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Review

The effects of sublethal doses of essential oils and their constituents on antimicrobial susceptibility and antibiotic resistance among food-related bacteria: A review

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ABSTRACT

Background: Essential oils (EOs) and their individual constituents (ICs) have demonstrated strong and wide-spectrum antimicrobial activities against food-related bacteria. Frequently, the concentrations of EOs or ICs required to inhibit bacteria in foods are higher than those that are organoleptically acceptable, and their use at sublethal concentrations in combination with other preservation technologies has been proposed.

Scope and approach: Bacteria have the capacity to develop physiological responses to sublethal growth conditions, which can lead to decreased susceptibility toward the same or other stressing agents, including resistance to antibiotics. Despite this knowledge, studies regarding the efficacy of EOs and ICs have mostly focused on doses that inactivate target bacteria in laboratorial media or in real foods, while less is known about the implications of their use at sublethal concentrations on bacterial susceptibility to antimicrobials, physical processes and antibiotic resistance. This review presents the available studies focused on the effects of the exposure of food-related bacteria to EOs or ICs at sublethal concentrations on alterations in susceptibility to homologous or heterologous stressing agents, including antibiotic-resistance, and related physiological responses in bacteria experiencing these stresses.

Key findings and conclusions: The findings of the available studies show that changes in susceptibility to antimicrobials and physical procedures as well as in antibiotic resistance vary with the target bacterium and the applied stress condition. Moreover, the observed stress-induced physiological responses have been varied. Notably, for the majority of the studies, the exposure of food-related bacteria to these stresses has not resulted in relevant changes in the sensitivity of the bacteria to antimicrobials, physical processes or antibiotics, which indicate that changes in bacterial sensitivity are not a major problem when considering the use of EOs or ICs in food preservation.

1. Introduction

In food processing, it is important that proper measures are taken to ensure the safety and stability of products for the duration of their shelf life. However, modern food consumer trends have made reaching these goals much more difficult. Consumers require high quality, preservative-free and safe but mildly processed foods with an extended shelf life (Brull & Coote, 1999; Burt, 2004).

The existing concerns regarding food processing have compelled researchers and industry to consider the development of new and effective food preservation technologies that may be applied in combination or in sequential steps (the Hurdle principle; Leistner, 1978) using sublethal treatments to provide a hostile environment for microbial survival and growth (Abee & Wouters, 1999; Barry-Ryan, 2015; Delves-Broughton, 2012). Such technologies must have broad-spectrum antimicrobial activity and a low risk of inducing decreased bacterial sensitivity due to their continuous and prolonged use, as well as a low toxicity to consumers and the environment (Luz, Gomes Neto, Tavares, Magnani, & de Souza, 2012a; Pazhani et al., 2004). To this end, one possibility has been the use of essential oils (EOs) and the compounds found therein as alternative antimicrobial food preservatives. EOs and their individual constituents (ICs) are considered naturally occurring food antimicrobials, together with other compounds that

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originate from microbial, plant and animal sources (Davidson & Harrison, 2002; Davidson, 2001).

Essential oil is the product obtained from natural raw materials of plant origin (e.g., flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) by steam distillation, mechanical processing of the epicarp of citrus fruits or dry distillation after the separation of the aqueous phase (if present) by physical processes (ISO 9235:2013). Antimicrobial properties of EOs are primarily associated with their functions against microorganisms in naturally occurring plant defense systems (Maldonado, Schieber, & Gänzle, 2015). The main reported locations and mechanisms of the antibacterial action of EOs and their ICs are the following: membrane destabilization and disruption (Lambert, Skandamis, Coote, & Nychas, 2001; Gomes Neto et al., 2015); inhibition of membrane-localized metabolic events (Cox, Mann, & Markham, 2001; Picone et al., 2013); damage to membrane proteins (Ultee, Kets, Alberda, Hoekstra, & Smid, 2000); depletion of proton motive force (Ultee, Kets, & Smid, 2001); and leakage of cytoplasmic constituents, metabolites and ions (Gustafson et al., 1998; Azerêdo, Stamford, de Figueiredo, & de Souza, 2012.

The available literature regarding the efficacy of EOs and ICs has chiefly reported on the efficacy of specific doses required to inactivate cells comprising single or mixed microbial populations in laboratorial media, food-mimicking media or food matrices (Calo, Crandall, O'Bryan, & Ricke, 2015; Chueca, Pagán, & Garcia-Gonzalo, 2014; Espina, Gelaw, de Lamo-Castellvi, Pagán, & Garcia-Gonzalo, 2013; Evrendilek, 2015; Prakash, Kedia, Mishra, & Dubey, 2015). These findings have reinforced the potential use of EOs or ICs in food preservation due to their capacity to inhibit a wide variety of pathogenic and/or food-spoiling bacteria.

Notably, the concentrations of EOs or ICs required (when applied alone) to substantially inhibit bacterial growth in foods are often higher than the concentrations that are organoleptically acceptable (Azerêdo et al., 2011; Gutierrez, Barry-Ryan, & Bourke, 2009). Thus, the use of sublethal concentrations of EOs or ICs in combination or in sequential application with other antimicrobial methods has been proposed to provide a balance between sensory acceptability and antimicrobial efficacy (Azerêdo et al., 2011; Gutierrez et al., 2009; Souza, Barros, Oliveira, & da Conceição, 2010). However, information regarding the possible effects of using EOs or ICs at sublethal concentrations on microbial sensitivity to antimicrobials or physical processes is still scarce, and most of the studies do not use combined strategies to inactivate bacteria. While EOs, as food antimicrobials, are primarily effective because they interfere with microbial homeostasis and growth/survival by imposing stress on microbial cells, one must consider that bacterial cell responses to sublethal stresses may result in decreased sensitivity toward the same stressor and even to other apparently unrelated stressors (Bikels-Goshen, Landau, Saguy, & Shapira, 2010; Gould, 1996; Russel, 2003). Bacteria challenged with sublethal stresses may undergo significant physiological changes that may enhance their ability to survive the imposed hostile conditions, and regardless of the specific mechanism associated with the mounted adaptive response, genetic regulatory modifications are commonly involved (Davidson & Harrison, 2002; Doyle et al., 2006).

The concern with stress responses in bacteria induced by food antimicrobials has been continued because of the (i) increasing incidence of bacteria resistant to clinically relevant antibiotics; (ii) increasing reliance on antimicrobials as a primary strategy to control pathogens in foods; (iii) evidence that decreased sensitivity to food antimicrobials, biocides and other food preservation techniques may be induced by exposure of bacteria to certain sublethal stresses (Davidson & Harrison, 2002; Doyle et al., 2006); and (iv) evidence that sublethal stress-related responses may induce increased virulence and lower infectious doses in bacteria (Samelis, Ikeda, & Sofos, 2003; Stopforth et al., 2005).

A number of studies have shown that bacterial cells challenged with sublethal stressing conditions can develop decreased sensitivity to a variety of classical antimicrobials or physical processes used to preserve foods, including organic acids (Chang & Cronan Junior, 1999), heat (Álvarez - Ordóñez, Fernández, López, Arenas, & Bernardo, 2008; Jørgensen, Hansen, & Knøchel, 1999; Skandamis, Yoon, Stpforth, Kendall, & Sofos, 2008), low temperatures (Evans, McClure, Gould, & Russell, 1998), salt (Guerzoni, Lanciotti, & Cocconcelli, 2001), gamma irradiation (Ayari, Dussault, Millette, Hamdi, & Lacroix, 2009) and biocides (Joynson, Forbes, & Lambert, 2002; Loughlin, Jones, & Lambert, 2002). Therefore, caution should be exercised in light of the potential for bacterial responses after continuous exposure to sublethal amounts of EOs or ICs. Along with antimicrobial efficacy, concern for the risk associated with the use of such an antimicrobial should include the ability of the bacteria to become less sensitive to it and even to other antimicrobials, physical processes or clinically relevant antibiotics (Aiello & Larson, 2003; Gomes Neto et al., 2015; Luz et al., 2013).

In this context, this review presents the findings of available studies on (1) the effects of exposure to sublethal concentrations of EOs or ICs on changes in sensitivity of food-related bacteria to antimicrobials and physical processes used in foods as well as to antibiotics and (2) the stress-related physiological responses in bacterial cells when exposed to these conditions. In this study, a sublethal concentration is considered the concentration of an EO or IC in growth media that is not high enough to kill/inactivate the target bacterial cells but may exert inhibitory effects on outgrowth with changes in growth kinetics (Andersson & Hurghes, 2014).

Discussion of changes in bacterial responses to antimicrobial compounds/substances, physical processes or antibiotics, by necessity, should include a description of the terms of antimicrobial tolerance and antibiotic resistance from a functional perspective. Bacterial tolerance to antimicrobials or to physical processes is a consequence of physicochemical or environmental stressing conditions that affect the antimicrobial-induced killing effects, and often does not affect the ability of the antimicrobial to interact with its target (Bayles, 2007; Russel, 2002, 2003), and is normally a transient (reversible physiological state) and non-heritable phenotype. Bacterial-mediated antibiotic resistance is a heritable trait that occurs by mutation (intrinsic resistance) or horizontal gene transfer (acquired resistance) and includes mutations at the drug target site, enzymatic degradation, uncoupling of antimicrobial agent-target interaction, chromosome-mediated drug efflux and/or overexpression of target proteins (Aiello & Larson, 2003; Bayles, 2007; Russel, 2002; Sheldon, 2005). To date, there is not enough evidence to affirm surely that mechanisms involved in decreased sensitivity of bacterial cells that experience sublethal stresses imposed by EOs or ICs could be similar to those producing increased antimicrobial tolerance or antibiotic resistance.

Therefore, to better understand the changes that occur in bacterial cells following exposure to sublethal concentrations of EOs or ICs presented in this review, the term "increased/decreased resistance" is applied only when the evaluated response refers to alterations in inhibitory effects of antibiotics, while "increased/decreased susceptibility" is applied to alterations in response to inhibitory effects of EOs, ICs, biocides or physical processes. Furthermore, in the current absence of meaningful MIC cutoff points (as they exist to antibiotics) for assessing bacterial sensitivity to EOs or ICs, the term "reduced or increased susceptibility" may be more appropriate when discussing bacterial sensitivity to these substances, as previously applied to some biocides (Aiello, Larson, & Levy, 2007).