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THE ANTIMICROBIAL ACTION OF *PSEUDOMONAS AERUGINOSA* BY-PRODUCTS IN THE CONTROL OF SINGLE AND MIXED BIOFILMS

Susana Lopes[1], Idalina Machado[1] and Maria Olívia Pereira[1]
[1]Centre of Biological Engineering
Email: supat@deb.uminho.pt, mopereira@deb.uminho.pt

KEYWORDS

Biofilm, Gram-negative and -positive bacteria, *Pseudomonas* aeruginosa, by-products, antimicrobial activity.

ABSTRACT

Since bacteria are continuously acquiring resistance to conventional chemical agents, it is urgently needed the development of new strategies for biofilm control. It is well recognised that certain microorganisms represent an important source of novel biologically active compounds, with pronounced antibacterial activity, as secondary metabolites. Such substances are accepted to be essential for their producers, inhibiting other bacteria that compete for common resources. The main goal of this work was to investigate the antimicrobial effect of secondary metabolites secreted by *P. aeruginosa* on planktonic and sessile growth of several pathogens, in order to later use those molecules as bio-regulation agents. *P. aeruginosa* supernatants had potential as anti-biofilm agents but only against staphylococcal biofilms since they failed in disturb other biofilm consortia that encompassed Gram- bacteria. This trait makes them quite ineffective chemical countermeasures against real biofilms.

INTRODUCTION

In nature, bacteria live by interacting and communicating with each other, regardless they belong to the same (intraspecies) or different species (interspecies). One of the major mechanisms of cell-cell communication in bacteria involves the synthesis and release of chemical molecules called diffusible signal molecules (Waters and Bassler, 2005). These signals can be cell-density related (quorum sensing - QS) or produced by bacteria at different stages of growth. Primary and secondary metabolites are recognized to contribute to a wealth of interactions between organisms (Duan *et al*., 2009) and can include a variety of nutrients, toxic or neutral metabolic byproducts, antibiotics and other signaling molecules. Such products are released and accumulated in the surrounding environment during bacterial growth (Fuqua and Greenberg, 2002) and can induce expression of certain genes and/or physiological changes in neighbouring cells (Fuqua *et al*., 1996; Parsek and Greenberg, 2005). The properties of these signals and the response elicited by them are important in ensuring bacterial survival and propagation in natural environments where hundreds of bacterial species coexist (Jayaraman and Wood, 2008) Responses of bacteria to chemical signals are quite varied and can include synergistic and/or antagonistic effects. Most research into interspecies bacterial interactions has focused on the beneficial aspects of these relationships that may include coaggregation (Rickard *et al*., 2003; Sharma *et al*., 2005) and conjugation (Ghigo, 2001). These positive interactions give advantages to microorganisms through the transfer of chemical signals, exchange of genetic information, growth promotion and increase of metabolic activity (Shank and Kolter, 2009), and protection from adverse environmental conditions (Leriche *et al*., 2003). Positive interactions among competitors can even contribute to biodiversity (Gross, 2008). However, not all interactions are beneficial, since antagonistic interactions play an important role in bacterial species predominance. Competition for substrate is considered to be the major evolutionary driving force in the microbial world (Simões *et al*., 2007). Negative interactions can give rise to sporulation, suppression of respiration (Hoffman *et al.,* 2006), growth inhibition through the production, for instance, of antimicrobial compounds, as antibiotics (Tait and Sutherland, 2002; Rao *et* al., 2005).

In order to investigate the effect derived from the bacterial release of secondary metabolites, *P. aeruginosa* by-products were evaluated on planktonic and sessile growth of several pathogens, in order to later use those molecules as bio-regulation agents. The role of such molecules was evaluated in cell suspensions and in biofilms of single and dual-species cultures formed by important human-associated pathogens.

METHODS

Supernatants from two *Pseudomonas aeruginosa* planktonic cultures (isolated: PaI and from collection: Pa) were recovered, filtered and stored for further experiments.

These supernatants were then tested on their own and on *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Escherichia coli* lawns.

After observing the results from the disk diffusion tests, the antimicrobial action of the supernatants from both *P. aeruginosa* strains was also assessed in single *Staphylococcus* (*aureus* and epidermidis) biofilm formation and in polymicrobial biofilms formed by those *Staphylococcus* species together with Gram- bacteria (*P. aeruginosa* and *E. coli*).

Supernatants were differently applied: as biofilm growth media complement and as biofilm disruption agents.

RESULTS

Both supernatants inhibited only Gram+ species lawns, being the more remarkable inhibition halos obtained with the isolated *P. aeruginosa* supernatant.

Concerning biofilms, metabolites from both strains can be considered anti-staphylococcal biofilms agents, since their single and mixed biofilm growth was significantly disturbed by both supernatants, regardless their mode of application. However, when staphylococcal species are entrapped in polymicrobial biofilms with *E. coli* and *P. aeruginosa*, supernatants did not exhibit noticeable anto-biofilm activity, mainly hen applied against established biofilms. In general, all mixed biofilms accumulated more mass and had more metabolic activity when submitted to the supernatants aggression.

REFERENCES

Duan K, Silbey CD, Davidson CJ, Surette MG (2009) Chemical interactions between organisms in microbial communities. *Contrib Microbiol* 16: 1-17.

Hoffman LR, Déziel E, D`Argenio DA, Lépine F, Emerson J, McNamara S, Gibson RL, Ramsey BW, Miller SI (2006) Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of *Pseudomonas aeruginosa*. PNAS (www.pnas.org) vol. 103, no. 52.

Fuqua C and Greenberg EP (2002) Listening in on bacteria acyl-homoserine lactone signalling. *Nat Rev in Molec Cell Biol* 3: 685-695.

Fuqua WC, Winans SC, Greenberg EP (1996) Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum sensing transcriptional regulators. *Ann Rev Microbiol* 50: 727:751.

Ghigo JM (2001) Natural conjugative plasmids induce bacterial biofilm development. *Nat* 412: 442-445.

Gross K (2008) Positive interactions among competitors can produce species-rich communities. *Ecol Lett* 11: 929:936.

Jayaraman A and Wood TK (2008) Bacterial quorum sensing: signals, circuits, and implications for biofilms and disease. *Annu Rev Biomed Eng* 10: 145-167.

Kolenbrander PE (2000) Oral microbial communities: biofilms, interactions, and genetic systems. *Annu Rev Microbiol* 54:413-37.

Leriche V, Briandet R, Carpentier B (2003) Ecology of mixed biofilms subjected daily to a chlorinated alkaline solution: spatial distribution of bacterial species suggests a protective effect of one species to another. *Environ Microbiol* 5: 64-71.

Parsek MR and Greenberg EP (2005) Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends in Microbiol* 13: 27-33.

Rao D, Webb JS, Kjelleberg S (2005) Competitive interactions in mixed-species biofilms containing the marine bacterium *Pseudoalteromonas tunicata. Appl Environ Microbiol* 71: 1729-1736.

Rickard AH, Gilbert P, High NJ, Kolenbrander PE, Handley PMcBain AJ, Ledder RG, Handley, PS, Gilbert PS (2003) Coaggregation between freshwater bacteria within biofilm and planktonic communities. *FEMS Microbiol Lett* 220: 133-140.

Shank EA and Kolter R (2009) New developments in microbial interspecies signaling. *Curr Opinion in Microbiol* 12:205-214.

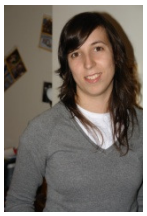
Sharma A, Inagaki S, Sigurdson W, uramitsu HK (2005) Synergy between *Tannerella forsythia and Fusobacterium nucleatum* in biofilm formation. *Oral Microbiol Immunol* 20: 39-42.

Simões LC, Simões *M, Vieira, MJ (2007) Micro*bial interactions in drinking water biofilms. In: *Biofilms: Coming of age*, Manchester, pp 43-52.

Tait K and Sutherland LW (2002) Antagonistic interactions amongst bacteriocin-producing enteric bacteria in dual-species biofilms. *J Appl Microbiol* 93: 345-352.

Waters CM and Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* 21: 319-346.

AUTHOR BIOGRAPHIES



SUSANA LOPES was born in Braga, Portugal, and went to the University of Minho (Braga), where she studied Biological Engineering and obtained her degree in 2007. She worked in 2009 on a fellowship called "Action & Resistance – Caracterização do modo de acção de biocidas emergentes e identificação dos principais mecanismos de resistência bacteriana", before beginning her PhD research work, which subject is "Assessment of Biofilm susceptibility by microorganism involved in Cystic Fibrosis using traditional and novel high-throughput platforms".