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Research review paper

Microorganisms under high pressure — Adaptation, growth and biotechnological potential

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ABSTRACT

Hydrostatic pressure is a well-known physical parameter which is now considered an important variable of life, since organisms have the ability to adapt to pressure changes, by the development of resistance against this variable. In the past decades a huge interest in high hydrostatic pressure (HHP) technology is increasingly emerging among food and biosciences researchers. Microbial specific stress responses to HHP are currently being investigated, through the evaluation of pressure effects on biomolecules, cell structure, metabolic behavior, growth and viability. The knowledge development in this field allows a better comprehension of pressure resistance mechanisms acquired at sub-lethal pressures. In addition, new applications of HHP could arise from these studies, particularly in what concerns to biotechnology. For instance, the modulation of microbial metabolic pathways, as a response to different pressure conditions, may lead to the production of novel compounds with potential biotechnological and industrial applications. Considering pressure as an extreme life condition, this review intends to present the main findings so far reported in the scientific literature, focusing on microorganisms with the ability to withstand and to grow in high pressure conditions, whether they have innated or acquired resistance, and show the potential of the application of HHP technology for microbial biotechnology.

Contents

1. Introduction

Hydrostatic pressure is a key physical parameter in the biosphere, ranging from 0.1 MPa (atmospheric pressure) at sea level to more than 110 MPa in ocean depths. The ability to adapt to pressure changes is a characteristic of life and it has influenced the evolution and distribution of both microorganisms and macroorganisms (Bartlett, 2002; Somero, 1990; Yayanos, 1986).

High hydrostatic pressure (HHP) is an emerging technology, traditionally employed in ceramics, steel and super alloy production, extrusion, and synthetic materials (Hoover et al., 1989). In the last 2 decades a main and increasingly successful industrial application of HHP is the

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non-thermal pasteurization of foods, which intends to extend the shelf-life of food products, without substantial modification of its nutritional, functional and organoleptic properties. In the past years, several new applications of HHP have emerged, along with the boost of information and knowledge in this field, generated by researchers of many different scientific disciplines. This widespread approach reflected in the wealth of the equipment, which now ranges from laboratory and industrial scale HHP generators to systems that are able to invade the HHP biosphere, such as submersibles used to sample piezophilic organisms in the deep sea (Aertsen et al., 2009). While laboratory scale generators have vessels with sample volume as low as 8 mL and a maximum achievable pressure of approximately 800 MPa (Aertsen et al., 2009), the vessels of industrial scale equipment can reach a volume of 420 L and a hydrostatic pressure intensity of 600 MPa (Hiperbaric — High Pressure Processing, 2012). As the most HHP practical applications are related to the exposition of biological systems to pressure (which may aim or not its destruction), the unique effects of this treatment are currently being investigated at different levels, ranging from biopolymers, enzymes and viruses to microorganisms, mammalian cells and tissues (Aertsen et al., 2009; Bartlett, 2002; Knorr et al., 2006). In a general approach, all pressure effects are a consequence of the volume decrease, which causes several structural modifications and disturbs the equilibrium of chemical reactions (Mentre et al., 1999). Therefore, as a result of the HHP treatment, organisms will perform specific stress responses in an attempt to adapt and survive. However, depending on the treatment intensity, pressure may cause disruption the of cell structure and, in consequence the organism destruction. Inactivation of microorganisms by lethal levels of HHP is already well studied and understood, mainly in the context of preservation and safety of foods (as the industrial use of HHP at room temperature or under refrigeration for non-thermal pasteurization) (Norton and Sun, 2008; Ramirez et al., 2009; Rastogi et al., 2007; Sousa et al., forthcoming). The latter reference (a review) includes information about three of the main world HHP industrial equipment builders, as well as a summary of the main landmarks on HHP pasteurization of foods and its commercialization, since its start in the early nineties in Japan to the recent entrance in this business sector of some multinational companies.

In the last years, the use of the variable pressure has raised interest for several types of applications in biotechnology, lato sensu speaking, as e.g., to modify the properties of macromolecules like cellulose (Figueiredo et al., 2010) and food proteins (Correia et al., 2011; Knorr et al., 2006), carry out efficient protein disaggregation, unfolding and refolding (Ferrão-Gonzales et al., 2000; Foguel et al., 2003), modulate physiological processes (Saraiva and Rodrigues, 2011), and enhance enzyme activity in the presence of ionic liquids (Salvador et al., 2010). In addition, HHP has been investigated as an efficient physical tool to modify food polymers, such as starches (Knorr et al., 2006). The gelatinization of starch under pressure has significant differences from heat gelatinization, bringing novel and different characteristics and functionalities to pressure gelatinized starch (Doona et al., 2006).

HHP has also been used in the context of vaccine development, since pressure causes inactivation of viruses and other infectious agents, preserving better its immunogenic properties (Silva et al., 2004). This technique was already applied in the development of vaccines for some infectious agents, such as yellow fever's virus (called 17DD virus) (Gaspar et al., 2008) and food-and-mouth disease's virus (called FMDV) (Ishimaru et al., 2004).

The concept of sub-lethal HHP is gaining relevance and, in what concerns to microorganisms, piezophiles and piezotolerant are now seen as having considerable potential use in biotechnology, but the difficulties in the cultivation of these organisms have limited their application (Simonato et al., 2006). These extremophiles may not only serve as a source of HHP resistant enzymes but also provide important knowledge regarding the structural adaptations necessary to withstand HHP (Abe and Horikoshi, 2001; Aertsen et al., 2009; Kawano et al., 2004). However it is important to note that innate piezophiles/piezotolerants are not the only types of microorganisms with capacity to withstand high pressure, since many microbial strains are documented to develop resistance against this parameter, while the wild type strain did not display this characteristic. These resistant strains may show a great interest in several areas, due to the possible acquisition of new desirable characteristics, obtained by reduction or even suppression of some metabolic pathways or utilization of new ones caused by pressure. The study and development of this subject can bring numerous interesting industrial applications (Aertsen et al., 2009; Hörmann et al., 2006). This paper intends to review the main scientific findings so far reported in literature on microorganisms that acquired resistance to pressure and microorganisms' growth under pressure, envisaging new possible biotechnological applications. It is important to highlight that this paper does not review the pressure resistance mechanisms of dormant bacterial spores, which are completely different from those observed in vegetative cells.

2. Pressure effects

Mesophilic microorganisms and piezophiles are different in terms of HHP stress response. Whereas piezophiles from deep sea are adapted to these pressures, mesophilic microorganisms aren't normally exposed to high pressure and their growth is sensitive to these conditions (Bidle and Bartlett, 2001; Nakasone et al., 2002). High pressure exerts many effects on living organisms, affecting not only cell structural organization but also its metabolic processes, which makes it difficult to pinpoint pressure effects in cell growth and viability (Bartlett, 2002). Table 1 presents the key pressure sensitive processes described for *Escherichia coli* in literature.

With increasing pressure, all the important cell functions are successively compromised and it turns impossible to withstand and survive at these hostile conditions, leading to loss of cell viability, at pressures around 200 MPa, in the case of *E. coli*. However, it is noteworthy that some authors have isolated piezotolerant strains of *E. coli* (after pressure shock treatments) capable of growing at much higher pressure values. These studies are described in this paper in Section 4 — "Mutants with Acquired Resistance to HHP".

Other authors (Park et al., 2003) found that the leakage of UV-absorbing compounds (from *Staphylococcus aureus*, *Bacillus subtilis*, *Fusarium oxysporum*, and *Fusarium sporotrichioides*) increases with pressure increment, being that the effect is more evident for fungi than for bacteria, with these results indicating different levels of cell disruption.

In general, all pressure effects arise from a single influence, which corresponds to the volume reduction of the biological system, favoring the acquisition of more compact structural forms. Besides the structural alterations in biomolecules, pressure also disturbs the equilibrium of (bio)chemical reactions (Mentre et al., 1999).

Concerning the HHP effects on lipid membranes, it is known that these structures are particularly pressure sensitive (above certain thresholds depending on the microorganisms' nature), because of its high compressible potential (Winter and Jeworrek, 2009). In general,

Table 1
Pressure sensitive processes detected in *E. coli* (Oger and Jebbar, 2010).

Process	Pressure abolishing process (MPa)	References
Motility	10	Meganathan and Marquis (1973)
Substrate transport	26	Landau (1967)
Cell division	20–50	ZoBell (1970), Zobell and Cobet (1962, 1964)
Growth	50	Yayanos and Pollard (1969)
DNA replication	50	Yayanos and Pollard (1969)
Translation	60	Gross et al. (2005), Yayanos and Pollard (1969)
Transcription	77	Yayanos and Pollard (1969)
Viability	200	Pagán and Mackey (2000)

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