro intestinal tract (GIT). They need to survive to: 1) the harsh conditions of the stomach secretion generating a fasting pH of 1.5, increasing to pH 3 to 5 during food intake; 2) the bile excreted by liver in small intestine represents another challenge for bacteria entering the GIT. Bile salts also seem to induce an intracellular acidification so that many resistance mechanisms are common for bile and acid stress. Indeed, the protonated form of
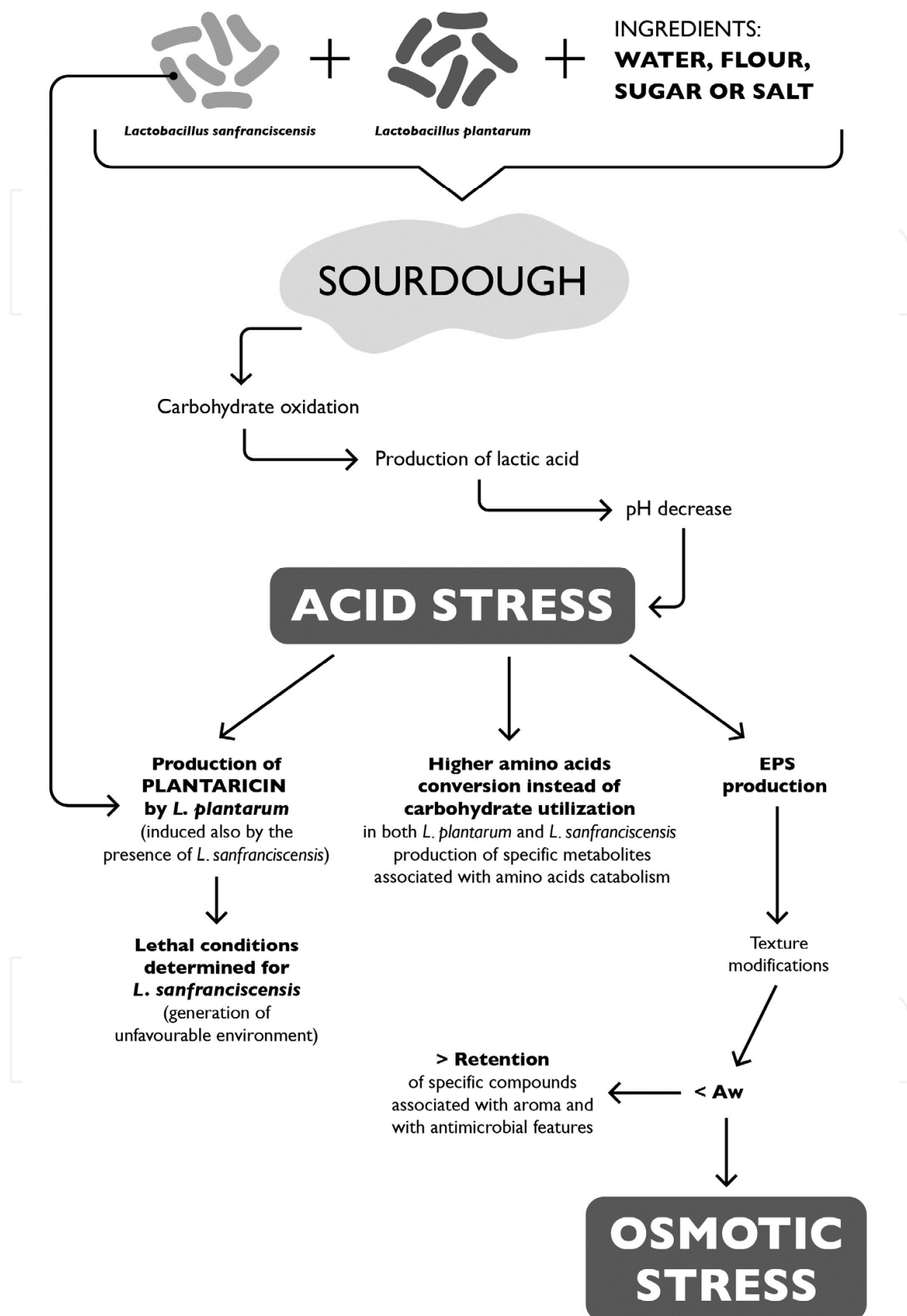
Figure 3. Sourdough fermentation dynamics. Case of possible parallel phenomena interesting acid and osmotic stress.

bile salts is thought to exhibit toxicity through intracellular acidification in a manner similar to those of organic acids like the lactic acid produced by the lactobacilli themselves. For a detailed overview of acid, bile, and other stress resistance mechanisms of lactobacilli, the reader is referred to more extensive review [113]. 3) In analogy to the stresses encountered by intestinal pathogens, they also encounter oxidative and osmotic stress in GI tract. 4) Interactions with other microbes and 5) Interactions with cells of the host immune system and the various antimicrobial products that they produce can also impose a serious threat for the probiotic microbes. Analogously to what described in food LAB, the phenomenon of cross-adaptation is often observed, i.e., that adaptation to one stress condition also protects against another stress factor, implying some common mechanisms. In this respect, also for probiotic LAB non-actively-growing stationary-phase cells are generally more resistant to various stressors than early-log-phase cells.

5.1. Maintaining integrity of the cell envelope

The different macromolecules constituting the cell membranes and cell walls of lactobacilli have been shown to contribute to maintaining cell integrity during stress to various degrees. For example, low pH caused a shift in the fatty acid composition of the cell membrane of an oral strain of *L. casei*. Similarly, bile salts have been shown to induce changes in the lipid cell membrane of *Lactobacillus reuteri* CRL1098.

The role of EPS in acid and bile resistance is less clear. However, EPS production has not been studied in detail after exposure to bile. In fact, to our knowledge, phenotypic analyses of dedicated *Lactobacillus* mutants affected in EPS biosynthesis genes have not yet been performed. Homopolysaccharides (HoPSs) from *L. reuteri* have been reported to have a more established role in stress resistance by the maintenance of the cell membrane in the physiological liquid crystalline phase under adverse conditions.

5.2. Repair and protection of DNA and proteins

A number of proteins that play a role in the protection or repair of macromolecules such as DNA and proteins also seem to be essential for acid and bile resistance. Intracellular acidification can result in a loss of purines and pyrimidines from DNA. Bile acids have also been shown to induce DNA damage and the activation of enzymes involved in DNA repair. Perhaps even more vital in the general stress response are chaperones that intervene in numerous stresses for important tasks such as protein folding, renaturation, protection of denatured proteins, and removal of damaged proteins.

5.3. Two-component and other regulatory systems

Mechanisms to specifically sense the presence of certain stress factors and regulate gene expression in response to these stimuli are also crucial for bacterial survival under adverse conditions. Although these mechanisms are not well characterized for lactobacilli, they often involve two-component regulatory systems (2CRSs). 2CRSs allow bacteria to sense and respond to changes in their environment after receiving an environmental signal through transmembrane sensing domains of the histidine protein kinase (HPK).

6. Methodological approaches to study the effects of stress on LAB

The study of stress responses by LAB is getting closer and closer to the different "omic" fields: genomic, proteomic and metabolomic. Other traditional approaches regarding the membrane cells composition and modifications, both from a structural (cellular fatty acids composition by gas-chromatographic method) and morphological (membrane and wall modification by electronic microscopy) point of view are still used.

Genes implicated in LAB stress responses are numerous and the levels of characterization of their actual role and regulation differ widely between species. The studies concerning stress responses in LAB sometimes benefit from the knowledge already acquired in other bacteria. For example, parts of the studies on heat response have been focused on specific genes because of their major role demonstrated in other microorganisms [17]. The cheapest and easiest way to study a stress response in LAB is to follow some specific genes related to stresses such as heat shock, salts and acids [114, 115]. This type of study is useful especially if the entire genome sequence of some LAB is still unknown. However, nowadays the study of whole trascriptome (the total set of RNAs) is one of the most exhaustive ways to study modifications of gene expression as a result of a stress condition. The transcriptome of a cell contains information about the biological state of the cell and the genes that play a role under specific circumstances. The principal technique used to study the trascriptome is microarray [116].

DNA microarray technology has been used in numerous experiments to analyze gene expression: one example is the evaluation of the general stress response of *B. subtilis* [117] or the investigation of the transcription profiles of *L. plantarum* grown in steady-state cultures that varied in lactate/lactic acid concentration, pH, osmolarity [66, 104]. This approach is useful also to study the behaviour of bacteria in a real food system. Hüfner et al. [5] studied the global transcriptional response of *L. reuteri* to sourdough environment, showing a significant changes of mRNA levels for 101 genes involved in diverse cellular processes, from carbohydrate and energy metabolism, to cell envelope biosynthesis, exopolysaccharide production, stress responses, signal transduction and cobalamin biosynthesis.

The gene expression dynamics of *L. casei* during fermentation in soymilk when grown up to lag phase, late logarithmic phase, or stationary phase were also studied. Comparisons of different transcripts close to each other revealed 162 and 63 significantly induced genes, in the late logarithmic phase and stationary phase, whose expression was at least threefold up-regulated and down-regulated, respectively. Approximately 38.4% of the up-regulated genes were associated with amino acid transport and metabolism, followed by genes/gene clusters involved in carbohydrate transport and metabolism, lipid transport and metabolism, and inorganic ion transport and metabolism [118].

The study of trascriptome is a good approach that gives a good overview of the changes that can occur inside a stressed bacterium. A limitation of this technique is that it is expensive and requires that the genome sequences of the organisms under study should be available for designing the oligonucleotides for the microarray [119].

A different but, at the same time, related point of view regards the study of proteins and proteome. The most common method to obtain this information is to extract total proteins and separate them by a sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) followed by a western blotting (in the first case) or a two dimesional electrophoresis (2D-E) analysis (in the second case). Also in this case if the study is focused on a single protein, it is necessary to know before the characteristic of the target protein to optimize the analytical conditions. 2D-Electrophoresis can provide more than 10000 detectable protein spots in a single gel run. Thus, proteins with post-translational modifications (PTMs), such as processing, phosphorylation and glycosylation, can be easily detected as separate spots. A spot separated by 2D-E theoretically consists of an almost homogeneous protein, and thus can be identified following digestion with a sequence-specific protease by peptide mass fingerprinting (PMF) approaches, typically using matrix-assisted laser desorption ionization (MALDI)- time-of-flight (TOF) mass spectrometers. The same level of automation is also available for proteomic approaches involving tandem mass spectrometry (MS-MS) analysis, extremely useful when studying organisms with incomplete or partial genomic information [120].

This kind of approach was used to investigate the cell surface proteins of a typical strain of *L. casei* in response to acidic growth conditions [121]. They demonstrated that growth of *L. casei* under acidic conditions caused molecular changes at the cell surface in order to accomplish an adaptive strategy, resulting in slower growth at low pH. Moreover, the proteomic approach was useful to study the heat shock response respectively on *L. helveticus* PR4 and *L. plantarum* [26, 31]. The cold adaptation of *Lactococcus piscium* strain CNCM I-4031 was studied with the same approach [122]. This analysis could be also performed to compare the effects that new technologies produce on bacteria comparing with the normal stress conditions. In fact, the HHP stress response of *L. sanfranciscensis* was compared with cold, heat, salt, acid and starvation stresses responses [18].

Due to increasingly available bacterial genomes in databases, proteomic tools have recently been used to screen proteins expressed by microorganisms in food, in order to better understand their metabolism *in situ*. While up to now the main objective has been the systematic identification of proteins, the next step will be to bridge the gap between identification and quantification of these proteins [123]. Proteomics has also been used to analyse the proteins released during the ripening of Emmentaler cheese. In an innovative study, proteomics was used to prepare a reference map of the different groups of proteins found in cheese [124]. These authors were able to categorize these proteins into five classes: those involved in proteolysis, glycolysis, stress response, nucleotide repair and oxidation-reduction. In addition, information was obtained regarding the peptidases released into the cheese during ripening process. This study enabled the Authors to differentiate between the various casein degradation mechanisms present, and to suggest that the streptococci within the cheese matrix are involved in peptide degradation and together with the indigenous lactobacilli contribute to the ripening process. Using proteomics these Authors were able to get a greater understanding of the microbial succession involved in the ripening of Emmentaler cheese, which information could not have been obtained using other protein

separation techniques. This example illustrates the power of proteomics as a tool for analyzing the composition of a complex mixture of proteins and peptides [119].

The global identification of stress-induced proteins in a given organism has technical limitations. Membrane proteins, for example, are rarely detected by this method. Secondly, it may be that changes in membrane proteins composition result from long-term adaptation processes, while short-term responses may primarily be accounted for the activation (and/or stabilization) of proteins already present. The latter hypothesis is valid especially in the case of transport systems, although for some of the systems studied a transcriptional induction has also been observed [17]. The use of this technique is not as widespread as that of DNA microarrays due to the challenges associated with the purification and separation of complex mixtures of proteins found in cell extracts. At the same time the study of the only transcriptome should take into consideration that a lot of post-transcriptional processes may act on RNA (ex. RNA interference, polyadenilation ecc) [125].

As reported above, the stress responses of LAB are studied also through the analysis of membrane composition, structure and integrity. Not unexpectedly, in fact, the cell membrane plays an important role in stress resistance. First of all, the membrane itself can change in adaptation to environmental conditions and these changes contribute to the protection of the bacteria [17]. The adaptive response to sub-lethal acid and cold stresses in *L. helveticus* and *L. sanfranciscensis* has been analyzed (as described above) [14]. The extraction and identification by GC-MS of lipid fatty acids and free fatty acids could give an overview of the membrane fluidity state. In the same article they developed a gas chromatographic method to separate and quantify the cell cyclopropane fatty acids lactobacillic (C19cyc11) and dehydrosterculic (C19cyc9) demonstrating different responses of the strains tested in terms of cyclopropane fatty acids production, probably due to the different original optimal environment. The comparison between the wild type and the acid-resistant mutant *L. casei* LBZ-2 evidenced in the latter higher membrane fluidity, higher proportions of unsaturated fatty acids, and higher medium chain length. In addition, cell integrity analysis showed that the mutant maintains a more intact cellular structure and lower membrane permeability after environmental acidification [126].

The last but not least approach used to study the stress response of LAB is the metabolic one. The study of the metabolites released, as a consequence of the stress exposure, can contribute to the understanding of the mechanisms that regulate the microbial interactions and the metabolic alterations induced by stress conditions. Moreover, these approaches can be exploited to identify which technological conditions induce microorganisms to produced desirable metabolites [4, 15].

With this perspective the use of GC-MS-SPME as a potent and easy tool to study the generation of volatile metabolite compounds such as flavoring molecules or aroma precursors was widely adopted [9,11-13, 15] and contributed to rationalize the process and optimize the products. In particular, the effects of HPH on different species of *Lactobacillus* involved in dairy product fermentation and ripening, monitoring the changes in volatile compounds as indicators of metabolic profiles has been studied [11].

Analysing the oxidative and heat stresses in *L. helveticus* two new 2[5H]-furanones released by this strain both as a possible signalling molecules and as possible important flavouring compounds has been identified by GC-MS-SPME [9]. On the contrary the study of non-volatile metabolites can be performed by normal chromatographic technique (HPLC), especially for amino acids and sugars [15], or by Fast Protein Liquid Chromatography (FPLC) separation for peptides, followed by a mass spectrometry identification [127]. An NMR approach to evaluate the effects on the growth of *L. plantarum* raising the medium molarity by high concentrations of KCl or NaCl and iso-osmotic concentrations of non-ionic compounds was performed [128].

Since all the techniques described above, if used alone, do not allow a total comprehension of stress responses, a lot of studies are trying to combine two or more approaches together. Combined transcriptomic and proteomic analyses were used to evaluate the glucose-limited chemo-stat in *Enterococcus faecalis* V583 [129] or to study the effect of bile salts in the growth of *L. casei* [130]. A combined physiological and proteomic approach, instead, was followed to unravel lactic-acid-induced alterations in *L. casei* [131].

Therefore it is possible to understand, from the references above, that techniques used to study the stress responses of LAB are taking more and more "omic" approach. This comports an accumulation of a huge number of data that it is not easy to manage and to compare. For this reason the use of new programs of data analysis is required. One of these approaches could be the use of heat maps, a technique born as a tool to understand microarray results [66]. Nowadays it could be useful also to manage the data from other fields: in fact, a heat maps was used to show the correlation between metabolites produced, the relative gene expression of specific genes and stress conditions [15]. The same useful tool, combined with other statistical analyses, has been also applied [132].

7. Conclusion

It is known that LAB can adapt to stress with different mechanisms widely studied in model and real systems. An overview of those responses has been described and reported in this chapter.

Stress not only induces changes enabling better survival, but also different performances in a system. In fermented food, the knowledge of the mechanisms that regulate LAB metabolic changes and their effects gain importance especially when those responses can be exploited in order to improve the food properties [4]. In particular, fermented foods are dynamic systems subjected to continuous evolution of their physico-chemical characteristics. The complex fluctuation of the food environment itself, during processing, is stress source for every microorganism involved and the changes that affect the fermented food habitats, can be perceived by LAB as stress.

In this chapter examples of the dynamic fluctuation effect on LAB metabolism have been described in order to outline that every reaction can cause a waterfall of metabolic events influencing the sensorial quality, the shelf-life and the bioactive compounds production of fermented foods.

The subjects of those events are LAB, indicating the importance of metabolism of these microorganisms in food. The cell physiology is crucial to ensure that cells are well suited to survival during downstream processes and that they exhibit high performances.

The production and exploitation of naturally adapted strains can be interesting for companies because of the absence of ethical and legal concerns. The adapted strains are not considered genetically modified microorganisms (GMOs) and therefore they can be applied in food processing without legal restrictions and, more important, without affecting the consumer perception, currently (in Europe) not ready to introduce in his diet foods produced with GMOs.

Individual stresses used in food processing and preservation may render probiotic LAB more resistant to further and different stresses, including those encountered in the human body, e.g. those encountered during gastro-intestinal passage (pH of the stomach, exposure to bile salts in small intestine etc.). A positive correlation has been recently observed between EPS production and resistance to bile salt and low pH stress in *Bifidobacterium* species isolated from breast milk and infant faeces [128].

This knowledge can open interesting perspectives to improve at the same time the performances of LAB, the quality of fermented food and the health-promoting properties of the LAB used.

Moreover, it will be interesting to identify the gastrointestinal tract also as a complex and dynamic system in which LAB need to adapt to adverse conditions, responding with metabolic shifts provided with interesting technological an healthy features.

The "omics" technologies could be particularly useful for identifying the mechanism leading to LAB stress responses. These approaches could also help to identify the mechanisms for cell fitness and stress adaptation that will be needed to develop more generic and science based technologies [7].

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8. References

[1] Poolman B, Ruhdal J, Gruss A (2011) LAB Physiology and energy metabolism. In: Ledeboer A, Hugenholtz J, Kok J, Konings W, Wouters J. Thirty years of research on lactic acid bacteria.Rotterdam: 24 Media Labs. pp. 77-101.

[2] Ledeboer A, Hugenholtz J, Kok J, Konings W, Wouters J (2011) In: Ledeboer A, Hugenholtz J, Kok J, Konings W, Wouters J. Thirty years of research on lactic acid bacteria.Rotterdam: 24 Media Labs. pp. v-vi.

[3] Marles-Wright J, Lewis R (2007) Stress response of bacteria. Curr. Opin. Struct. Biol. 17:755–760.

[4] Serrazanetti DI, Guerzoni ME, Corsetti A, Vogel RF (2009) Metabolic impact and potential exploitation of the stress reactions in lactobacilli. Food Microbiol. 26:700–711.

[5] Hüfner E, Britton RA, Roos S, Jonsson H, Hertel C (2008) Global transcriptional response of *Lactobacillus reuteri* to the sourdough environment. Syst. Appl. Microbiol. 31:323–338.

[6] Heller KJ (2001) Probiotic bacteria in fermented foods: product characteristics and starter organisms. Am. J. Clin. Nutr. 73:374S–379S.

[7] Lacroix C, Yildirim S (2007) Fermentation technologies for the production of probiotics with high viability and functionality. Curr. Opin. Biotechnol. 18:176–183.

[8] Serrazanetti DI (2009) Effects of acidic and osmotic stresses on flavor compounds and gene expression in *Lactobacillus sanfranciscensis*. Ph.D. thesis. University of Teramo, Teramo, Italy.

[9] Ndagijimana M, Vallicelli M, Cocconcelli PS, Cappa F, Patrignani F, Lanciotti R, Guerzoni ME (2006) Two 2[5H]-furanones as possible signaling molecules in *Lactobacillus helveticus*. Appl. Environ. Microbiol. 72:6053–6061.

[10] Vannini L, Ndagijimana M, Saracino P, Vernocchi P, Corsetti A, Vallicelli M, Cappa F, Cocconcelli PS, Guerzoni ME (2007) New signaling molecules in some Gram-positive and Gram-negative bacteria. Int. J. Food Microbiol. 120:25–33.

[11] Lanciotti R, Patrignani F, Iucci L, Saracino P, Guerzoni ME (2007) Potential of high-pressure homogenization in the control and enhancement of proteolytic and fermentative activities of some *Lactobacillus* species. Food Chem. 102:542–550.

[12] Guerzoni ME, Vernocchi P, Ndagijimana M, Gianotti A, Lanciotti R (2007) Generation of aroma compounds in sourdough: effects of stress exposure and lactobacilli–yeasts interactions. Food Microbiol. 24:139–148.

[13] Vernocchi P, Ndagijimana M, Serrazanetti DI, Gianotti A, Vallicelli M, Guerzoni ME (2008) Influence of starch addition and dough microstructure on fermentation aroma production by yeasts and lactobacilli. Food Chem. 108:1217–1225.

[14] Montanari C, Sado-Kamdem SL, Serrazanetti DI, Etoa FX, Guerzoni ME (2010) Synthesis of cyclopropane fatty acids in *Lactobacillus helveticus* and *Lactobacillus sanfranciscensis* and their cellular fatty acids changes following short term acid and cold stresses. Food Microbiol. 27:493–502.

[15] Serrazanetti DI, Ndagijimana M, Sado SL, Corsetti A, Vogel RF, Ehrmann M, Guerzoni ME (2011) Acid stress-mediated metabolic shift in *Lactobacillus sanfranciscensis* LSCE1. Appl .Environ. Microbiol. 77:2656-2666.

[16] Klaenhammer TR, Barrangou R, Buck BL, Azcarate-Peril MA, Altermann E (2005) Genomic features of lactic acid bacteria effecting bioprocessing and health. FEMS Microbiol. Rev. 29:393–409.

[17] van de Guchte M, Serror P, Chervaux C, Smokvina T, Ehrlich SD, Maguin E (2002) Stress responses in lactic acid bacteria. Antonie Leeuwenhoek. 82:187–216.

[18] Hörmann S, Scheyhing C, Behr J, Pavlovic M, Ehrmann M, Vogel RF (2006) Comparative proteome approach to characterize the high-pressure stress response of *Lactobacillus sanfranciscensis* DSM 20451[T]. Proteomics. 6:1878–1885.

[19] Guerzoni ME, Lanciotti R, Cocconcelli PS (2001) Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and thermal stresses in *Lactobacillus helveticus*. Microbiol. 147:2255–2264.

[20] Pavlovic M, Hörmann S, Vogel RF, Ehrmann MA (2008) Characterisation of a piezotolerant mutant of *Lactobacillus sanfranciscensis*. Z. Naturforsch. 63:791–797.

[21] Burns P, Patrignani F, Serrazanetti DI, Vinderola GC, Reinheimer JA, Lanciotti R, Guerzoni ME. (2008) Probiotic crescenza cheese containing *Lactobacillus casei* and *Lactobacillus acidophilus* manufactured with High Pressure-Homogenized Milk. J Dairy Sci. 91:500–512.

[22] Patrignani F, Burns P, Serrazanetti DI, Vinderola G, Reinheimer J, Lanciotti R, Guerzoni ME (2009) Suitability of high pressure-homogenized milk for the production of probiotic fermented milk containing *Lactobacillus paracasei* and Lactobacillus acidophilus. J. Dairy Res. 5:1-9.

[23] Gouesbert G, Jan G, Boyaval P (2002) Two-dimensional electrophoresis study of *Lactobacillus delbrueckii* subsp. *bulgaricus* thermotolerance. Appl. Environ. Microbiol. 68:1055–1063.

[24] Desmond C, Fitzgerald GF, Stanton C, Ross RP (2004) Improved stress tolerance of GroESL-overproducing *Lactococcus lactis* and probiotic *Lactobacillus paracasei* NFBC 338. Appl. Environ. Microbiol. 70:5929–5936.

[25] Corcoran BM, Ross RP, Fitzgerald GF, Dockery P, Stanton C (2006) Enhanced survival of GroESL-overproducing *Lactobacillus paracasei* NFBC 338 under stressful conditions induced by drying. Appl. Environ. Microbiol. 72:5104–5107.

[26] Di Cagno R, De Angelis M, Limitone A, Fox PF, Gobbetti M (2006) Response of *Lactobacillus helveticus* PR4 to heat stress during propagation in cheese whey with a gradient of decreasing temperatures. Appl. Environ. Microbiol. 72:4503–4514.

[27] Laplace JM, Sauvageot N, Harke A, Auffray Y (1999) Characterization of *Lactobacillus collinoides* response to heat, acid and ethanol treatments. Appl. Microbiol. Biotechnol. 51:659–663.

[28] Schmidt G, Hertel C, Hammes WP (1999) Molecular characterisation of the dnaK operon of *Lactobacillus sakei* LTH681. Syst. Appl. Microbiol. 22:321–328.

[29] Zink R, Walker C, Schmidt G, Elli M, Pridmore D, Reniero R (2000) Impact of multiple stress factors on the survival of dairy lactobacilli. Sci. Aliment. 20:119–126.

[30] Prasad J, McJarrow P, Gopal P (2003) Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. Appl. Environ. Microbiol. 69:917–925.

[31] De Angelis M, Gobbetti M (2004) Environmental stress responses in *Lactobacillus*: a review. Proteom. 4:106–122.

[32] Bucio A, Hartemink R, Schrama JW, Verreth J, Rombouts FM (2005) Survival of Lactobacillus plantarum 44a after spraying and drying in feed and during exposure to gastrointestinal tract fluids in vitro. J. Gen. Appl. Microbiol. 51:221–227.

[33] Castaldo C, Siciliano RA, Muscariello L, Marasco R, Sacco M (2006) CcpA affects expression of the groESL and dnaK operons in *Lactobacillus plantarum*. Microb. Cell Fact. 5:35.

[34] Gardiner GE, O'Sullivan E, Kelly J, Auty MAE, Fitzgerald GF, Collins JK, Ross RP, Stanton C (2000) Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Appl. Environ. Microbiol. 66:2605–2612.

[35] Earnshaw RG, Appleyard J, Hurst RM (1995) Understanding physical inactivation processes: combined preservation opportunities using heat, ultrasound and pressure. Int. J. Food Microbiol. 28:197–219.

[36] Teixeira P, Castro H, Mohacsi-Farkas C, Kirby RJ (1997) Identification of sites of injury in *Lactobacillus bulgaricus* during heat stress. Appl. Microbiol. 83:219–226.

[37] Hansen PJ, Drost M, Rivera RM, Paula-Lopes FF, AI-Katananit YM, Krininger CE, Chase CC (2001) Adverse impact of heat stress on embryo production: causes and strategies for mitigation. Theriogenol. 55:91–103.

[38] Kilstrup M, Jacobsen S, Hammer K, Vogensen FK (1997) Induction of heat shock proteins DnaK, GroEL, and GroES by salt stress in *Lactococcus lactis*. Appl. Environ. Microbiol. 63:1826–1837.

[39] KimWS, Dunn NW (1997) Identification of a cold shock gene in lactic acid bacteria and the effect of cold shock on cryotolerance. Curr. Microbiol. 35:59–63.

[40] KimWS, Khunajakr N, Dunn NW (1998) Effect of cold shock on protein synthesis and on cryotolerance of cells frozen for long periods in *Lactococcus lactis*. Cryobiol. 37:86–91.

[41] Wouters JA, Jeynov B, Rombouts FM, de Vos WM, Kuipers OP, Abee T (1999) Analysis of the role of 7 kDa cold-shock proteins of *Lactococcus lactis* MG1363 in cryoprotection. Microbiol. 145:3185–3194.

[42] Derzelle S, Hallet B, Francis KP, Ferain T, Delcour J, Hols P (2000) Changes in cspL, cspP, and cspC mRNA abundance as a function of cold shock and growth phase in *Lactobacillus plantarum*. J. Bacteriol. 182:5105–5113.

[43] Fonseca F, Béal C, Corrieu G (2001) Operating Conditions That Affect the Resistance of Lactic Acid Bacteria to Freezing and Frozen Storage. Cryobiol. 43:189–198.

[44] Zhang G, Fan M, Li Y, Wang P, Lv Q (2012) Effect of growth phase, protective agents, rehydration media and stress pretreatments on viability of *Oenococcus oeni* subjected to freeze-drying Afr. J Microbiol. Res. 6:1478-1484.

[45] Bâati L, Fabre-Gea C, Auriol D, Blanc PJ (2000) Study of the cryotolerance of *Lactobacillus acidophilus*: effect of culture and freezing conditions on the viability and cellular protein levels. Int. J. Food. Microb. 59:241–247.

[46] Panoff JM, Thammavongs B, Guéguen M. (2000) Cryotolerance and cold stress in lactic acid bacteria. Sci. Aliment. 20:105-110.

[47] Panoff JM, Thammavongs B, Laplace JM, Hartke A, Boutibonnes P, Auffray Y (1995) Cryotolerance and Cold Adaptation in *Lactococcus lactis* subsp. *lactis* IL1403. Cryobiol. 32:516–520.

[48] Grogan DW, Cronan JE (1986) Characterization of *Escherichia coli* mutants completely detective in synthesis of cyclopropane fatty acids. J. Bacteriol. 166:872.

[49] Grogan D, Cronan J (1997) Cyclopropane ring formation in membrane lipids of bacteria. Microbiol. Mol. Biol. Rev. 61:429-441.

[50] Raynaud S, Perrin R, Cocaign-Bousquet M, Loubiere P (2005) Metabolic and Transcriptomic Adaptation of *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* in Response to Autoacidification and Temperature Downshift in Skim Milk. Appl. Environ. Microbiol. 71:8016-8023.

[51] Wolf G, Arendt EK, Pfähler U, Hammes WP (1990) Heme-dependent and hemeindependent nitrite reduction by lactic acid bacteria results in different N-containing products. Int. J. Food Microbiol. 10:323–329.

[52] Duwat P, Cesselin B, Sourice S, Gruss A (2000) *Lactococcus lactis*, a bacteriamodel for stress responses and survival. Int. J. Food Microbiol. 55:83–86.

[53] Rochat T, Gratadoux JJ, Gruss A, Corthier G, Maguin E, Langella P, van de Guchte M (2006) Production of a heterologous nonheme catalase by *Lactobacillus casei*: an efficient tool for removal of H_2O_2 and protection of *Lactobacillus bulgaricus* from oxidative stress in milk. Appl. Environ. Microbiol. 72:5143–5149.

[54] Yamamoto Y, Poyart C, Trieu-Cuot P, Lamberet G, Gruss A, Gaudu P (2005) Respiration metabolism of Group B Streptococcus is activated by environmental haem and quinone and contributes to virulence. Mol. Microbiol. 56:525–534.

[55] Vido K, Diemer H, Van Dorsselaer A, Leize E, Juillard V, Gruss A, Gaudu P (2005) Roles of thioredoxin reductase during the aerobic life of *Lactococcus lactis*. J. Bacteriol. 187:601–610.

[56] Rezaïki L, Cesselin B, Yamamoto Y, Vido K, van West E, Gaudu P, Gruss A (2004) Respiration metabolism reduces oxidative and acid stress to improve long-term survival of *Lactococcus lactis*. Mol. Microbiol. 53:1331–1342.

[57] Lechardeur D, Cesselin B, Fernandez A, Lamberet G, Garrigues C, Pedersen M, Gaudu P, Gruss A (2011) Using heme as an energy boost for lactic acid bacteria. Curr. Opin. Biotechnol. 22:143-149.

[58] Brooijmans RJW, de Vos WM, Hugenholtz J (2009) *Lactobacillus plantarum* WCFS1 Electron Transport Chains. Appl. Environ. Microbiol. 75:3580-3585.

[59] Lushchak VI (2001) Oxidative stress and mechanisms of protection against it in bacteria. Biochem. Moscow. 66:476–489.

[60] Hertel C, Schmidt G, Fischer M, Oellers K, Hammes WP (1998) Oxygendependent regulation of the expression of the catalase gene katA of *Lactobacillus sakei* LTH677. Appl. Environ. Microbiol. 64:1359–1365.

[61] Miyoshi A, Rochat T, Gratadoux JJ, Le Loir Y, Costa Oliveira S, Langella P, Azavedo V (2003) Oxidative stress in *Lactococcus lactis*. Genet. Mol. Res. 2:348–359.

[62] Bruno-Bárcena JM, Andrus JM, Libby SL, Klaenhammer TR, Hassan HM (2004) Expression of a heterologous manganese superoxide dismutase gene in intestinal lactobacilli provides protection against hydrogen peroxide toxicity. Appl. Environ. Microbiol. 70:4702–4710.

[63] Jänsch A, Korakli M, Vogel RF, Ganzle MG (2007) Glutathione reductase from *Lactobacillus sanfranciscensis* DSM20451[T]: contribution to oxygen tolerance and thiol exchange reactions in wheat sourdoughs. Appl. Environ. Microbiol. 73:4469–4476.

[64] Rallu F, Gruss A, Ehrlich SD, Maguin E (2000) Acid- and multistress-resistant mutants of *Lactococcus lactis*: identification of intracellular stress signals. Mol. Microbiol. 35:517–528.

[65] Cotter PD, Hill C (2003) Surviving the acid test: responses of Gram-positive bacteria to low pH. Microbiol. Mol. Biol. Rev. 67:429–453.

[66] Pieterse B, Leer RJ, Schuren FHJ, van der Werf MJ (2005) Unravelling the multiple effects of lactic acid stress on *Lactobacillus plantarum* by transcription profiling. Microbiol. 151:3881–3894.

[67] Piuri M, Sanchez-Rivas C, Ruzal SM (2005) Cell wall modifications during osmotic stress in *Lactobacillus casei*. J. Appl. Microbiol. 98:84–95.

[68] Piuri M, Sanchez-Rivas C, Ruzal SM (2003) Adaptation to high salt in *Lactobacillus*: role of peptides and proteolytic enzymes. J. Appl. Microbiol. 95:372–379.

[69] Jordan S, Hutchings MI, Mascher T (2008) Cell envelope stress response in Gram-positive bacteria. FEMS Microbiol. Rev. 32:107–146.

[70] Kets EPW, Teunissen PJM, Bont JAM (1996) Effect of Compatible Solutes on Survival of Lactic Acid Bacteria Subjected to Drying. Appl. Environ. Microbiol. 62:259-261.

[71] Ward DE, van der Wejden CC, van der Merwe MJ, Westerhoff HV, Claiborne A, Snoep JL (2000) Branched-chain α-keto acid catabolism via the gene products of the bkd operon in *Enterococcus faecalis*: A new, secreted metabolite serving as a temporary redox sink. J Bacteriol. 182:3239–3246.

[72] Considine KM, Kelly AL, Fitzgerald GF, Hill C, Sleator RD (2008) High-pressure processing – effects on microbial food safety and food quality. FEMS Microbiol. Lett. 281:1–9.

[73] Jofré A, Champomier-Vergès M, Anglade P, Baraige F, Martín B, Garriga M, Zagorec M, Aymerich T (2007) Protein synthesis in lactic acid and pathogenic bacteria during recovery from a high pressure treatment. Res. Microbiol. 158:512–520.

[74] Tabanelli G (2011) Use of sub-lethal high pressure homogenization (HPH) treatments to enhance functional properties of Lactic acid bacteria probiotic strains. Ph.D. thesis. University of Bologna, Bologna, Italy.

[75] Chung HJ, Bang W, Drake MA (2006) Stess response of *Escherichia coli*. Compr Rev Food Sci F. 5:52-64

[76] Burns P, Vinderola G, Molinari F, Reinheimer J (2008) Suitability of whey and buttermilk for the growth and frozen storage of probiotic lactobacilli. Int. J. Dairy Technol. 61:156-164.

[77] Sieuwerts S, de Bok FAM, Hugenholtz J, van Hylckama Vlieg JET (2008) Unraveling Microbial Interactions in Food Fermentations: from Classical to Genomics Approaches. Appl. Environ. Microbiol. 74:4997-5007

[78] Caplice E, Fitzgerald GF (1999) Food fermentations: role of microorganisms in food production and preservation. Int. J. Microbiol. 50:131-149

[79] Di Cagno R, De Angelis M, Calasso M, Vincentini O, Vernocchi P, Ndagijimana M, De Vincenzi M, Dessì MR, Guerzoni ME, Gobbetti M (2010) Quorum sensing in sourdough *Lactobacillus plantarum* DC400: induction of plantaricin A (PlnA) under co-cultivation with other lactic acid bacteria and effect of PlnA on bacterial and Caco-2 cells. Proteomics. 10:2175-2190.

[80] Lorenz MC, Cutler NS, Heitman J (2000) Characterization of Alcohol-induced Filamentous Growth in *Saccharomyces cerevisiae*. Mol. Biol. Cell 11:183–199.

[81] Hansen PJ, Drost M, Rivera RM, Paula-Lopes FF, AI-Katananit YM, Krininger CE, Chase CC (2001) Adverse impact of heat stress on embryo production: causes and strategies for mitigation. Theriogenology. 55: 91–103.

[82] Giraffa G (2004) Studying the dynamics of microbial populations during food fermentation. FEMS Microbiol. Rev. 28:251-260.

[83] Johnson EA (2002) Microbial Adaptation and Survival in Foods. In: Microbial Stress Adaptation and Food Safety. Ed. A.E. Yousef and V.K Juneja. CRC Press

[84] Davidson PM (2001) Chemical preservatives and natural antimicrobial compounds, p. 593–627. In: Food Microbiology: Fundamentals and Frontiers, 2nd ed. M.P. Doyle.

[85] Hüfner E, Hertel C (2008) Improvement of raw sausage fermentation by stress-conditioning of the starter organism *Lactobacillus sakei*. Curr. Microbiol. 57:490-496.

[86] Hüfner E, Markieton T, Chaillou S, Crutz-Le Coq AM, Zagorec M, Hertel C (2007) Identification of *Lactobacillus sakei* genes induced during meat fermentation and their role in survival and growth. Appl. Environ. Microbiol. 73:2522-2531.

[87] Marceau A, Zagorec M, Chaillou S, Mera T, Champomier-Verges MC (2004) Evidence for involvement of at least six proteins in adaptation of *Lactobacillus sakei* to cold temperatures and addition of NaCl. Appl. Environ. Microbiol. 70:7260-7268.

[88] Kamdem S, Patrignani F, Guerzoni ME (2007) Shelf-life and safety characteristics of Italian Toscana traditional fresh sausage (Salsiccia) combining two commercial ready-to-use additives and spices. Food Control. 18: 421 - 429.

[89] Schneider M (1988) Zur mikrobiologie von sauerkraut bei der vergärung in verkaufsfertigen kleinbehältern. Dissertation Hohenheim University, Stuttgart.

[90] Pederson CS, Albury MN (1969) The sauerkraut fermentation. New York State Agricultural Experiment Station Technical Bulletin 824, Geneva, New York.

[91] Ruiz-Barba JL, Garrido-Fernàndez A, Jiménez-Diaz R (1991) Bactericidal action of oleuropein extracted from green olives against *Lactobacillus plantarum*. Let. Appl. Microbiol. 12:65-68.

[92] Ciafardini G, Marsilio V, Lanza B, Possi N (1994) Hydrolysis of oleuropein by *Lactobacillus plantarum* strains associated with olive fermentation. Appl. Environ. Microbiol. 60:4142-4147

[93] Lavermicocca P, Valerio F, Lonigro SL, De Angelis M, Morelli L, Callegari ML, Rizzello CG, Visconti A (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. 71:4233–4240.

[94] Ruiz-Barba JL, Jiménez-Diaz R (1995) Availability of essential B-group vitamins to *Lactobacillus plantarum* in green olive fermentation brines. Appl. Environ. Microbiol. 61:1294-1297.

[95] O'Sullivan E, Condon S (1997) Intracellular pH is a major factor in the induction of tolerance to acid and other stresses in *Lactococcus lactis*. Appl. Environ. Microbiol. 63:4210–4215.

[96] Sanders JW, Venema G, Kok J (1999) Environmental stress responses in *Lactococcus lactis*. FEMS Microbiol. Rev. 23:483–501.

[97] Guerzoni ME, Gianotti A, Serrazanetti DI (2011) Fermentation as a tool to improve healthy properties of bread". In V. R. Preedy, R. R. Watson, & V. B. Patel, (Eds.), Flour and breads and their fortification in health and disease prevention (pp.385-393). London, Burlington, San Diego: Academic Press, Elsevier.

[98] Gänzle MG, Schwab C (2009). Ecology of exopolysaccharide formation by lactic acid bacteria: sucrose utilisation, stress tolerance, and biofilm formation. In: Ulrich, M. (Ed.), Bacterial Polysaccharides – Current Innovation and Trends. Horizon Press.

[99] Welman AD, Maddox IS (2003) Exopolysaccharides from lactic acid bacteria: perspectives and challenges. Trends Biotechnol. 21:269-274

[100] Lim EM, Ehrlich SD, Maguin E (2000). Identification of stress-inducible proteins in *Lactobacillus delbrueckii* subsp. *bulgaricus*. Electrophoresis. 21:2557–2561.

[101] Streit F, Corrieu G, Beal C (2007) Acidification improves cryotolerance of *Lactobacillus delbrueckii* subsp. *bulgaricus* CFL1. J. Biotechnol. 128:659–667.

[102] Streit F, Delettre J, Corrieu G, Beal C (2008) Acid adaptation of *Lactobacillus delbrueckii* subsp. *bulgaricus* induces physiological responses at membrane and cytosolic levels that improves cryotolerance. J. Appl. Microbiol. 105:1071–1080.

[103] Liu SQ, Asmundson RV, Gopal PK, Holland R, Crow VL (1998) Influence of Reduced Water Activity on Lactose Metabolism by *Lactococcus lactis* subsp. *cremoris* at Different pH Values. Appl. Environ. Microbiol. 64:2111-2116

[104] Hartke A, Bouche S, Giard JC, Benachour A, Boutibonnes P, Auffray Y (1996) The lactic acid stress response of *Lactococcus lactis* subsp. *lactis*. Curr. Microbiol. 33:194–199.

[105] Broadbent JR, Lin C (1999). Effect of heat shock or cold shock treatment on the resistance of *Lactococcus lactis* to freezing and lyophilization. Cryobiology. 39: 88–102.

[106] Behr J, Gänzle MG, Vogel RF (2006) Characterization of a highly hop-resistant *Lactobacillus brevis* strain lacking hop transport. Appl. Environ. Microbiol. 72:6483-92.

[107] Atrih A, Rekhif N, Michel M, Lefebvre G (1993) Detection and characterization of a bacteriocin produced by *Lactobacillus plantarum* C19. Can. J. Microbiol. 39:1173-1179.

[108] Jiménez-Diaz R, Rios-Sànchez RM, Desmazeaud M, Ruiz-Barba JL, Piard JC (1993) Plantaricin S and T, two new bacteriocins produced by *Lactobacillus plantarum* LPCO10 isolated from a green olive fermentation. Appl. Environ. Microbiol. 59:1416-1424.

[109] Klaenhammer TR (1988) Bacteriocins of lactic acid bacteria. Biochim. 70:337-349.

[110] Neysens P, De Vuyst L (2005) Carbon dioxide stimulates the production of amylovorin L by *Lactobacillus amylovorus* DCE 471, while enhanced aeration causes biphasic kinetics of growth and bacteriocin production. Int. J. Food Microbiol. 105:191–202.

[111] Davidson PM, Harrison MA (2002) Microbial Adaptation to Stresses by Food Preservatives, in Microbial Stress Adaptation and Food Safety. Ed. A.E. Yousef and V.K Juneja. CRC Press

[112] Vrancken G, De Vuyst L, Rimaux T, Allemeersch J, Weckx S (2011) Adaptation of *Lactobacillus plantarum* IMDO 130201, a Wheat Sourdough Isolate, to Growth in Wheat Sourdough Simulation Medium at Different pH Values through Differential Gene Expression. Appl. Environ. Microbiol. 77:3406–3412

[113] Lebeer S, Vanderleyden J, De Keersmaecker SCJ (2008) Genes and Molecules of Lactobacilli Supporting Probiotic Action. Microbiol. Mol. Biol. R. 72:728–764

[114] Jobin MP, Garmyn D, Divies C, Guzzo J (1999) Expression of the *Oenococcus oeni* trxA gene is induced by hydrogen peroxide and heat shock. Microbiol. 145:1245-1251.

[115] Capozzi V, Arena MP, Crisetti E, Spano G, Fiocco D (2011) The hsp 16 Gene of the Probiotic *Lactobacillus acidophilus* Is Differently Regulated by Salt, High Temperature and Acidic Stresses, as Revealed by Reverse Transcription Quantitative PCR (qRT-PCR) Analysis. Int. J. Mol. Sci. 12:5390-5405.

[116] Pieterse B (2006) Transcriptome analysis of the lactic acid and NaCl-stress response of *Lactobacillus plantarum* Ph.D. thesis. Wageningen University, Wageningen, The Netherlands

[117] Petersohn A, Brigulla M, Haas S, Hoheisel JD, Volker U, Hecker M (2001) Global Analysis of the General Stress Response of *Bacillus subtilis*. J. Bacteriol. 183:5617-5631.

[118] Wang JC, Zhang WY, Zhong Z, Wei AB, Bao QH, Zhang Y, Sun TS, Postnikoff A, Meng H, Zhang HP (2012) Transcriptome analysis of probiotic *Lactobacillus casei* during fermentation in soymilk. J. Ind. Microbiol. Biotech. 39:191-206.

[119] Anandan S (2006) Genomic and Proteomic Approaches for Studying Bacterial Stress Responses. In: Advances in Microbial Food Safety. V. Juneja, J.P. Cherry and M.H Tunick eds. ACS Books.

[120] Renzone G, D'Aambrosio C, Arena S, Rullo R, Ledda L, Ferrara L, Scaloni A (2005) Differential proteomic analysis in the study of prokaryotes stress resistance. Ann. Ist. Super. Sanità. 41:459-468.

[121] Nezhad MH, Knight M, Britz ML (2012) Evidence of changes in cell surface proteins during growth of *Lactobacillus casei* under acidic conditions. Food. Sci. Biotech. 21:253-260.

[122] Garnier M, Matamoros S, Chevret D, Pilet MF, Leroi F, Tresse O (2010) Adaptation to Cold and Proteomic Responses of the Psychrotrophic Biopreservative *Lactococcus piscium* Strain CNCM I-4031. Appl. Environ. Microbiol. 76:8011–8018.

[123] Jardin J, Mollé D, Piot M, Lortal S, Gagnaire V (2012) Quantitative proteomic analysis of bacterial enzymes released in cheese during ripening. Int. J. Food Micro. 155:19–28.

[124] Gagnaire V, Piot M, Camier B, Vissers JPC, Gwenael J, Leonil J (2004) Survey of bacterial proteins released in cheese: a proteomic approach. Int. J. Food Microbiol. 94:185-201.

[125] Güell M, Yus E, Lluch-Senar M, Serrano L (2011) Bacterial transcriptomics: what is beyond the RNA horiz-ome? Nat. Rev. Microbiol. 9:658-669.

[126] Wu C, Zhang J, Wang M, Du G, Chen J (2012) *Lactobacillus casei* combats acid stress by maintaining cell membrane functionality. J. Ind. Microbiol. Biotechnol. Accepted

[127] Coda R, Rizzello CG, Pinto D, Gobbetti M (2012) Selected Lactic Acid Bacteria Synthesize Antioxidant Peptides during Sourdough Fermentation of Cereal Flours, Appl. Environ. Microbiol. 78:1087-1096.

[128] Glaasker E, Tjan FSB, Ter Steeg PF, Konings WN, Poolman B (1998) Physiological Response of *Lactobacillus plantarum* to Salt and Nonelectrolyte Stress. J. Bacteriol. 180:4718-4723.

[129] Mehmeti I, Faergestad EM, Bekker M, Snipen L, Nes IF, Holo H (2012) Growth Rate-Dependent Control in *Enterococcus faecalis*: Effects on the Transcriptome and Proteome, and Strong Regulation of Lactate Dehydrogenase. Appl. Environ. Microbiol. 78:170-176.

[130] Alcántara C, Zúñiga M (2012) Proteomic and transcriptomic analysis of the response to bile stress of *Lactobacillus casei* BL23. Microbiol. Accepted

[131] Wu C, Zhang J, Chen W, Wang M, Du G, Chen J (2011) A combined physiological and proteomic approach to reveal lactic-acid-induced alterations in *Lactobacillus casei* and its mutant with enhanced lactic acid tolerance. Appl. Microbiol. Biotechnol. 93:707-722.

[132] Jozefczuk S, Klie S, Catchpole G, Szymanski J, Cuadros-Inostroza A, Steinhauser D, Selbig J, Willmitzer L (2010) Metabolomic and transcriptomic stress response of *Escherichia coli*. Mol. Sys. Biol. 6:364.