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Review

The current knowledge on the application of anti-biofilm enzymes in the food industry

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ABSTRACT

Biofilms are encountered on nearly all wet surfaces, with their development being often unwanted due to the serious problems they can cause in different fields, including in the food sector. They are recognized as the preferential microbial lifestyle due to the numerous advantages for the embedded cells. Biofilm cells are highly resistant to stress conditions, particularly to antimicrobials, as their complex and compact structure hampers the penetration of antimicrobials and the access to the deep positioned cells. The increased resistance to the currently employed control strategies emphasizes the urgent need of new alternative and/or complementary eradication approaches. To this direction, the use of enzymes is an interesting alternative anti-biofilm approach due to their capability to degrade crucial components of the biofilm matrix, cause cell lysis, promote biofilm disruption and interrupt the cell-to-cell signaling events governing biofilm formation and maintenance. This review provides an overview of the enzymes used for biofilm control, their targets and examples of effective applications.

Contents

1. Introduction

Biofilms are microbial communities attached to either biotic or abiotic surfaces and embedded in a self-produced hydrated polymeric matrix (Cos, Toté, Horemans, & Maes, 2010; Costerton, Lewandowski, Caldwell, Korber, & Lappin-Scott, 1995; Simões, 2011; Stoodley, Sauer, Davies, & Costerton, 2002). This sessile state represents an outstanding survival strategy for microorganisms, as it protects them against various environmental stresses (e.g. starvation, dehydration) and antimicrobial agents (e.g. antibiotics and biocides) (Costerton et al., 1995; Mah &

Abbreviations: AHLs, Acyl homoserine lactones; AI-2, Autoinducer-2; AIPs, Autoinducing peptides; AIs, Autoinducers; CFU, Colony forming units; DNase, Deoxyribonuclease; eDNA, Extracellular DNA; EPS, Extracellular polymeric substances; QS, Quorum sensing; SS, Stainless steel; US, Ultrasounds.

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O'Toole, 2001). Although biofilm formation may play an important advantageous role in many processes (e.g. biodegradation of environmental pollutants, plant growth promotion, maintenance of the microbial balance within the human body), it can also cause significant problems in clinical setting and several industries (Bridier et al., 2015; Donlan, 2002; Giaouris et al., 2014; Percival, Malic, Cruz, & Williams, 2011). In fact, biofilms are responsible for persistent human infections, dissemination of pathogens, product contamination, obstruction and corrosion of metallic pipes, decrease of heat transfer efficiency, increase of fluid frictional resistance and other equipment damages, which represent a significant economic and public health concern (Beech, 2004; Cloete, Jacobs, & Brözel, 1998; Gilbert, McBain, & Rickard, 2003; Shi & Zhu, 2009). The biofilm resistance and the consequent failure of the conventional methods to eradicate biofilm-enclosed microorganisms can be explained by: (i) the physicochemical diffusion barrier generated by the presence of an extracellular polymeric matrix; (ii) an altered microbial metabolic state (reduced growth rate/dormant state) in part due to nutrient/oxygen limitation; (iii) the expression of specific resistance genes; and (iv) the differentiation of cells into phenotypic variants less susceptible to treatments (e.g. presence of persister cells) (Anderson & O'Toole, 2008; Gilbert et al., 2003; Stewart, 2002).

In the food industry, aggressive chemicals, such as sodium hydroxide or sodium hypochlorite, together with clean-in-place techniques are often used to mitigate undesirable biofilm effects. However, such approaches are not always effective for biofilm control, particularly with respect to the inactivation of the inner cell layers of these aggregates and their removal from the surfaces. At the same time, the chemicals used for biofilm control can corrode materials and machinery, endanger users and negatively impact the environment (Gilbert et al., 2003). Among the newly developed biofilm prevention and control approaches are the ones focusing on the intrinsic cellular processes involved in biofilm establishment and maturation, such as motility, cell-to-cell aggregation, production of extracellular polymeric substances (EPS) and intercellular communication (quorum sensing, QS) (Cegelski et al., 2009; Huang & Stewart, 1999; Landini, Antoniani, Burgess, & Nijland, 2010). Therefore, a relevant strategy for removing biofilms from industrial systems is to employ enzymes. Indeed, these have been used for the treatment of biofilms formed in food areas (Anand, Singh, Avadhanula, & Marka, 2014; Lequette, Boels, Clarisse, & Faille, 2010).

2. Anti-biofilm enzymes

Enzymes are natural catalysts capable of accelerating chemical reactions without being consumed (Shanmugam & Sathishkumar, 2009). Undoubtedly, the cellular metabolism depends on these proteins and even minor molecular modifications can have vital metabolic consequences, affecting the complexity of the network of chemical reactions (Cabral, Gama, & Aires-Barros, 2003). Several factors can interfere with the activity and specificity of enzymes, such as temperature, pH, substrate, presence and/or absence of activators, co-factors or inhibitors (Cabral et al., 2003; Copeland, 2000). The possible applications of these biological molecules are endless, including their use in the industries of foods and beverages, detergents, drugs, textiles, pulp, paper and animal feed (Bajpai, 1999; Kirk, Borchert, & Fuglsang, 2002). Enzymes can be classified in six main classes: i) oxidoreductases (e.g. alcohol dehydrogenase, glucose oxidase, heme oxygenase, catalase, dihydrofolate reductase, phenylalanine hydroxylase, etc) that catalyse redox reactions and transfer oxygen or hydrogen atoms; ii) transferases (e.g. lipid kinase, transaldolase, phosphomutase, acyl-, methyl-, glucosyl-, phosphoryl-, transferase, etc) that allow the transfer of an atom or a group of atoms from one molecule to another; iii) hydrolases (e.g. serine protease, pectinesterase, glycosylase, pyrophosphatase, aminopeptidase, oligoribonuclease, etc) that catalyse hydrolytic reactions; iv) lyases (e.g. pyruvate decarboxylase, hydratase, aldolase, synthase, etc) that catalyse reactions by removing an atom or a group of atoms; v) isomerases (e.g. isomerase, epimerase and racemase) that catalyse reactions of rearrangement in a molecule; and vi) ligases or synthetases (e.g. synthetase and carboxylase) that can join two molecules together with a covalent bond (Aehle, 2004; Cabral et al., 2003; Shen & Chou, 2007).

The use of enzymes as anti-biofilm agents has increased in recent years (Taraszkiewicz, Fila, Grinholc, & Nakonieczna, 2013; Thallinger, Prasetyo, Nyanhongo, & Guebitz, 2013) with their application been successful in biofilm removal from industrial surfaces. Several applications have been described (Table 1) in an effort to reduce the problems associated to the presence of biofilms and to substitute the harmful and ineffective chemical biocides, thereby providing a greener alternative (Cortés, Consuegra, Sinisterra, & Mendez-Vilas, 2011; Srey, Jahid, & Ha, 2013). The application of enzymes for the cleaning of the food contact surfaces is approved by the regulatory agencies (Schmidt, 1997) and there is no evidence related to the interference of the enzymatic treatments with the food quality. Indeed, provided the surfaces are properly rinsed there is no possibility of food contamination or the risk for an enzyme to be considered an additional illegal additive (Troller, 1993).

2.1. Mode of action

The target of biofilm-disrupting enzymes is usually the EPS matrix surrounding the cells (Lequette et al., 2010; Xavier, Picioreanu, Rani, van Loosdrecht, & Stewart, 2005). However, their mode of action can greatly vary. Enzymes can: i) attack directly the biofilm components and degrade them; ii) induce cellular lysis; iii) interfere with the QS system; iv) or even catalyse the formation of antimicrobials (Augustin, Ali-Vehmas, & Atroshi, 2004; Cordeiro & Werner, 2011; Donlan, 2002; Simões, Simões, & Vieira, 2010; Thallinger et al., 2013). The action of enzymes is intrinsically related to the decrease of biofilm physical integrity, degrading matrix molecules into monomers that can be transported through the cell and further metabolized (Molobela, Cloete, & Beukes, 2010). As enzymes can act on the biofilm EPS, the structural components of this matrix should be ideally identified before any enzymatic application (Molobela et al., 2010). Carbohydrates, polysaccharides, proteins (frequently exhibiting amyloid-like properties), glycoproteins, lipids, phospholipids, glycolipids, and nucleic acids are usually identified as components of the EPS matrix (Branda, Vik, Friedman, & Kolter, 2005; Flemming & Wingender, 2010; Hobley, Harkins, MacPhee, & Stanley-Wall, 2015). The matrix composition and architecture is dependent on a number of extrinsic factors, including fluctuations in nutrient and gaseous levels and fluid shear (Simões et al., 2010). Moreover, a range of complex enzymatic and regulatory activities can be found within the matrix (Allison, 2003; Sutherland, 1999).

By using enzymes, the in-use biocides can be either replaced or their concentration can be significantly reduced since the enzymatic action on the EPS matrix favours the access of the chemicals to the cells (Cortés et al., 2011; Lequette et al., 2010; Srey et al., 2013). Given that biofilms can have heterogeneous composition, diverse types of enzymes are required to combat them and usually a mixture of enzymes should be applied, or combined with complementary treatments (Augustin et al., 2004; Kumar & Anand, 1998; Thallinger et al., 2013). There are four types of enzymes of particular interest for biofilm removal: anti-QS enzymes, oxidative enzymes (Thallinger et al., 2013), polysaccharide-degrading enzymes and proteolytic enzymes, (Johansen, Falholt, & Gram, 1997; Thallinger et al., 2013). These four types of enzymes belong to three of the main classes mentioned before: hydrolases, oxidoreductases and lyases (Fig. 1).

2.2. Anti-quorum sensing enzymes

The close proximity of cells in biofilms and the spatio-chemical conditions enables bacterial coexistence and the retaining matrix provides optimal conditions for QS phenomenon (Giaouris et al., 2015; Li & Tian, 2012). QS is a form of intercellular communication used by many species of bacteria in response to an increase in cell density. This complex gene regulatory system relies on the production, release and