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Artigo

Natural alternatives for reducing bacterial biofilms in food industry

Alternativas naturais para redução de biofilmes bacterianos em indústria de alimentos

Rodolfo Nunes Almeida[1], Priscila Renata Costa[1], Juliana Aparecida Santos de Oliveira[1], Marcondes Batista Ferreira[1], Otávio Augusto Martins[2], Fernanda Raghiante[1*]

Summary: Of the microorganisms often found in biofilms, bacteria are the predominant group. In the food industry, pathogenic biofilms considered of great importance in the context of food safety and public health, arousing the interest of researches, highlighting the use of essential oils due to their antimicrobial properties, with the purpose of making sanitizers that have less impact compared to chemical sanitizers that may eventually transfer chemical waste to food. The aim of this review was to present the process of forming bacterial biofilms in the food industry and to suggest some natural alternatives for its reduction. It is concluded that biofilm formation is natural and the search for alternative ways to reduce the chemical impact contaminating the environment and food, leads to natural solutions. This demonstrate the ability to act against various microorganisms and can be incorporated into sanitizers, detergents and infinite other possibilities.

Index terms: Food safety. Essential oil. Food microbiology.

Resumo: Dos micro-organismos frequentemente encontrados em biofilmes, as bactérias são o grupo predominante. Em indústria de alimentos, os biofilmes patogênicos são considerados de grande importância no contexto de segurança dos alimentos e saúde pública, despertando o interesse de pesquisas, em destaque para a utilização de óleos essenciais, devido às suas propriedades antimicrobianas, com a finalidade de elaborar sanitizantes naturais que provocam menor impacto se comparados aos sanitizantes que podem eventualmente transferir resíduos químicos aos alimentos. E diante disso, por meio de revisão, este trabalho teve como objetivo apresentar o processo de formação de biofilmes bacterianos em indústrias de alimentos e sugerir algumas alternativas naturais de sua redução. Conclui-se que a formação de biofilme é natural e a busca por formas alternativas de diminuir o impacto químico contaminante ao ambiente e aos alimentos conduzem a soluções naturais que demonstram capacidade de atuação frente à variados micro-organismos, podendo ser incorporados à sanitizantes, detergentes e infinitas outras possibilidades.

Termos para indexação: Segurança Alimentar. Óleos essenciais. Microbiologia de alimentos.

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* Autor correspondente. E-mail: fernanda.raghiante@iftm.edu.br
[1]Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, IFTM- *Campus* Uberlândia, Minas Gerais, Brasil.
[2] Universidade Estadual Paulista – UNESP - *Campus* Botucatu, São Paulo, Brasil.

Introduction

The ability to adapt to environmental stresses and metabolic diversity are fundamental characteristics of microorganisms, and of these, bacteria are the predominant group found in biofilm (JAY 2005). Bacterial biofilms are generally defined as bacterial cell aggregates attached to a surface and coated by a matrix of self-produced polymers (TREMBLAY et al. 2014). The replication rate, high reproduction rates, high adaptability and production of extracellular substances and structures, are the main characteristics of organisms with high biofilm production capacity (NITSCHKE & COSTA 2007).

Survival by proliferation and chronicity are allowed by bacterial existence in two basic states of life: as planktonic cells - also called free-living cells, important for the rapid proliferation and propagation of microorganisms into new territories, or as sessile cells - Also titled as biofilm, which characterize chronicity (TRENTIN et al. 2013).

The formation of biofilms causes phenotypic alterations of planktonic cells, which can be portrayed as strategies for microorganism survival in environments with adverse conditions (SAUER 2002; OLIVEIRA et al. 2010). In natural environments, 95 % to 99 % of microorganisms exist in the form of biofilms, and can be found in almost all substrates that have sufficient moisture and nutrients to support their growth (PENG et al. 2002).

The ability of numerous microorganisms to adhere, colonize and form biofilms on large varieties of contact surfaces of materials used in food processing plants (Lima et al. 2015) are of great concern to the food industry because of their potential in Resistant to antimicrobial and sanitizing treatments, as well as causing deterioration, loss of quality or spread of pathogens through cross-contamination (KASNOWSKI et al. 2010).

The aim of this review was to present the process of forming bacterial biofilms in the food industry and to suggest some natural alternatives for its reduction.

Methodology

The research methodology of this review is based on scientific books and articles. The scientific articles were acquired from the sites:

(a) www.pubmed.com;
(b) www.highwire.stanford.edu;
(c) www.scholar.google.com;
(d) www.scielo.br.

Discussion

Biofilm Development

The biofilm development can be divided into at least 4 stages, as observed in Figure 1: I) reversible binding; II) irreversible binding; III) maturation; IV) detachment, where the cells that detach themselves from the biofilm return to the planktonic growth mode, then closing the biofilm development cycle.

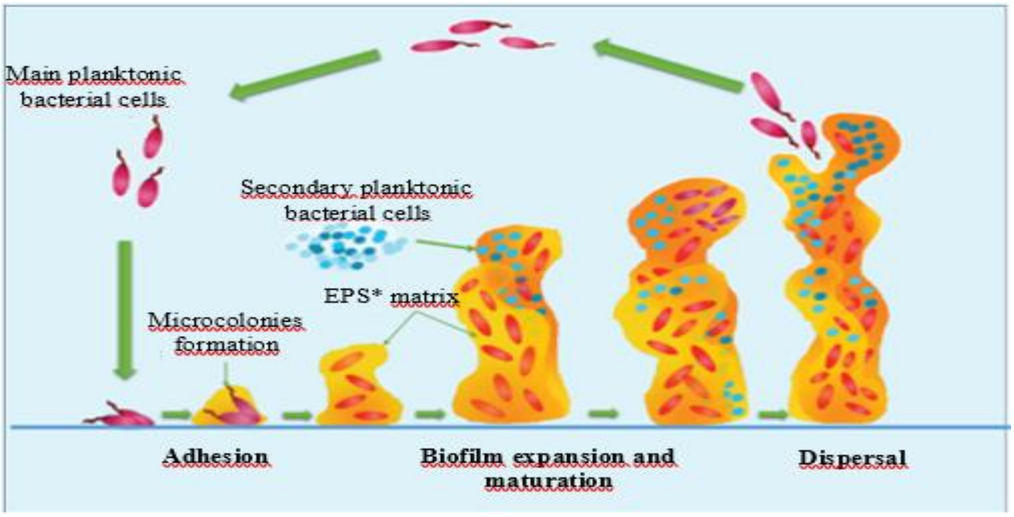

Figure 1 - Schematization of the development of biofilms. The adhesion phase corresponds to the stages of reversible binding and irreversible binding; The phase of expansion and maturation of the biofilm corresponds to the stage of maturation and the dispersion phase corresponds to the stage of detachment.
*Exopolysaccharide.
Source: Adapted from Tremblay et al. (2014).

However, at each stage of the biofilm, bacterial cells are physiologically distinct from cells in other stages because these processes are not necessarily synchronized in any biofilm, but are generally localized, so that at any moment a small area of the surface may contain biofilm at each stage of development and maturation (STOODLEY et al. 2002).

I. Reversible Connection

The complex process of bacterial adhesion, whether on an abiotic surface (inanimate surfaces such as plastics and metals) or biotic (animal or plant cells and tissues) is the first stage in the formation of biofilms (Dunne 2002).

As a general rule, primary adhesion (or reversible adhesion) between bacteria and abiotic surfaces is mediated by non-specific physico-chemical interactions, whereas adhesion to biotic surfaces is accompanied by molecular interactions mediated by specific binding of the receptor and ligand type To the antigen and antibody process (TRENTIN et al. 2013).

On abiotic surfaces, the initial attraction occurs randomly between planktonic bacterial cells and the surface, through Brownian motion and gravitational force, or, in a directed way, via chemotaxis and motility, through flasks and pili (O'Toole & Kolter 1998), During reversible adhesion, bacteria still exhibit Brownian motions and are easily removed by the application of minimal forces (OLIVEIRA et al. 2010).

The reversible adhesion stage is generally characterized by non-specific long-range physico-chemical interactions between the bacterium and the material, including hydrodynamic forces, electrostatic interactions, Van der Waals forces and hydrophobic interactions (DUNNE 2002).

II. Irreversible connection

The irreversible adhesion results from anchoring appendices (pili, flagellum, adhesin protein secretion) (Oliveira et al. 2010) and/or the production of extracellular polymeric substances - also known as exopolysaccharide matrix, EPS or glycocalyx - (Jay 2005), making the connections between the cells and the surface stronger (Figure 2).

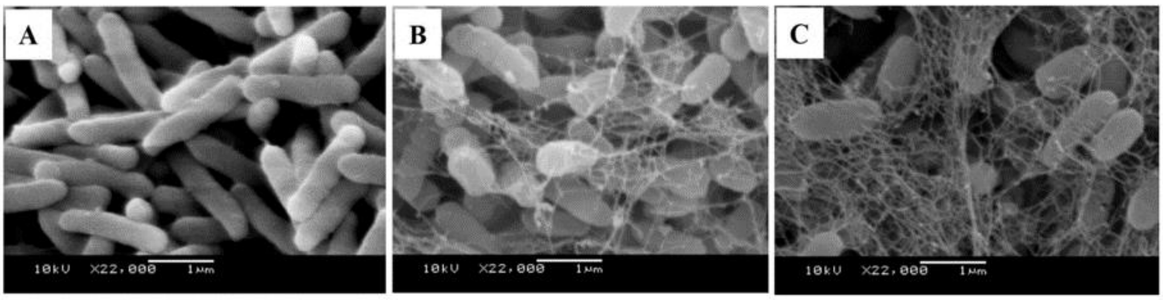

Figure 2 - Scanning electron microscopy images demonstrating the adhesion process (A) and biofilm formation with the progressive production of the exopolysaccharide matrix (B and C) in magnification of 22,000X.
Source: Trentin et al. (2013).

The fusion between appendages of the bacterium and the surface substrate may involve specific interactions, hydrogen bonds, hydrophobic interactions and covalent and ionic bonds, being difficult to remove due to the application of strong mechanical force or chemical interruption of the adhesion forces by the application of enzymes, detergents, surfactants, sanitizers or heat (OLIVEIRA et al. 2010).

III. Maturation

Biofilms are primarily formed by bacterial populations included in a matrix of exopolysaccharides originating from a microbial secretion (EPS) adhered to one another or to surfaces (Jay 2005; Costerton et al. 1999), whose cells express genes in a pattern (Sauer et al. 2002, p. 1140) that differ profoundly from that in planktonic life, among which are proteins involved in metabolism, translation, membrane transport and / or secretion and gene regulation (STOODLEY et al. 2002).

Biofilms contain, in addition to sessile microorganisms, particles of proteins, lipids, phospholipids, carbohydrates, minerals and vitamins, among others, forming deposits where the primary colonizing microorganisms continue to grow. An association with several other primarily planktonic microorganisms, evolving into a polymicrobial biofilm (JAY 2005; MACÊDO 2000).

Sutherland (2001) points out that water is the most significant fraction in the composition of the biofilm matrix, reaching 97 %, whereas microorganisms represent only 2 to 5%, although they excrete polymeric substances predominant in the organic matter of the dry mass of the biofilm, responsible for the morphology, structure, cohesion and functional integrity of the same. Although polysaccharides predominate in their composition, the matrix can also consist of proteins, such as glycoproteins, phospholipids and nucleic acids. In general, mature biofilms appear morphologically as mushroom-like structures, surrounded by the exopolysaccharide matrix (EPS), permeated by water channels that function as a primitive circulatory system for delivery of nutrients into the biofilm and removal of (TRENTIN et al. 2013; STOODLEY et al. 2002).

IV. Detachment

In general, upon reaching a certain critical mass and dynamic equilibrium, the outermost layers of the biofilm initiate the release of rapidly dispersing planktonic cells, colonizing new surfaces and organizing new biofilms elsewhere (KASNOWSKI et al. 2010).

Three types of dispersion processes may occur: expansive - when cells of a microcolony undergo lysis and resume motility, and are then released from the structure; Fragmentation - where fragments of extracellular matrix containing microorganisms are released; And superficial - occurs by the growth of the biofilm itself (Macêdo 2000).

Within a biofilm community, microorganisms are capable of share nutrients and are protected from harmful environmental factors such as desiccation, antibiotics, antifungal and host immune systems - in biotic biofilms (Tortora et al. 2012). While Ronner & Wong (1993) considered as a biofilm a cell count of 10^3 to 10^5 per cm^2, already Andrade et al. (1998) considered as biofilm the minimum number of 10^7 cells adhered per cm^2 to the surface.

Biofilm and Food Industry

The occurrence of flaws in sanitation procedures in food industries can cause food residues to stick to equipment and surfaces (Araújo et al. 2013). There are different strategies for combating biofilm formation: preventing the initial adhesion of the microorganism, preventing microbial growth, inhibiting the polymer matrix synthesis or degrading the matrix (TREMBLAY et al. 2014).

The process of periodic sanitation of equipment in the industries includes cleaning and sanitizing, through the application of various chemical products, aiming to eliminate microorganisms, organic residues and minerals adhered to the surface, preventing planktonic cells from multiplying and forming biofilms (BARROS et al. 2015).

The use of methods that reduce or eliminate surface microorganisms from equipment on which raw and processed food may come in contact, include physical methods such as handwashing and high pressure sprays as well as chemical methods such as the use of hypochlorites, iodophors and quaternary ammonium compounds (Araújo et al. 2013). These microorganisms are more resistant to antimicrobial agents than planktonic microorganisms, and can persist and survive even after a variety of sanitization processes, representing a source of food contamination and food poisoning (OLIVEIRA et al. 2010).

In general, in food processing plants, cleaning with detergents precedes sanitization, because the presence of organic matter reduces the effectiveness of the sanitizing agent, and it should be noted that dead spaces, joints, valves, seals and surfaces corroded by Time of use are the most opportune areas for the development of biofilms in the processing line (ARAÚJO et al. 2013).

Pathogenic biofilms in the food industry are considered of great importance in the context of food safety and public health, arousing the interest of research, highlighting the use of essential oils extracted from medicinal plants due to their antimicrobial properties, with the purpose of making sanitizers natural and biological agents to control microbial growth, as an alternative to the traditional use of synthetic agents, detergents and sanitizers that cause negative impacts to the environment and may eventually transfer chemical residues to food (LIMA et al. 2015).

Bara & Vanetti (1998) tested the antibacterial activity of medicinal plants, aromatics and natural dyes against strains of *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Listeria monocytogenes* and *Yersinia enterocolitica*, where Rosemary (*Lippia sidoides*) presented growth inhibitory activity in 100 % against *Salmonella* Typhimurium, *S. aureus*, *L. monocytogenes* and *Y. enterocolitica*; *Curcuma longa* at concentrations of 1 % and 2 %, showed a 40.74 % effect on the growth of *S. aureus* and *L. monocytogenes* strains, whereas in the presence of Carmin extracted from Conchonilla (*Coccos cacti*) at 1 % concentration, the growth of *Salmonella* Typhimurium was the most inhibited.

Silveira et al. (2005) investigated the antimicrobial activity of extracts of Guariroba (*Syagrus oleracea*) and Buriti (*Mauritia vinifera*) fruits against ATCC strains of Gram positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). S. *oleracea* extracts showed inhibition of Gram negative strains (*P. aerugionosa* and *E. coli*), but it was not able to significantly inhibit the *E. faecalis* strain. In contrast, extracts of *M. vinifera* were highly inhibitory to *P. aeruginosa* and *S. aureus*, but were not able to significantly inhibit strains of *E. coli* and *E. faecalis*.

Wiest et al. (2009) studied the in vitro inhibition and inactivation of *Salmonella* spp. with extracts of plants with ethnographic indicative medicinal or seasoning using 86 plants with indications of effects on microorganisms. Compared to *Salmonella* spp., 8 samples showed the highest inhibitory activity: Leek-porro (*Allium porrum*), Alho-nira (*Allium tuberosum*), Macela (*Achyrocline satureoides*), Chilli pepper (*Capsicum frutescens*), Yerba mate (*Ilex paraguariensis*), Oregano (*Origanum applii*), Sage (*Salvia officinalis*) and Chinchilla (*Tagetes minuta*).

Schuh et al. (2016) associated the lemon grass extract (*Cymbopogon flexuosus*) at the concentration of 0.78 % in the preparation of a detergent solution applied in a mixed biofilm of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, where the *P. aeruginosa* strain proved to be more resistant to the treatment with the detergent solution of essential oil. However, there was a significant reduction of *S. aureus* cells and reduction of 55.43 % of organic material, which demonstrated that the solution was effective in minimizing the adhesion of the association of biofilm forming bacteria.

Final Considerations

Biofilm formation is natural, common among the various species of microorganisms, in great prominence to bacteria, as a form of chronicity. It is a concern incorporated into the routine of the food industry, which, in order to reduce or even eradicate the contamination of its processing plants using chemicals, can eventually contaminate the food that passes through it. Research into alternative ways to reduce the chemical impact of contamination on the environment and food leads to natural solutions, isolated from plants, condiments, dyes and other products originated from nature, which demonstrate the ability to act against various microorganisms, and can be incorporated into sanitizers, detergents and endless other possibilities.

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