

Scientific Reports, 2017, vol.7

Targeting microbial biofilms using Ficin, a nonspecific plant protease

Baidamshina D., Trizna E., Holyavka M., Bogachev M., Artyukhov V., Akhatova F., Rozhina E., Fakhrullin R., Kayumov A.

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© The Author(s) 2017. Biofilms, the communities of surface-attached bacteria embedded into extracellular matrix, are ubiquitous microbial consortia securing the effective resistance of constituent cells to environmental impacts and host immune responses. Biofilm-embedded bacteria are generally inaccessible for antimicrobials, therefore the disruption of biofilm matrix is the potent approach to eradicate microbial biofilms. We demonstrate here the destruction of *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms with Ficin, a nonspecific plant protease. The biofilm thickness decreased two-fold after 24 hours treatment with Ficin at 10 µg/ml and six-fold at 1000 µg/ml concentration. We confirmed the successful destruction of biofilm structures and the significant decrease of non-specific bacterial adhesion to the surfaces after Ficin treatment using confocal laser scanning and atomic force microscopy. Importantly, Ficin treatment enhanced the effects of antibiotics on biofilms-embedded cells via disruption of biofilm matrices. Pre-treatment with Ficin (1000 µg/ml) considerably reduced the concentrations of ciprofloxacin and bezalkonium chloride required to suppress the viable *Staphylococci* by 3 orders of magnitude. We also demonstrated that Ficin is not cytotoxic towards human breast adenocarcinoma cells (MCF7) and dog adipose derived stem cells. Overall, Ficin is a potent tool for staphylococcal biofilm treatment and fabrication of novel antimicrobial therapeutics for medical and veterinary applications.

<http://dx.doi.org/10.1038/srep46068>

References

- [1] Yang, L. et al. Combating biofilms. *Fems Immunology and Medical Microbiology* 65, 146-157, doi: 10.1111/j.1574-695X.2011.00858.x (2012).
- [2] Richards, J. J. & Melander, C. Controlling Bacterial Biofilms. *Chembiochem* 10, 2287-2294, doi: 10.1002/cbic.200900317 (2009).
- [3] Donlan, R. M. Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases* 8, 881-890 (2002).
- [4] Worthington, R. J., Blackledge, M. S. & Melander, C. Small-molecule inhibition of bacterial two-component systems to combat antibiotic resistance and virulence. *Future Medicinal Chemistry* 5, 1265-1284, doi: 10.4155/fmc.13.58 (2013).
- [5] Barth, E., Myrvik, Q. M., Wagner, W. & Gristina, A. G. In vitro and In vivo comparative colonization of *staphylococcus-aureus* and *staphylococcus-epidermidis* on orthopedic implant materials. *Biomaterials* 10, 325-328, doi: 10.1016/0142-9612(89)90073-2 (1989).
- [6] Blackledge, M. S., Worthington, R. J. & Melander, C. Biologically inspired strategies for combating bacterial biofilms. *Current Opinion in Pharmacology* 13, 699-706, doi: 10.1016/j.coph.2013.07.004 (2013).

- [7] Costerton, J. W., Stewart, P. S. & Greenberg, E. P. Bacterial biofilms: A common cause of persistent infections. *Science* 284, 1318-1322, doi: 10.1126/science.284.5418.1318 (1999).
- [8] Gaddy, J. A. & Actis, L. A. Regulation of *Acinetobacter baumannii* biofilm formation. *Future Microbiology* 4, 273-278, doi: 10.2217/fmb.09.5 (2009).
- [9] Rogers, S. A., Huigens, R. W., Cavanagh, J. & Melander, C. Synergistic Effects between Conventional Antibiotics and 2-Aminoimidazole-Derived Antibiofilm Agents. *Antimicrobial Agents and Chemotherapy* 54, 2112-2118, doi: 10.1128/aac.01418-09 (2010).
- [10] Park, J. H., Lee, J. H., Cho, M. H., Herzberg, M. & Lee, J. Acceleration of protease effect on *Staphylococcus aureus* biofilm dispersal. *Fems Microbiology Letters* 335, 31-38, doi: 10.1111/j.1574-6968.2012.02635.x (2012).
- [11] Kaplan, J. B. Biofilm Dispersal: Mechanisms, Clinical Implications, and Potential Therapeutic Uses. *Journal of Dental Research* 89, 205-218, doi: 10.1177/0022034509359403 (2010).
- [12] Izano, E. A., Amarante, M. A., Kher, W. B. & Kaplan, J. B. Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *Applied and Environmental Microbiology* 74, 470-476, doi: 10.1128/aem.02073-07 (2008).
- [13] Donelli, G. et al. Synergistic activity of dispersin B and cefamandole nafate in inhibition of staphylococcal biofilm growth on polyurethanes. *Antimicrobial Agents and Chemotherapy* 51, 2733-2740, doi: 10.1128/aac.01249-06 (2007).
- [14] Darouiche, R. O., Mansouri, M. D., Gawande, P. V. & Madhyastha, S. Antimicrobial and antibiofilm efficacy of triclosan and DispersinB (R) combination. *Journal of Antimicrobial Chemotherapy* 64, 88-93, doi: 10.1093/jac/dkp158 (2009).
- [15] Alkawash, M. A., Soothill, J. S. & Schiller, N. L. Alginate lyase enhances antibiotic killing of mucoid *Pseudomonas aeruginosa* in biofilms. *Apmis* 114, 131-138, doi: 10.1111/j.1600-0463.2006.apm-356.x (2006).
- [16] Alipour, M., Suntres, Z. E. & Omri, A. Importance of DNase and alginate lyase for enhancing free and liposome encapsulated aminoglycoside activity against *Pseudomonas aeruginosa*. *Journal of Antimicrobial Chemotherapy* 64, 317-325, doi: 10.1093/jac/dkp165 (2009).
- [17] Bayer, A. S. et al. Effects of alginase on the natural-history and antibiotic-therapy of experimental endocarditis caused by mucoid *pseudomonas-aeruginosa*. *Infection and Immunity* 60, 3979-3985 (1992).
- [18] Nijland, R., Hall, M. J. & Burgess, J. G. Dispersal of Biofilms by Secreted, Matrix Degrading, Bacterial DNase. *Plos One* 5, doi: 10.1371/journal.pone.0015668 (2010).
- [19] Baker, P. et al. Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent *Pseudomonas aeruginosa* biofilms. *Science Advances* 2, doi: 10.1126/sciadv.1501632 (2016).
- [20] Leroy, C., Delbarre-Ladrat, C., Ghillebaert, F., Compere, C. & Combes, D. Effects of commercial enzymes on the adhesion of a marine biofilm-forming bacterium. *Biofouling* 24, 11-22, doi: 10.1080/08927010701784912 (2008).
- [21] Sharafutdinov, I. et al. HtrA Protease from *Bacillus subtilis* Suppresses the Bacterial Fouling of the Rat Skin Injuries. *Bionanoscience* 6, 564-567, doi: 10.1007/s12668-016-0281-2 (2016).
- [22] Schallenberger, M. A., Niessen, S., Shao, C. X., Fowler, B. J. & Romesberg, F. E. Type I Signal Peptidase and Protein Secretion in *Staphylococcus aureus*. *Journal of Bacteriology* 194, 2677-2686, doi: 10.1128/jb.00064-12 (2012).
- [23] Kristensen, J. B. et al. Antifouling enzymes and the biochemistry of marine settlement. *Biotechnology Advances* 26, 471-481, doi: 10.1016/j.biotechadv.2008.05.005 (2008).
- [24] Lequette, Y., Boels, G., Clarisse, M. & Faille, C. Using enzymes to remove biofilms of bacterial isolates sampled in the food-industry. *Biofouling* 26, 421-431, doi: 10.1080/08927011003699535 (2010).
- [25] Pavlukhina, S. V. et al. Noneluting Enzymatic Antibiofilm Coatings. *Acs Applied Materials & Interfaces* 4, 4708-4716, doi: 10.1021/am3010847 (2012).
- [26] Thallinger, B., Prasetyo, E. N., Nyanhongo, G. S. & Guebitz, G. M. Antimicrobial enzymes: An emerging strategy to fight microbes and microbial biofilms. *Biotechnology Journal* 8, 97-+, doi: 10.1002/biot.201200313 (2013).
- [27] Selan, L., Berlutti, F., Passariello, C., Comodiballanti, M. R. & Thaller, M. C. Proteolytic-enzymes-a new treatment strategy for prosthetic infections. *Antimicrobial Agents and Chemotherapy* 37, 2618-2621 (1993).
- [28] Harris, L. G., Nigam, Y., Sawyer, J., Mack, D. & Pritchard, D. I. *Lucilia sericata* Chymotrypsin Disrupts Protein Adhesin-Mediated Staphylococcal Biofilm Formation. *Applied and Environmental Microbiology* 79, 1393-1395, doi: 10.1128/aem.03689-12 (2013).
- [29] Longhi, C. et al. Protease treatment affects both invasion ability and biofilm formation in *Listeria monocytogenes*. *Microbial Pathogenesis* 45, 45-52, doi: 10.1016/j.micpath.2008.01.007 (2008).
- [30] Thurnheer, T. & Belibasakis, G. N. Incorporation of staphylococci into titanium-grown biofilms: an invitro "submucosal" biofilm model for peri-implantitis. *Clinical Oral Implants Research* 27, 890-895, doi: 10.1111/clr.12715 (2016).
- [31] Kayumov, A. R. et al. Inhibition of biofilm formation in *Bacillus subtilis* by new halogenated furanones. *Journal of Antibiotics* 68, 297-301, doi: 10.1038/ja.2014.143 (2015).

- [32] Kayumov, A. R. et al. New Derivatives of Pyridoxine Exhibit High Antibacterial Activity against Biofilm-Embedded Staphylococcus Cells. *Biomed Research International*, doi: 10.1155/2015/890968 (2015).
- [33] Braun, M., Zaremba, W. & Kietzmann, M. Enhancement of wound healing by an ointment containing proteolytic enzymes and vitamins. *Praktische Tierarzt* 88, 110-114 (2007).
- [34] Chambers, L. et al. Degradation of extracellular matrix components by defined proteinases from the greenbottle larva *Lucilia sericata* used for the clinical debridement of non-healing wounds. *British Journal of Dermatology* 148, 14-23, doi: 10.1046/j.1365-2133.2003.04935.x (2003).
- [35] Hogan, S., Stevens, N. T., Humphreys, H., O'Gara, J. P. & O'Neill, E. Current and Future Approaches to the Prevention and Treatment of Staphylococcal Medical Device-Related Infections. *Current Pharmaceutical Design* 21, 100-113 (2015).
- [36] Trizna, E. et al. Soluble and immobilized papain and trypsin as destroyers of bacterial biofilms. *Genes and Cells* 10, 106-112 (2016).
- [37] Sabirova, A. R. et al. A novel secreted metzincin metalloproteinase from *Bacillus intermedius*. *Febs Letters* 584, 4419-4425, doi: 10.1016/j.febslet.2010.09.049 (2010).
- [38] Trizna, E. Y. et al. Thio Derivatives of 2(5H)-Furanone As Inhibitors against *Bacillus subtilis* Biofilms. *Acta Naturae* 7, 102-107 (2015).
- [39] Trizna, E., Latypova, L., Kurbangalieva, A., Bogachev, M. I. & Kayumov, A. 2(5H)-Furanone Derivatives as Inhibitors of Staphylococcal Biofilms. *Bionanoscience* 6, 423-426, doi: 10.1007/s12668-016-0258-1 (2016).
- [40] Baugh, S., Phillips, C. R., Ekanayaka, A. S., Piddock, L. J. V. & Webber, M. A. Inhibition of multidrug efflux as a strategy to prevent biofilm formation. *Journal of Antimicrobial Chemotherapy* 69, 673-681, doi: 10.1093/jac/dkt420 (2014).
- [41] O'Toole, G. A., & Kolter, R. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Molecular Microbiology*. 28, 449-461, doi: 10.1046/j.1365-2958.1998.00797.x (1998).
- [42] Herigstad, B., Hamilton, M. & Heersink, J. How to optimize the drop plate method for enumerating bacteria. *Journal of Microbiological Methods*. 44, 121-129, doi: 10.1016/s0167-7012(00)00241-4 (2001).