Chapter 4

Listeria monocytogenes in Medical Research

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Abstract

Bacteria are known to produce compounds of high value such as secondary metabolites used in biotechnological applications. It is therefore worthwhile to think how to exploit a pathogenic bacterium, e.g., *Listeria monocytogenes*, to be an effective source of bioactive compound used in particular in medicinal purposes. *Listeria monocytogenes* is considered as an acute contaminated bacterium in foods and could be a causal agent of food-borne diseases. This bacterium is the causal agent of listeriosis, a grave disease, caused by eating contaminated food. Although, *L. monocytogenes* is a pathogenic microorganism that threatens the progress of food industry, it would be also a reservoir of secondary metabolites such as antibiotics and other metabolites of economic importance when appropriate strain improvement will be addressed. This section would discuss in brief the negative and positive features of *L. monocytogenes* as either a pathogenic bacterium or an important microorganism in medical research.

Keywords: productive strain, metabolic diseases, food-borne pathogens, valorization, biotechnological applications

1. Introduction

Listeria monocytogenes is a Gram-positive bacterium that is recognized as a facultative intracellular pathogen. An intracellular growth could therefore be observed among food and clinical strains of such bacterium [1].

L. monocytogenes is a well-known food-borne pathogen, which has been found in many fresh and processed foods, and it is widely distributed in nature. In fact, this organism is able to survive in extreme environments including elevated osmolarity, cold, and acid shocks. Understanding the key stress adaptation is important for a better control of *L. monocytogenes*

in food as well as in its resulted health diseases. Some authors have reported studies on the protein patterns expressed in response to salt shock in *L. monocytogenes* [2–4].

L. monocytogenes could enter the food chain and lead to severe disseminated infection, as listeriosis, and this feature is very likely due to its ability to survive in both reduced temperature and high-salt conditions [5].

L. monocytogenes can be transmitted to humans through ingestion of contaminated food, particularly ready-to-eat meat, seafood, and dairy products [6].

Listeria monocytogenes is therefore an interesting microorganism to be studied mainly in food and medical research.

The present study focuses on reviewing and describing some important features of *Listeria monocytogenes* from different published reports.

2. Adaptation to salt stress and protein patterns of *L. monocytogenes*

Listeria monocytogenes is able to tolerate salt stress. The mechanism of adaptation to increased salt concentration by this bacterium could be due to intracellular accumulation of compatible solutes. Indeed, the compatible solutes, such as carnitine and glycine betaine, protect the cell from deleterious effects of the external osmolarity and prevent water loss [7, 8].

Comparison of the salt-induced protein patterns of *L. monocytogenes* strain LO28 grown in a rich medium or in a chemically defined medium shows clear differences between these two media as reported by Duché et al. [2]. The later report revealed that the NaCl stress response of *L. monocytogenes* is a complex process. Indeed, there is synthesis of different proteins more or down-expressed in the presence of salt and either directly related or not to the salt stress response of *L. monocytogenes*. The protein pattern analysis revealed the synthesis of three proteins of the general metabolism (AckA, PdhD, and S6), which was modified after salt stress, but does not seem to be directly related to the salt stress response of *L. monocytogenes*. However, two proteins more expressed (GbuA and Ctc) in the presence of salt seem to be directly related with the response to salt stress of *L. monocytogenes*.

The report of Dussurget et al. [9] revealed the presence of an *L. monocytogenes*-specific putative gene encoding a bile salt hydrolase (BSH) and demonstrated that BSH is a novel PrfA-regulated *L. monocytogenes* virulence factor involved in the intestinal and hepatic phases of listeriosis [9].

Protein patterns of *L. monocytogenes* were also analyzed by proteomic analysis in comparison with mode of growth either in biofilm or in planktonic mode [10]. The results showed a significant variation of the protein patterns of *L. monocytogenes* between the two growth conditions. The study indicated in particular that the biofilm development is probably controlled by specific regulation of protein expression involved at various levels of cellular physiology [10].

3. Adaptation of *L. monocytogenes* to reduced temperature

Controlling *L. monocytogenes* in food has become a major preoccupation in the food industry and storage. For this end, many reports were investigated to determine the efficacy of natural drugs such as essential oils to inhibit the growth of such microorganism. In this regard, beef meat including plant essential oil such as that from lemon (*Citrus limon*) is an interesting target during refrigerated storage as contamination of beef meat by food spoilage and food-borne pathogens is considered one of the major problems to the progress of food industry. Indeed, the addition of such essential oil could substantially delay the growth of *L. monocytogenes* ATCC 19117 in raw minced beef meat under storage at 4°C [11].

Listeria monocytogenes is one of the most important psychrotrophic food pathogens, which is responsible for food-borne illnesses in particular listeriosis that has been known as one of the emerging zoonotic diseases nowadays [12, 13]. *L. monocytogenes* could be related to anaerobically packed cooked meat products and shelf-life failures of conserved foods.

Foods are exposed to contamination by several bacteria such as those reported as the causal agents of food-borne diseases, i.e., *Staphylococcus aureus*, *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* [14, 15].

The latter pathogen is the causal agent of listeriosis, a potential grave disease, and is often fatal in susceptible individuals, caused by eating contaminated food in particular with *Listeria monocytogenes* [16].

To prevent food contamination during the production, sale, and distribution and to extend the shelf-life time of raw and/or processed foods, additives should be used. However, the safety aspects of the synthetic additives could be paid attention, as these chemical preservatives are considered toxic and responsible for many carcinogenic and teratogenic attributes [17].

Natural products such as plants and herbs and naturally derived compounds are regarded as new alternatives to prevent the proliferation of pathogens, e.g., strains of *Listeria monocytogenes* in food [11, 18].

A particular interest has been focused on the potential application of plant essential oils such as those from lemon [11] and thyme [18] as safer additives for meat, in particular minced beef meat during refrigerated storage.

4. Some traits highlighted in *L. monocytogenes*

Food and clinical strains of *Listeria monocytogenes* were used in the report of Kanki et al. [1] to study specific alleles in particular the activities of listeriolysin O (LLO) and phospholipases PlcA and PlcB that are known to promote rupture of the phagocytic vacuole, besides initial intracellular bacterial growth in Caco-2 cells.

In fact, the aim of this report [1] is to investigate whether *Listeria monocytogenes* strains differ in their ability to escape from the primary phagosome after internalization into human intestinal epithelial cells.

The results showed differences among LLO and PrfA truncation mutants, but there are no differences in LLO activities between food and clinical strains of *Listeria monocytogenes* or among their serotypes. The same authors [1] concluded that LLO and PrfA mutants of *Listeria monocytogenes* exert a significant effect on intracellular growth, although it was unclear from this study whether PlcA and PlcB alleles affect escape from vacuoles.

Their study put down that low-virulence *L. monocytogenes* strains associated with escape ability from the primary vacuoles are not widely distributed among food strains [1].

Other reports of Camilli et al. [19] showed that the plcA gene of *Listeria monocytogenes* encoding a secreted phosphatidylinositol-specific phospholipase C (PI-PLC) plays important roles in pathogenesis.

L. monocytogenes is responsible for food-borne infections that can cause many health perturbations, e.g., septicemia and meningitis in immunocompromised individuals as well as stillbirth and miscarriage in pregnant women [6].

L. monocytogenes invades intestinal epithelial cells after passing through the stomach, via internalin A (InlA), which interacts with the E-cadherin receptor on the epithelial cell surface [20]. During invasion, *L. monocytogenes* cells become engulfed within a phagocytic vacuole constituting the primary phagosome. Phospholipases PlcA and PlcB, encoded by *plcA* and *plcB*, respectively, and listeriolysin O (LLO), encoded by *hly*, are responsible to promote rupture of the internalized vacuole and bacterial escape into the cytoplasm [21]. Indeed, LLO favors introduction of pores in the membrane of the phagosome, and phospholipases (PlcA and PlcB) help the damage of the membrane, leading the vacuoles to lyse [22].

The replication of *Listeria monocytogenes* occurs within the cytoplasm, and its motility is mediated by ActA that induces actin to polymerize forming actin comet tails around the bacteria. Upon reaching the plasma membrane, *Listeria monocytogenes* invades and internalizes into neighboring cells and disseminates the infection to other cells; such dissemination is called cell-to-cell spread [22]. When invading neighboring cells, *L. monocytogenes* could be localized in a double-membrane (secondary) vacuole, which can be lysed by LLO, PlcA, and PlcB [23]. Camargo et al. [22] denoted that the *Listeria* genes encoding virulence factors for intestinal translocation, namely, *hly*, *plcA*, *plcB*, *actA*, and their regulator *prfA*, reside in the *Listeria* pathogenicity island 1 (LIPI-1).

The virulence gene *inlA* requires activation of the PrfA, which is the virulence regulator for transcription. *L. monocytogenes* expresses the virulence factors and crosses the intestinal barrier to be then carried by the lymph or blood to the mesenteric lymph nodes, spleen, and liver [20].

Many mutant strains of *L. monocytogenes* are widely distributed among food strains especially those of truncated InlA [22]. In fact, mutants of *inlA* with nucleotide substitutions introduce that premature stop codons (PMSCs) express an inactive, truncated InlA.

PMSCs in *inlA* could be presented in isolates of serotypes 1/2a, 1/2b, and 1/2c, but not 4b, and *inlA* PMSCs are most commonly present in food isolates, with few detected in clinical isolates [24]. Orsi et al. [24] indicated that the heterogeneity among the serotypes of clinical isolates of *L. monocytogenes* (e.g., predominance of serotype 4b) may be explained by *inlA* PMSCs.

L. monocytogenes would comprise also hypervirulent and hypovirulent clones [25]. Except for *L. monocytogenes* hypovirulent food isolates with *inlA* PMSCs, attenuated *L. monocytogenes* strains could be isolated from food environments and harbored mutations in the genes encoding PrfA, InlB, PlcA, LLO, and ActA [26, 27].

In addition to being an important pathogen for humans and animals, *L. monocytogenes* is also being regarded and developed as a novel vaccine platform, in particular for tumor immunotherapy [28].

The infection of *L. monocytogenes* has naturally triggered robust CD8+ T-cell responses due essentially to its constitutive intracellular life cycle [29].

L. monocytogenes could be promising bacteria for immunotherapy platform, which could be illustrated by the >15 active or completed clinical trials when using attenuated *L. monocytogenes* for the treatment of a variety of cancers (http://clinicaltrials.gov).

The exact mechanisms by which *L. monocytogenes* triggers cell-mediated immunity remain unclear, and rigorous efforts are needed to further exploit *L. monocytogenes* as an effective agent for cancer therapy.

During infection, *L. monocytogenes* targets antigen-presenting cells, and it delivers antigens directly to the class I major histocompatibility complex (MHC) presentation pathway, due to its cytosolic localization. This bacterium is genetically tractable, facilitating pathogen attenuation for clinical safety as well as the ability to engineer the pathogen to express tumor antigens of interest [28].

There are two different *L. monocytogenes*-based immunotherapeutic platforms from Aduro BioTech [30] and Advaxis [31] indicating that cytosolic access is necessary for triggering cell-mediated immunity, while cell-to-cell spread of the pathogen is not, thereby ensuring vaccine safety [30, 31].

L. monocytogenes infection is a complex process that could affect the death pathways of different host cells, including programmed and non-programmed cell death.

The review of McDougal and Sauer [32] highlighted the mechanisms of modulation of cell death and its implications on *L. monocytogenes* acute infection, as well as the generation of adaptive immunity. Indeed, in this review [32], the influences of host cell death pathways were discussed, including necrosis and necroptosis, apoptosis, and inflammasome-mediated pyroptosis on both *L. monocytogenes* virulence and *L. monocytogenes*-induced immunity.

Thus, it is important to understand how cell death modulation during *L. monocytogenes* infection could lead to novel insight into therapeutic approaches for the treatment of infection and the development of vaccine strains as cancer immunotherapies. The role of *L. monocytogenes*

as a tumor immunotherapy platform would open other questions on how critical cell death pathways are that influence the priming and quality of cell-mediated immune responses [32].

Other reports from Buyck et al. [33] described the presentation, diagnosis, and treatment of *Listeria monocytogenes* sepsis in an older patient and presented a short literature review about listeriosis and the importance of safe food practices [33].

5. Current and future developments

The starting point for this chapter is the characterization of *Listeria monocytogenes* as a virulent organism and as a possible turn of such pathogen to be effective as preventive or curative agent such as vaccine.

As any other organisms/microorganisms, *Listeria monocytogenes* could follow a strategy to have an improved strain of such bacterium. For this end, screening for productive strains and strain improvement in biotechnological organisms of *Listeria monocytogenes* would be the focus of this part of the current chapter. *Listeria monocytogenes* could be beneficial bacteria in modern industrial microbiology and biotechnology, and the strategy used to achieve such purpose would be summarized below.

On the one hand, it is important to scratch for sources of microorganisms, e.g., strains of *Listeria monocytogenes* used in biotechnology. To achieve such goal, two important steps to be borne in mind are (a) literature search and culture collection supply and (b) isolation de novo of organisms producing metabolites of economic importance. Some general screening methods are described as three important points: (i) enrichment with the substrate utilized by the organism being sought, (ii) enrichment with toxic analogues of the substrate utilized by the organism being sought, and (iii) testing microbial metabolites for bioactive activity. The later point would involve such as:

- Testing for antimicrobial activity
- Testing for enzyme inhibition
- Testing for morphological changes in fungal test organisms
- Conducting animal tests on the microbial metabolites

On the other hand, it is now important to focus on strain improvement when the above steps were clearly understood and investigated.

It is also important to open a parenthesis talking about the fact that:

The ability of any organism, e.g., *Listeria monocytogenes*, to make any particular product, e.g., bioactive compounds, is based on its capability for the secretion of a particular set of enzymes. Enzymes are the key factors of organisms' life. Moreover, the production of the enzymes depends ultimately on the genetic makeup of the organisms. How to improve organism

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strains? The answer of this question could be put down with five condition procedures as follows:

1. Regulating the enzymes' activities secreted by the organism
2. Increasing the permeability of the organism in the case of searching metabolites secreted extracellularly, so that the microbial products can find this way more easily outside the cell
3. Selecting strains from natural variants
4. Modifying the existing genetic apparatus in a producing organism
5. Using recombinant DNA technology or genetic engineering

The two latter possible procedures, namely, modification of the existing genetic apparatus without the introduction of foreign DNA and introducing new genetic properties into the organism being sought by using foreign DNA, will be discussed below in general for any industrial organism.

The manipulation of the genome of industrial organisms for the purpose of strain improvement may be done either by manipulation not involving foreign DNA or that involving foreign DNA.

On the one hand, the general procedure of the genome manipulations not involving foreign DNA or bases could be predicated on conventional mutation. Indeed, the nature of conventional mutation can be originated from physical agents with ionizing radiations and/or ultraviolet light, besides chemical mutagens.

These chemical mutagens, for instance, may be divided into three groups:

i. Chemical mutagens that act on DNA of resting or nondividing organisms (chemicals acting on resting DNA) such as nitrous acid, alkylating agents, NTG (nitrosoguanidine), and nitrogen mustards
ii. DNA analogues which may be incorporated into DNA during replication (base analogues)
iii. Chemical mutagens that cause frameshift mutations (also known as intercalating agents)

Whatever mutagens are used (physical or chemical agents), the choice of either mutagen should satisfy the final effective purpose, and it is also important to bear in mind the practical isolation of mutants. For this end, some general related practices should be taken appropriately such as exposing organisms to the mutagen, selection for mutants, and screening.

Moreover, the isolation of auxotrophic mutants can be of great importance in modern industrial microbiology and biotechnology as these mutants are frequently used in industries for the production, for instance, of essential amino acids. We can note that in contrast to the wild-type or prototrophic organisms that possess all the enzymes needed to synthesize all growth requirements, auxotrophic mutants are those which lack the enzymes to manufacture certain required nutrients; consequently, such nutrients must therefore be added to the growth medium.

On the other hand, the general procedure of strain improvement methods involving foreign DNA or bases could be predicated on many genetic-changing methods such as transduction, transformation, conjugation, parasexual recombination, protoplast fusion, site-directed mutation, and metabolic engineering.

6. Conclusion

In summary, this chapter highlighted primarily that *Listeria monocytogenes* is a well-known pathogenic bacterium particularly to humans and animals causing severe infections especially those from food-borne such as listeriosis. In fact, *L. monocytogenes* could enter the food chain and lead to food-borne illness even at refrigerated temperatures. The pervasiveness of this food spoilage microorganism is due, in part, to its ability to tolerate environments including reduced temperatures, elevated osmolarity, and acid shocks. Consequently, an adequate surveillance system for safe food practices, handling, and storage needs to be established to control *L. monocytogenes* and, thus, should be taken into consideration for healthier world.

Although *L. monocytogenes* is a pathogenic microorganism that threatens the progress of food industry, it would be also a reservoir of secondary metabolites such as antibiotics and other metabolites of economic importance when appropriate strain improvement is addressed. Furthermore, understanding the mechanism of action of *L. monocytogenes* with regard to the infection as well as the immunity could provide critical insights into novel therapeutics for the treatment of infection, as well as the development of vaccine strains as tumor immunotherapies. Thus, it could also refine our use of pathogenic microbes such as *L. monocytogenes* as beneficial microorganisms used in vaccines and cancer immunotherapy.

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