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REVIEW PAPER

Fungal-bacterial biofilms: their development for novel biotechnological applications

Gamini Seneviratne · J. S. Zavahir · W. M. M. S. Bandara · M. L. M. A. W. Weerasekara

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Abstract The attachment of microbes on biotic or abiotic surfaces to form biofilm structures has a great impact on biodegradation and biosynthesis in nature. Various interactions in such biofilms and their extracellular polymeric substances (EPS) layer make them considerably different in physiology and action, compared to that of their individual microbes in planktonic (free swimming) mode of growth. Expression of new genes is up-regulated in the biofilm cells, due in part to the cellular interactions, compared with the planktonic cells. Formation of fungal-bacterial biofilms (FBB) by bacterial colonization on biotic fungal surface gives the biofilm enhanced metabolic activities compared to monocultures, and perhaps multi-species bacterial or fungal biofilms on abiotic surfaces. Incorporation of a N_2-fixing rhizobial strain to the FBB to form fungal-rhizobial biofilms (FRB) has been shown to improve potential biofilm applications in N-deficient settings and in the production of biofilmed inocula for biofertilizers and biocontrol in plants. Their applications in agricultural and environmental settings, enzyme technology, drug discovery studies and energy research are being investigated. Thus, it has already been shown that the use of the FBB is a promising technology for many applications. This review deals with the different areas in which FBB/FRB have been seen to be applied with successful results as well as the numerous emerging avenues in which they show promising potential.

Keywords Fungal-bacterial biofilms · Fungal-rhizobial biofilms · Rhizobial biofilms · Biofilms · Biotechnology

Introduction

A great attention has been focused on the role of microbes in the degradation and synthesis, known as biodegradation and biosynthesis (Dobbins et al. 1992; Oppermann-Sanio and Steinbüchel 2002) in lieu of physical and chemical methods. These processes occur in association with biotic or abiotic surfaces. Certain microbes can attach to the surfaces and differentiate to form complex, multi-cellular communities called biofilms. A biofilm consists of microbial cells (algal, fungal, bacterial and/or other microbial) and an extracellular biopolymer these cells produce, known as EPS, which provides structure and protection to the community. These communities can be found in medical, industrial and natural environments. They can also be engineered in vitro for various biotechnological applications (Seneviratne 2003).

The microbes undergo profound changes during their transition from planktonic organisms to cells that are part of a complex, surface-attached biofilm. Recent genetic and molecular approaches used to study bacterial biofilms have identified genes and regulatory circuits important for initial cell-surface interactions, biofilm maturation, and the return of biofilm microorganisms to a planktonic mode of growth (O'Toole et al. 2000). Biofilms have a unique pattern of gene expression which is different from their non-biofilm-forming stages (Vilain and Brözel 2006). For example, expression of genes for polysaccharide production is up-regulated in biofilm cells compared with planktonic cells in liquid media (Davies et al. 1993). Then, high cellular levels of cyclic di-GMP are produced, which promote

G. Seneviratne (✉) · J. S. Zavahir · W. M. M. S. Bandara · M. L. M. A. W. Weerasekara
Biological Nitrogen Fixation Project, Institute of Fundamental Studies, Hantana Road, Kandy 20400, Sri Lanka
e-mail: gaminis@ifs.ac.lk

increased synthesis of the EPS, and hence biofilm formation and aggregative behavior (Dow et al. 2007). Its low cellular levels promote motility. Thus, the biofilms are considerably different in physiology and action, compared to that of their individual microbes in the planktonic mode of growth.

Biotechnological applications of bacterial or fungal biofilms

Biofilms encompass a spectrum of applications in the medical setting, mainly categorized as causing infections and diseases in humans, often occurring from contamination of devices from urinary catheters to microscopes (Costerton et al. 1999). Studies on *Candida* biofilms have shown an improvement in drug resistance as a result of highly specific, surface-induced gene expression (Baillie and Douglas 2000), which provide the means to design novel therapies for biofilm-based infections (Chandra et al. 2001) and to control their formation on implanted devices such as indwelling catheters or prosthetic heart valves (Douglas 2002). Apart from medical applications, biofilms have tremendous practical importance in environmental settings. The microbes attached to particles of contaminated soils and aquatic sediments help degrade soil-bound contaminants occurring from chemical releases into the environment. By mimicking this, biofilm reactors have been designed to promote microbial growths that are effective for treating environmental wastes such as sewage, industrial waste streams or contaminated groundwater (Chen and Chen 2000; Soares et al. 2003; Goel et al. 2003). Biofilms can also be used to produce a wide variety of biochemicals that are then purified and utilized for public utility, including medicines, food additives or chemical additives for cleaning products. Biofilm cellulolytic enzyme activity and productivity have been evaluated, being up to 40 and 55%, respectively higher than that attained by planktonic cultures, in the production of cellulases by *Aspergillus niger* (Villena and Gutiérrez-Correa 2003). In this manner the biofilms have potential application in various spheres of enzyme technology. Production of anti-microbial compounds, using microbial biofilms attached to bioreactor surfaces has been studied (Yan et al. 2003). The significance of biofilms on food or food contact surfaces and their ability to protect food-borne pathogens from environmental stresses has been reported (Trachoo 2003). Biological corrosion has been decreased by using beneficial biofilms through the inhibition of bacteria which corrode metals (Zuo and Wood 2004). *Saccharomyces cerevisiae* forms biofilms in packed bed continuous bioreactors to produce ethanol from molasses (Tyagi and Ghose 1982; Demirci et al. 1997). Thus, the biofilms can be beneficially employed for a variety of biotechnological applications.

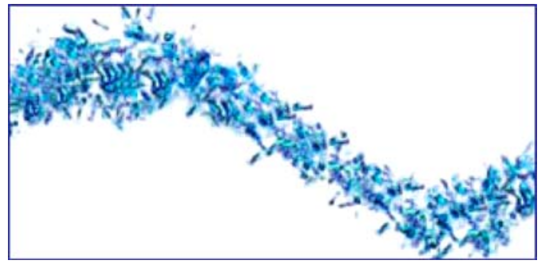

Fig. 1 A phase-contrast microscopic view of a fungal filament attached by bacterial cells forming a Fungal-Bacterial Biofilm (FBB). Magnification: 2,000×

Fungal-bacterial biofilms

The FBB differ from purely bacterial or fungal biofilms, since the fungi act as the biotic surface to which the bacteria adhere (Fig. 1). In the case of non-filamentous fungi, both bacteria and fungi can act as the biotic surface. In their attachment, cell aggregation and competitive inhibition for attachment sites take place in the biofilm (Wargo and Hogan 2006). It is also reported that the surface polysaccharides play an important role in the colonization of bacterial biofilms by non-filamentous fungi (e.g. *Candida albicans*) and vice-versa (Wargo and Hogan 2006). The physical interactions within the biofilms are key factors in their enhanced performance, compared to those attached to abiotic surfaces. These biofilms have been observed to have better growth and colonization abilities than their monocultures. For example, in an industrial flowing water system, interactions between some fungi and bacteria showed significantly higher rates of colonization and growth over single cultures in a complex seven-species model community (Elvers et al. 1998). De Boer et al. (2005) showed that new bacterial niches in the soil were created in the presence of fungi, due to the bacterial consumption of fungal exudates by attaching to the fungal surface. Such cooperation for metabolic products was also reported for two bacteria in a biofilm (Christensen et al. 2002). Thus, the interactions between microbes are important for metabolic cooperation among them. These interactions also offer greater protection to the biofilms' constituent microbes, particularly for the bacterial species, where they were seen to be less susceptible to biocide treatment than planktonic cells of the same organism (Elvers et al. 2002).

Biotechnological potential of fungal-bacterial biofilms

Numerous recent studies have shown a promising trend in the applications of the FBB in diverse fields. With the first in vitro development and observation of interactions between common non-mycorrhizal soil fungi and rhizobia, forming biofilms (Seneviratne and Jayasinghearachchi 2003), a series of studies were conducted to demonstrate the potential applications of these for various purposes. It was observed that the interaction fixed N_2 biologically, as revealed by nitrogenase activity and N accumulation, which was not observed when the Rhizobium sp. was grown as a monoculture (Jayasinghearachchi and Seneviratne 2004a). The rhizobial strain used here was *Bradyrhizobium elkanii* SEMIA 5019, a soybean-nodulating strain with a high N_2-fixing capability. Naturally-occurring biofilms are sometimes N-deficient for optimal action, as was demonstrated by the increased microbial efficiency of phosphorus solubilization when an N-source was added to the system (Singh and Amberger 1998). Existence of a diazotroph in a biofilm overcomes the N-deficiency, as revealed in the study of Seneviratne and Jayasinghearachchi (2005).

Application of a developed biofilmed inoculant of the FRB was seen to significantly increase N_2 fixation in soybean by ca. 30%, compared to a Rhizobium alone inoculant (conventional inoculant) (Jayasinghearachchi and Seneviratne 2004b). Further, co-inoculation of plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi in rain-fed wheat fields produced the highest protein contents of grains compared to their monocultures (Roesti et al. 2006). In a review by Bashan (1998) on microbial inocula in agriculture, mixed inoculation with arbuscular-mycorrhizal fungi and diazotrophic bacteria has been reported to generate synergistic interactions with the possible consequences of a significant increase in growth, in the phosphorus content of the plants, enhanced mycorrhizal infection, and an improvement in the uptake of mineral nutrients such as phosphorus, nitrogen, zinc, copper and iron. These inocula stimulate plant growth through a range of mechanisms that improve nutrient acquisition and inhibition of fungal plant pathogens (Artursson et al. 2006; Toljander et al. 2006; Biró et al. 2000). Such microbial associations between bacteria and arbuscular mycorrhizal fungi have been observed to occur naturally in the soil (Artursson and Jansson 2003), which provide evidence for their potential of successful establishment if applied as inocula to the soil.

The FBB of beneficial endophytes were observed to produce higher acidity and plant growth-promoting hormones than their mono- or mixed cultures with no biofilm formation (Bandara et al. 2006). The higher acidity is generally important for pathogen suppression. As such, the conventional practice of plant inoculation with monocultures or mixed cultures of effective microbes may not give the highest microbial effect, which may only be achieved by the biofilmed inocula. Thus, production and application of such biofilmed inocula seem to be important for improved plant production.

The FRB used in enhancing soybean N_2 fixation above, increased N and P availabilities and showed a high nitrogenase activity even under a very high soil NO_3^- concentration, compared to its monocultures, when applied directly to the soil (Seneviratne and Jayasinghearachchi 2005). As such, diverse forms of the biofilmed inocula can serve for the future demand of augmented crop productivity with increased N_2 fixation and mineral uptake. In the conventional inoculant technology of microbial monocultures, a major problem yet to be addressed is the poor survival of the introduced microorganisms in the soil due to various environmental stress factors. The biofilmed inocula were observed to help their Rhizobia survive at high salinity (400 mM NaCl) and tannin concentrations (0.4 mM tannic acid) by 10^5- and 10^{12}-fold, respectively compared to the rhizobial monocultures (Seneviratne G, unpublished). Further, their higher tolerance for low pH, Cr and predation by earthworms than the monocultures was also noted (Jayasinghearachchi HS, unpublished). It has been reported that the formation of microcolonies and the production of toxins are effective mechanisms that may allow bacterial biofilms (e.g. *Pseudomonas aeruginosa*) to resist protozoan grazing and to persist in the environment (Matz et al. 2004). Similar observations of Burmolle et al. (2006) revealed that in multispecies biofilms the synergistic interactions cause an enhancement of biofilm formation and increased resistance to antimicrobial agents. Bacterial cells are protected from antimicrobial agents in biofilms through the formation of persister cells; a highly protected state adopted by a small fraction of the outermost cells of a biofilm (Roberts and Stewart 2005).

The biofilmed inocula can also be used for successful establishment of introduced beneficial microorganisms in plants for biocontrol etc. This was confirmed in vitro by a *Pleurotus ostreatus-Pseudomonas fluorescens* biofilm that increased endophytic colonization of tomato by *P. fluorescens*, a biocontrolling agent, by over 1000%, compared to inoculation with *P. fluorescens* alone (Jayasinghearachchi and Seneviratne 2006a). The inoculation of the edible mushroom *P. ostreatus* with a rhizobial strain showed that this association fixed N_2 through biofilm formation and increased the protein content of the mushroom by 147% (Jayasinghearachchi & Seneviratne 2004a). Thus, the inoculation of mushrooms with compatible rhizobia can further increase the nutritive value of mushrooms in its industry.

The biofilmed inocula can be effectively used in biosolubilization of rock phosphate. This was demonstrated by developing biofilms from *Penicillium* spp., *P. ostreatus* and *Xanthoparmelia mexicana*, a lichen fungus, which increased P solubilization up to ca. 230%, compared to the fungus alone cultures (Jayasinghearachchi and Seneviratne 2006b; Seneviratne and Indrasena 2006).

Combination of *Penicillium frequentans* and *Bacillus mycoides* formed a biofilmed inoculant which increased the biodegradability of degradable polyethylene by *P. frequentans*, by ca. 14-fold (Seneviratne et al. 2006). Inoculation of fungal-bacterial co-cultures into polycyclic aromatic hydrocarbon (PAH)-contaminated soil resulted in significantly improved degradation of high-molecular-weight PAHs, compared with the indigenous microbes and soil amended with only axenic inocula (Boonchan et al. 2000).

The FRB above was tested for its potential of generating bioactive compounds (Zavahir and Seneviratne 2007). The biofilm was observed to increase the number of compounds produced, by ca. 12-fold in comparison to its bacterial or fungal monocultures. This technology can be manipulated by using different microbes and culture conditions to produce diverse compounds for the discovery of novel drugs. The FRB described in this review have brought a soybean-nodulating rhizobial strain a long way from its home through an array of biotechnological applications. Biofertilizers in agriculture, rock phosphate solubilization and drug discovery are only a handful of the avenues of potential applications, with many more to be discovered. Therefore, there is a great scope for developing a biofilm technology to produce eco-friendly, beneficial FRB for various applications.

Conclusions and future directions

Studies reported in this review have shown that the FBB/FRB are more effective in their biological performance than monocultures, and perhaps multi-species bacterial or fungal biofilms on abiotic surfaces. The soil application of the FRB as biofilmed inocula appears to be important if soil fertility is to be sustained in nutrient-depleted lands, as well as survival of rhizobia is to be improved in the soil in the absence of their hosts. However, applications of this biotechnology are scarce, because it is still under-studied. More research should be done in the future in order to optimize such biofilmed inocula for various biotechnological applications, where microbes are involved. Selection of combinations of microbes for the highest efficiency is a key in this technology. Depending on the application, engineering aspects such as growing these inocula on the surfaces etc. should also be developed.

A major hurdle in microbial biofuel production is the ethanol susceptibility of microbes used in the production system (Service 2007). This may be overcome by using biofilmed inocula, which show higher environmental stress tolerance. A microbial fuel cell containing a mixed bacterial biofilm has shown a five-fold higher rate and efficiency in converting glucose to electricity (Rabaey et al. 2003). This power output may be further increased if substituted with the FBB as they may have higher metabolic efficiencies than bacterial biofilms. Therefore, potential applications of the biofilmed inocula for improved 'green' energy production as well as biodegradation of synthetic and perhaps natural polymers hazardous to the environment and drug discovery demand immediate research efforts to put them into practice. That will help address a number of major crises that we are yet to be prepared to face in the near future.

References

Artursson V, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8:1–10

Artursson V, Jansson JK (2003) Use of bromodeoxyuridine immunocapture to identify active bacteria associated with arbuscular mycorrhizal hyphae. Appl Environ Microbiol 69:6208–6215

Baillie GS, Douglas LJ (2000) Matrix polymers of *Candida* biofilms and their possible role in biofilm resistance to antifungal agents. J Antimicrob Chemother 46:397–403

Bandara WMMS, Seneviratne G, Kulasooriya SA (2006) Interactions among endophytic bacteria and fungi: effects and potentials. J Biosci 31:645–650

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729–770

Biró B, Köves-Péchy K, Vörös I et al (2000) Interrelations between *Azospirillum* and *Rhizobium* nitrogen-fixers and arbuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile, AMF-free or normal soil conditions. Appl Soil Ecol 15:159–168

Boonchan S, Britz ML, Stanley GA (2000) Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. Appl Environ Microbiol 66:1007–1019

Burmolle M, Webb JS, Rao D et al (2006) Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. Appl Environ Microbiol 72:3916–3923

Chandra J, Kuhn DM, Mukherjee PK et al (2001) Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J Bacteriol 183:5385–5394

Chen CY, Chen SD (2000) Biofilm characteristics in biological denitrification biofilm reactors. Water Sci Technol 41:147–154

Christensen BB, Haagensen JAJ, Heydorn A et al (2002) Metabolic commensalism and competition in a two-species microbial consortium. Appl Environ Microbiol 68:2495–2502

Costerton JW, Philip SS, Greenberg EP (1999) A common cause of persistent infections. Science 284:1318–1322

Davies DG, Chakrabarty AM, Geesey GG (1993) Exopolysaccharide production in biofilms: Substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. Appl Environ Microbiol 59:1181–1186

De Boer W, Folman LB, Summerbell RC et al (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29:795–811

Demirci A, Pometto AL, Ho KLG (1997) Ethanol production by *Saccharomyces cerevisiae* in biofilm reactors. J Ind Microbiol Biotechnol 19:299–304

Dobbins DC, Aelion CM, Pfaender F (1992) Subsurface, terrestrial microbial ecology and biodegradation of organic chemicals: a review. CRC Crit Rev Environ Control 22:67–136

Douglas LJ (2002) *Candida* biofilms and their role in infection. Trends Microbiol 11:30–36
Dow JM, Fouhy Y, Lucey J et al (2007) Cyclic di-GMP as an intracellular signal regulating bacterial biofilm formation. In: Kjelleberg S, Givskov M (eds) The biofilm mode of life: mechanisms and adaptations. Horizon Bioscience, Norwich, pp 71–94
Elvers KT, Leening K, Moore CP et al (1998) Bacterial-fungal biofilms in flowing water photo-processing tanks. J Appl Microbiol 84:607–618
Elvers KT, Leening K, Moore CP et al (2002) Binary and mixed population biofilms: time-lapse image analysis and disinfection with biocides. J Ind Microbiol Biotechnol 29:331–338
Goel A, Müller MB, Sharma M et al (2003) Biodegradation of nonylphenol ethoxylate surfactants in biofilm reactors. Acta Hydroch Hydrob 31:108–119
Jayasinghearachchi HS, Seneviratne G (2004a) Can mushrooms fix atmospheric nitrogen? J Biosci 23:293–296
Jayasinghearachchi HS, Seneviratne G (2004b) A *bradyrhizobial-Penicillium* spp. biofilm with nitrogenase activity improves N_2 fixing symbiosis of soybean. Biol Fertil Soils 40:432–434
Jayasinghearachchi HS, Seneviratne G (2006a) A mushroom-fungus helps improve endophytic colonization of tomato by *Pseudomonas fluorescenc* through biofilm formation. Res J Microbiol 1:83–89
Jayasinghearachchi HS, Seneviratne G (2006b) Fungal solubilization of rock phosphate is enhanced by forming fungal-rhizobia biofilms. Soil Biol Biochem 38:405–408
Matz C, Bergfeld T, Rice SA et al (2004) Microcolonies, quorum sensing and cytotoxicity determine the survival of *Pseudomonas aeruginosa* biofilms exposed to protozoan grazing. Environ Microbiol 6:218–226
Oppermann-Sanio F, Steinbüchel A (2002) Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production. Naturwissenschaften 89:1432–1904
O'Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Ann Rev Microbiol 54:49–79
Rabaey K, Lissens G, Siciliano SD et al (2003) A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnol Lett 25:1531–1535
Roberts ME, Stewart PS (2005) Modelling protection from antimicrobial agents in biofilms through the formation of persister cells. Microbiology 51:75–80
Roesti D, Gaur R, Johri BN et al (2006) Plant growth stage, fertilizer management and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria affect the rhizobacterial community structure in rain-fed wheat fields. Soil Biol Biochem 38:1111–1120
Seneviratne G (2003) Development of eco-friendly, beneficial microbial biofilms. Curr Sci 85:1395–1396
Seneviratne G, Indrasena IK (2006) Nitrogen fixation in lichens is important for improved rock weathering. J Biosci 31:639–643
Seneviratne G, Jayasinghearachchi HS (2003) Mycelial colonization by bradyrhizobia and azorhizobia. J Biosci 28:243–247
Seneviratne G, Jayasinghearachchi HS (2005) A rhizobial biofilm with nitrogenase activity alters nutrient availability in a soil. Soil Biol Biochem 37:1975–1978
Seneviratne G, Tennakoon NS, Weerasekara MLMAW et al (2006) Polyethylene biodegradation by a developed *Penicillium–Bacillus* biofilm. Curr Sci 90:20–21
Service RF (2007) Cellulosic ethanol: biofuel researchers prepare to reap a new harvest. Science 315:1488–1491
Singh CP, Amberger A (1998) Organic acids and phosphorus solubilization in straw composted with rock phosphate. Biores Technol 63:13–16
Soares A, Guieysse B, Mattiasson B (2003) Biodegradation of nonylphenol in a continuous packed-bed bioreactor. Biotech Lett 25:927–933
Toljander JF, Artursson V, Paul LR et al (2006) Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species. FEMS Microbiol Lett 254:34–40
Trachoo N (2003) Biofilms and the food industry. Songklanakarin J Sci Technol 25:807–815
Tyagi RD, Ghose TK (1982) Studies on immobilized *Saccharomyces cerevisiae*. I. analysis of continuous rapid ethanol fermentation in immobilized cell reactor. Biotechnol Bioeng 24:781–795
Vilain S, Brözel VS (2006) Multivariate approach to comparing whole-cell proteomes of *Bacillus cereus* indicates a biofilm-specific proteome. J Proteome Res 5:1924–1930
Villena GK, Gutiérrez-Correa M (2003) *Aspergillus niger* biofilms for cellulases production: some structural and physiological aspects. Rev Peru Biol 10:78–87
Wargo MJ, Hogan DA (2006) Fungal—bacterial interactions: a mixed bag of mingling microbes. Curr Opin Microbiol 9:359–364
Yan W, Boyd KG, Adams DR et al (2003) Biofilm-specific cross-species induction of antimicrobial compounds in Bacilli. Appl Environ Microbiol 69:3719–3727
Zavahir JS, Seneviratne G (2007) Potential of developed microbial biofilms in generating bioactive compounds. Res J Microbiol 2:397–401
Zuo R, Wood TK (2004) Inhibiting mild steel corrosion from sulfate-reducing and iron-oxidizing bacteria using gramicidins-producing biofilms. Appl Microbiol Biotechnol 65:747–753