microPublication BIOLOGY

3/25/2024 - *Open Access*

An apparent lack of synergy between degradative enzymes against *Staphylococcus aureus* biofilms

Jeremy R. Ellis[1,2], Paul A. Rowley[1§]

[1]Department of Biological Sciences, University of Idaho, Moscow, Idaho, United States

[2]The Johns Hopkins University School of Medicine, Johns Hopkins University, Baltimore, Maryland, United States

[§]To whom correspondence should be addressed: prowley@uidaho.edu

Abstract

Enzymes combat bacterial infections by degrading biomolecules to disperse *Staphylococcus aureus* biofilms. Commercial enzyme mixtures, like cellulase and pepsin, show concentration-dependent dispersion, but low concentrations lack synergy. Only the sequential addition of pepsin followed by *Arthrobacter luteus* zymolyase 20T displays synergy, effectively dispersing biofilms. Purified zymolyase 100T outperforms zymolyase 20T but lacks synergy with pepsin. This study underscores the complexity of enzymatic biofilm dispersal, highlighting the need for tailored approaches based on enzyme properties and biofilm composition.

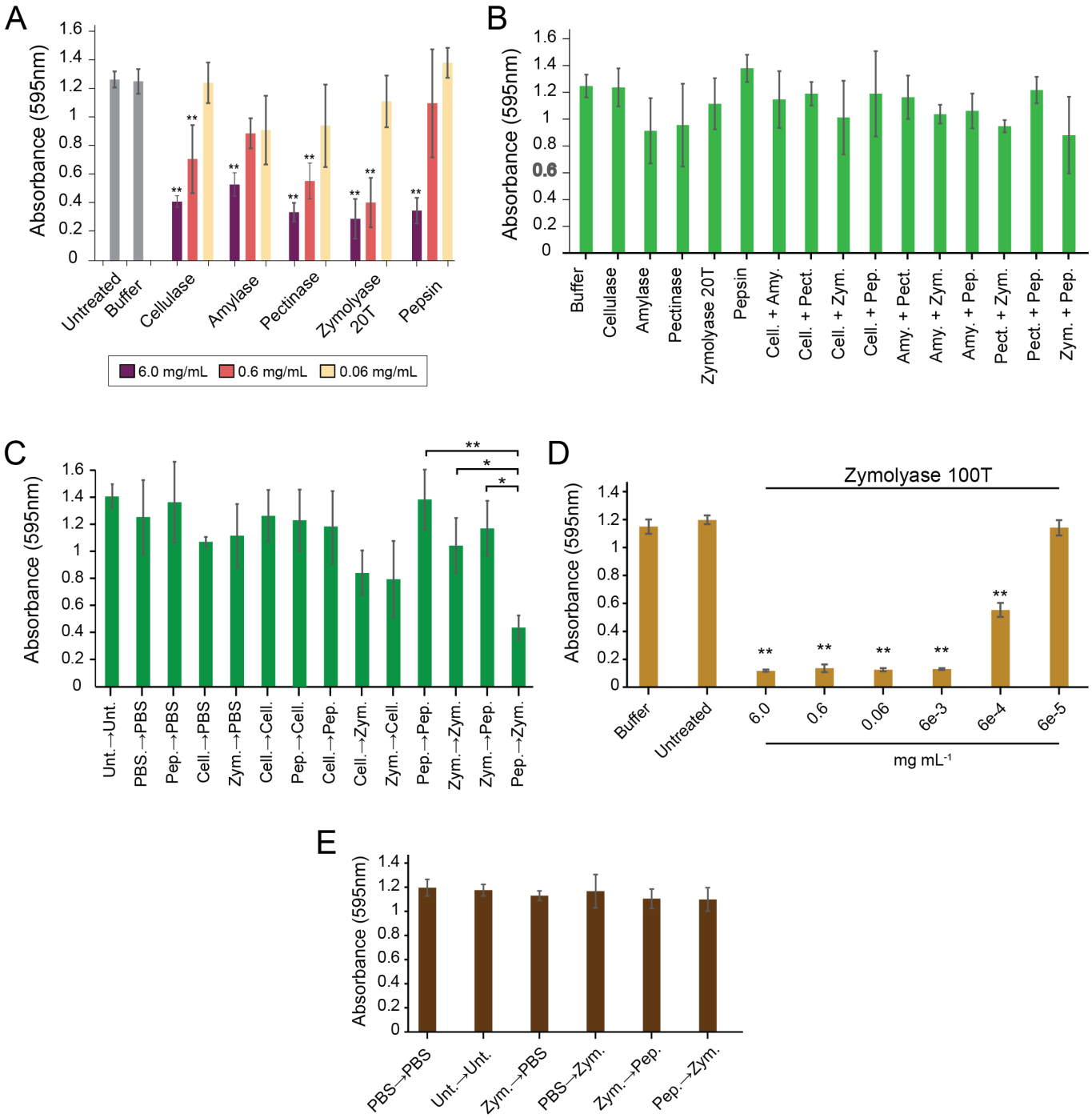

Figure 1. Commercially available enzymes are not synergistic for biofilm dispersal, but sequential treatment with pepsin followed by zymolyase 20T or zymolyase 100T leads to biofilm dispersal.:

A. *S. aureus* biofilms grown on polystyrene were treated with three concentrations (6, 0.6, and 0.06 mg mL^{-1}) of commercial enzymes (cellulase, amylase, pectinase, zymolyase, and pepsin) and 50 U/mL for DNase I.. B. *S. aureus* biofilms grown on polystyrene were treated with 1:1 mixtures of either 0.06 mg mL^{-1} cellulase (cell.), amylase (Amy.), pectinase (Pect.), zymolyase 20T (Zym.), pepsin (Pep.) or 50 U/mL DNase I. (n = 3). C. *S. aureus* biofilms grown on polystyrene were treated sequentially with the indicated enzymes for one hour per treatment phase. Different sequential combinations of cellulase (Cell.), zymolyase 20T (Zym.), and pepsin (Pep.)) were added to a final enzyme concentration of 0.06 mg mL^{-1}. Unt, untreated; PBS, phosphate-buffered saline. D. *S. aureus* biofilms grown on polystyrene were treated with zymolyase 100T at different concentrations for one hour. E. *S. aureus* biofilms grown on polystyrene were treated sequentially with zymolyase

100T (Zym.; 6e-5 ug mL^{-1}) and pepsin (Pep.; 0.06 mg mL^{-1}). Biofilms were stained with 0.1% crystal violet, and the absorbance was measured at 595 nm (* $p<0.05$, ** $p<0.01$, Tukey test) (n = 3, biological replicates).

Description

Biofilms can be constructed by many different microorganisms as an extracellular scaffold that enables surface colonization. The secretion of extracellular polysaccharides and proteins enables surface attachment and the formation of biofilm microcolonies (Karatan et al., 2009; Petrova et al., 2016). These mature biofilms can be hard to remove and enable the dispersal of planktonic bacterial cells to colonize new locations. In situations where bacterial colonization is undesirable, biofilms can be a serious challenge and endanger the functionality and longevity of critical infrastructure as well as human health (Bryers, 2008; Carvalho, 2018; Cugini et al., 2019; Flemming et al., 2010).

Major components of biofilms are exopolysaccharides (EPS) consisting of homopolysaccharides (i.e cellulose) or heteropolysaccharides (e.g. alginate, Pel, Psl). Digestion of EPS by glycoside hydrolases (GHs) is a strategy that is effective at the removal of bacterial biofilms from surfaces and wounds (Donelli et al., 2007; Ellis et al., 2023; Jee et al., 2020; Kamali et al., 2021; Kaplan et al., 2018; Kaur et al., 2020; Kovach et al., 2020; Lakshmi et al., 2022; Redman et al., 2020; Stiefel et al., 2016). Many different GHs can degrade EPS constituents of biofilms, such as cellulase, amylase, dispersin B, alginate lyase, and xylosidase, resulting in partial biofilm disruption. In addition to GHs, proteases have also been used to disperse biofilms effectively (Boles et al., 2008; Lauderdale et al., 2010). Degradation of biofilms by enzymes allows more effective surface cleaning and increases the efficacy of antibiotics and bacteriophage treatment (Darouiche et al., 2009; Donelli et al., 2007; Fleming et al., 2017; Lu et al., 2007; Pestrak et al., 2019). However, not every hydrolytic enzyme is effective at biofilm dispersion, which suggests that the molecular complexity of biofilms and the substrate specificity of enzymes dictates the effectiveness of enzymatic biofilm dispersal (Ellis et al., 2023; Redman et al., 2020).

Effective biofilm dispersion has been observed with mixtures of GHs and degradative enzymes (Ellis et al., 2023; Fleming et al., 2017; Jee et al., 2020; Kaur et al., 2020). The strategy of using multiple GHs would likely enable the simultaneous disassembly of multiple EPS that would cause a loss of biofilm structural integrity. Targeting different EPS enables the dispersal of complex biofilms that are likely to exist in polymicrobial infections that may be resistant to the action of a single GH. We recently demonstrated that a commercial preparation of cellulase contained a complex mixture of at least two GHs that have different substrate specificities (Ellis et al., 2023). This “cellulase” mixture degraded the pure substrates carboxymethylcellulose, amylose, and pectin but also efficiently dispersed *Staphylococcus aureus* biofilms. This was in stark contrast to purified recombinant cellulases that were specific in their degradation of carboxymethylcellulose and unable to disperse biofilms. Similarly, the combination of high concentrations of cellulase and amylase mixtures also showed some improvement in biofilm-dispersing activities (Fleming et al., 2017). Altogether, these data suggest that mixtures of different degradative enzymes can be more effective than purified enzymes. This motivated a systematic approach to understanding how different combinations of degradative enzymes can be used to improve biofilm dispersal. In this study, we investigated the synergistic action of selected GHs, and proteases to disperse biofilms.

A lack of synergy using commercial enzymes to disperse S. aureus biofilms.

GHs can disperse biofilms as measured by crystal violet staining and colony-forming units (Figure S1A). Therefore, we tested the effectiveness of cellulase, amylase, pectinase, zymolyase, and pepsin in dispersing *S. aureus* biofilm grown on polystyrene as measured by staining with crystal violet (Figure 1A). It was observed that all of these degradative enzymes had concentration-dependent dispersion of biofilms with the most pronounced dispersal at high concentrations of each enzyme (6 mg mL^{-1}). Pepsin and pectinase were found to disperse biofilms at high concentrations despite their low enzymatic activity at pH 7.4. However, we have previously demonstrated the activity of pectinase on pectin at neutral pH (Ellis et al., 2023). To test for potential synergistic activity present between enzymes, we mixed different enzymes at a 1:1 w/w ratio at concentrations that did not exhibit significant biofilm dispersing activity when used alone (0.06 mg mL^{-1} for all enzymes). The rationale was that synergy between enzymes would show improved biofilm dispersion compared to the individual enzymes. However, after incubation with *S. aureus* biofilms on polystyrene, no dispersal with any combination of enzymes was observed (Figure 1B). Enzyme combinations were also mixed at ratios of 1:2, 1:10, and 1:100 with a final enzyme concentration 0.06 mg mL^{-1}, but were also ineffective at biofilm dispersal (Figure S1B)

The sequential addition of pepsin and zymolyase 20T causes biofilm dispersion.

While there was no significant synergy between enzymes when mixed, we were concerned that adding protease could affect the activity of GHs. Therefore, we also assayed whether sequentially adding enzymes at low concentrations would demonstrate synergy. Pepsin and Zymolayse 20T were assayed as they caused observable biofilm dispersion (Figure 1A), and cellulase was included as we had previously characterized the GHs in this enzyme mixture (Ellis et al., 2023). After one hour

of treatment, the first enzyme solution was removed, and the second enzyme was added and incubated for an additional one hour. Consistent with prior experiments, we did not observe any improved biofilm-dispersing activities for most combinations of enzymes. However, the altered treatment regime did reveal that adding pepsin followed by zymolase 20T caused a significant dispersion of *S. aureus* biofilms compared to adding either of the individual enzymes, PBS buffer only, and untreated biofilms (Figure 1C). Importantly, reversing the order of addition so that zymolase 20T was added first, followed by pepsin, did not result in biofilm dispersal.

Zymolyase 100T is effective at dispersing biofilms but lacks synergy with pepsin.

Zymolyase 20T is a commercially available preparation of multiple enzymes precipitated by ammonium sulfate from cultures of the bacteria *Arthrobacter luteus*. This preparation is typically used to digest cell wall components of multiple different species of fungi (Kitamura et al., 1971). Many different enzymatic activities are present in these commercial preparations, including ß-glucanase, protease, and mannanase. Enzymes associated with ß-glucanase and protease activities have been successfully separated by additional steps of protein purification (Kitamura, 2014). Indeed, zymolyase 100T is a preparation derived from 20T that has been purified by ß-1,3-glucan affinity chromatography to enrich the ß-glucanase activity (Kitamura, 1982). We find that the 100T preparation appears to have a similar composition of proteins compared to 20T by SDS-PAGE gel electrophoresis (Figure S1C), but is significantly more effective at dispersing biofilms of *S. aureus* (Figure 1D). Specifically, 100T causes significant biofilm dispersal at a concentration of 0.0006 mg mL^{-1} compared to 0.6 mg mL^{-1} for 20T, which is likely explained by the increased concentration of ß-glucanase in the preparation due to affinity purification. However, the sequential addition of pepsin followed by 100T failed to elicit synergy, as was observed with 20T (Figure 1E).

Discussion and Conclusion

GHs have been shown to efficiently degrade bacterial biofilms, which holds future promise for combatting recalcitrant bacterial infections that are resistant to antibiotics. The motivation behind the current study was to identify combinations of enzymes to disperse biofilms more efficiently when compared to single enzymes. As biofilms are often composed of many different polymers, including proteins, carbohydrates, and nucleic acids, it seemed reasonable that combining biofilm-degrading enzymes would lead to an enhancement of dispersal. This would be analogous to approaches to the deconstruction of complex plant cell wall carbohydrates for biofuel production (Kubicek et al., 2016). Although we found that many enzymes were effective at the dispersal of biofilms at high concentrations, no pair of enzymes demonstrated synergy when combined at concentrations that would not cause dispersal. However, the sequential addition of pepsin followed by zymolyase 20T was unique in its ability to cause biofilm dispersion, whereas the reverse order of addition showed no effect. This result is similar to studies of plant cell wall digestion experiments that have determined that carbohydrate-binding modules can potentiate enzymatic degradation (Blake et al., 2006). Specifically, the binding of these carbohydrate-binding modules is thought to unmask carbohydrates of the plant cell wall that are susceptible to degradation by enzymes. Similarly, the action of non-hydrolytic accessory enzymes on cellulose can also enable more efficient depolymerization by cellulases (Kubicek et al., 2016). These requirements highlight the importance of order-of-addition for the deconstruction of complex organic substrates that could apply to the dispersal of biofilms. We draw an analogy between plant cell walls and bacteria biofilms as both are known to contain ordered structures of carbohydrates (Hartmann et al., 2019; Lawrence et al., 2007). In the current study, it is feasible that unmasking carbohydrates by the action of pepsin (or an unspecified contaminant of the commercial preparation), either by proteolysis or the binding of biofilms, could allow the hydrolysis of previously masked carbohydrates upon the addition of 20T. *S. aureus* is known to encode extracellular proteases that are thought to be required for biofilm formation and remodeling (Martí et al., 2010). Specifically, *S. aureus* proteases can cleave the extracellular *S. aureus* Biofilm Associated Protein (BAP), which is essential for adhesion and biofilm development (Cucarella et al., 2001). This is only one of many proteins that are integral to bacterial biofilms and could be targeted by proteolysis to alter biofilm structure (such as (Besingi et al., 2017; Gerven et al., 2015; Reichhardt, 2023)). However, we find that combining different degradative enzymes mostly does not enhance biofilm dispersion, despite prior observations that mixtures of GHs and other biologically active molecules can have an additive effect on dispersion (Donelli et al., 2007; Ellis et al., 2023; Jee et al., 2020; Kaur et al., 2020).

Our previous work shows that commercial GHs can contain a complex mixture of proteins with varying hydrolytic activities (Ellis et al., 2023). This adds uncertainty to the interpretation of the effects of degradative enzymes on biofilms, as it is difficult to pinpoint the relevant enzymatic activities required for dispersion. Like other commercial preparations of GHs and pepsin, zymolyase 20T is a crude mixture of different enzymes extracted by ammonium sulfate precipitation. Specifically, the manufacturer's specification for 20T detected the presence of significant ß-glucanase, mannanase, and protease activities. In the current study, there was a clear difference in the composition of zymolyase 20T and 100T that affected biofilm dispersing synergy with pepsin, even if there was no dramatic difference in the overall protein composition of the mixture. The vital component that enabled synergy with pepsin was likely removed after affinity purification of 100T using ß-1,3-glucan (Kitamura, 1982). Further analysis of the composition of 20T compared to 100T would be required to determine the molecule

in 20T responsible for synergy. It is also important to recognize that the purity of the pepsin used in the current study was not tested and could contain other biologically active compounds with the potential to influence synergy. It is well understood that biofilms vary in their composition, which would likely influence the effectiveness of enzymatic biofilm dispersion, adding an additional layer of complexity to using GHs for biofilm dispersal. This highlights the need to investigate the role of synergy in biofilm dispersal further with more diverse combinations of purified GHs and other degradative enzymes.

Methods

Propagation of microorganisms. The bacterial strains *S. aureus* SA31 and *P. aeruginosa* PAO1 were used for biofilm studies and maintained by growing in tryptic soy broth (TSB) and Luria broth (LB), respectively.

Enzymes used for biofilm dispersion. Enzymes used in this study were sourced from various companies; cellulase >60,000 U/g (*Aspergillus niger*) MP Biomedicals, catalog number 150583; amylase ~165,000 U/g (*Bacillus sp.*), MP Biomedicals, catalog number 100447; pectinase 400-800 U/g (*Rhizopus sp.*), Sigma-Aldrich, catalog number P2401; zymolyase 20T 20,000 U/g, (*Arthrobacter luteus*), Amsbio, catalog number 120491-1; zymolyase 100T ≥100,000 U/g (*Arthrobacter luteus*), United States Biological, catalog number Z1004; pepsin ≥250,000 U/g (Porcine gastric mucosa), Sigma-Aldrich, catalog number P7000. All enzymes were suspended in phosphate-buffered saline at pH 7.4 to the desired concentration. Both amylase and cellulase were activated by incubation at 37 °C for 30 min prior to use.

Polystyrene biofilm model. The *in vitro* polystyrene biofilm model used to measure the effectiveness of GHs at dispersion is described by Ellis et al.. Briefly, an overnight culture of *S. aureus*, cultivated in TSB + 1% dextrose broth, underwent a 1:100 dilution with sterile TSB + 1% dextrose broth. The diluted culture was dispensed into untreated sterile 96-well round-bottomed polystyrene plates (100 µL/well). Plates were sealed around the edges with parafilm and robust biofilms were allowed to develop for 48 hours at 37 °C. After incubation, the supernatants were removed, and the wells were blotted using paper towels. Subsequently, 125 µL of either the culture supernatant or the purified enzyme solution was added to each well. For the sequential treatment of biofilms with enzymes, the first enzyme treatment was allowed to bathe biofilms for 1 hour, after which the liquid was carefully aspirated. The second enzyme treatment was added for an additional hour. Crystal violet staining and subsequent quantification occurred only after the second treatment course was completed.

Analysis of Zymolyase 20T and 100T by SDS-PAGE. 10 µg of 20T and 100T zymolyase were run on a 10% SDS-PAGE gel and subsequently visualized with Coomassie staining.

Acknowledgements:

We would like to acknowledge the mentorship and support of Professor James J. Bull at the University of Idaho.

Extended Data

Description: Supporting information for the manuscript. Resource Type: Image. File: Synergy_supp_V2.png. DOI: 10.22002/g5eyh-a1429

References

Besingi RN, Wenderska IB, Senadheera DB, Cvitkovitch DG, Long JR, Wen ZT, Brady LJ. 2017. Functional amyloids in Streptococcus mutans, their use as targets of biofilm inhibition and initial characterization of SMU_63c. Microbiology 163: 488-501. DOI: 10.1099/mic.0.000443

Blake AW, McCartney L, Flint JE, Bolam DN, Boraston AB, Gilbert HJ, Knox JP. 2006. Understanding the Biological Rationale for the Diversity of Cellulose-directed Carbohydrate-binding Modules in Prokaryotic Enzymes. Journal of Biological Chemistry 281: 29321-29329. DOI: 10.1074/jbc.m605903200

Boles BR, Horswill AR. 2008. agr-Mediated Dispersal of Staphylococcus aureus Biofilms. PLoS Pathogens 4: e1000052. DOI: 10.1371/journal.ppat.1000052

Bryers JD. 2008. Medical biofilms. Biotechnology and Bioengineering 100: 1-18. DOI: 10.1002/bit.21838

de Carvalho CCCR. 2018. Marine Biofilms: A Successful Microbial Strategy With Economic Implications. Frontiers in Marine Science 5: 10.3389/fmars.2018.00126. DOI: 10.3389/fmars.2018.00126

Cucarella C, Solano C, Valle J, Amorena B, Lasa Ini, Penadés JR. 2001. Bap, a *Staphylococcus aureus* Surface Protein Involved in Biofilm Formation. Journal of Bacteriology 183: 2888-2896. DOI: 10.1128/jb.183.9.2888-2896.2001

Cugini C, Shanmugam M, Landge N, Ramasubbu N. 2019. The Role of Exopolysaccharides in Oral Biofilms. Journal of Dental Research 98: 739-745. DOI: 10.1177/0022034519845001

Darouiche RO, Mansouri MD, Gawande PV, Madhyastha S. 2009. Antimicrobial and antibiofilm efficacy of triclosan and DispersinB(R) combination. Journal of Antimicrobial Chemotherapy 64: 88-93. DOI: 10.1093/jac/dkp158

Donelli G, Francolini I, Romoli D, Guaglianone E, Piozzi A, Ragunath C, Kaplan JB. 2007. 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

Ellis JR, Bull JJ, Rowley PA. 2023. Fungal Glycoside Hydrolases Display Unique Specificities for Polysaccharides and Staphylococcus aureus Biofilms. Microorganisms 11: 293. DOI: 10.3390/microorganisms11020293

Fleming D, Chahin L, Rumbaugh K. 2017. Glycoside Hydrolases Degrade Polymicrobial Bacterial Biofilms in Wounds. Antimicrobial Agents and Chemotherapy 61: 10.1128/aac.01998-16. DOI: 10.1128/aac.01998-16

Flemming HC, Wingender J. 2010. The biofilm matrix. Nature Reviews Microbiology 8: 623-633. DOI: 10.1038/nrmicro2415

Van Gerven N, Klein RD, Hultgren SJ, Remaut H. 2015. Bacterial Amyloid Formation: Structural Insights into Curli Biogensis. Trends in Microbiology 23: 693-706. DOI: 10.1016/j.tim.2015.07.010

Hartmann, R., Singh, P. K., Pearce, P., Mok, R., Song, B., Díaz-Pascual, F., Dunkel, J., & Drescher, K. (2019). Emergence of three-dimensional order and structure in growing biofilms. *Nature Physics*, *15*(3), 251–256. doi: 10.1038/s41567-018-0356-9 DOI: doi: 10.1038/s41567-018-0356-9

Jee SC, Kim M, Sung JS, Kadam AA. 2020. Efficient Biofilms Eradication by Enzymatic-Cocktail of Pancreatic Protease Type-I and Bacterial α-Amylase. Polymers 12: 3032. DOI: doi: 10.3390/polym12123032

Kamali E, Jamali A, Izanloo A, Ardebili A. 2021. In vitro activities of cellulase and ceftazidime, alone and in combination against Pseudomonas aeruginosa biofilms. BMC Microbiology 21: 10.1186/s12866-021-02411-y. DOI: 10.1186/s12866-021-02411-y

Kaplan JB, Mlynek KD, Hettiarachchi H, Alamneh YA, Biggemann L, Zurawski DV, et al., Granick. 2018. Extracellular polymeric substance (EPS)-degrading enzymes reduce staphylococcal surface attachment and biocide resistance on pig skin in vivo. PLOS ONE 13: e0205526. DOI: 10.1371/journal.pone.0205526

Karatan E, Watnick P. 2009. Signals, Regulatory Networks, and Materials That Build and Break Bacterial Biofilms. Microbiology and Molecular Biology Reviews 73: 310-347. DOI: 10.1128/mmbr.00041-08

Kaur A, Rishi V, Soni SK, Rishi P. 2020. A novel multi-enzyme preparation produced from Aspergillus niger using biodegradable waste: a possible option to combat heterogeneous biofilms. AMB Express 10: 10.1186/s13568-020-00970-3. DOI: 10.1186/s13568-020-00970-3

Kitamura, K. (1982). Preparation Wall Lytic Enzyme from Arthrobacter luteus by its Adsorption on beta-Glucan. *Journal of Fermentation Technology*, *60*(3), 257–260.

Kitamura K. 1982. Re-examination of Zymolyase Purification. Agricultural and Biological Chemistry 46: 963-969. DOI: 10.1080/00021369.1982.10865178

Kitamura K, Kaneko T, Yamamoto Y. 1971. Lysis of viable yeast cells by enzymes of Arthrobacter luteus. Archives of Biochemistry and Biophysics 145: 402-404. DOI: 10.1016/0003-9861(71)90053-1

Kovach KN, Fleming D, Wells MJ, Rumbaugh KP, Gordon VD. 2020. Specific Disruption of Established *Pseudomonas aeruginosa* Biofilms Using Polymer-Attacking Enzymes. Langmuir 36: 1585-1595. DOI: 10.1021/acs.langmuir.9b02188

Kubicek CP, Kubicek EM. 2016. Enzymatic deconstruction of plant biomass by fungal enzymes. Current Opinion in Chemical Biology 35: 51-57. DOI: 10.1016/j.cbpa.2016.08.028

Lakshmi SA, Alexpandi R, Shafreen RMB, Tamilmuhilan K, Srivathsan A, Kasthuri T, et al., Pandian. 2022. Evaluation of antibiofilm potential of four-domain α-amylase from Streptomyces griseus against exopolysaccharides (EPS) of bacterial pathogens using Danio rerio. Archives of Microbiology 204: 10.1007/s00203-022-02847-4. DOI: 10.1007/s00203-022-02847-4

Lauderdale KJ, Malone CL, Boles BR, Morcuende J, Horswill AR. 2009. Biofilm dispersal of community-associated methicillin-resistant *Staphylococcus aureus* on orthopedic implant material. Journal of Orthopaedic Research 28: 55-61. DOI: 10.1002/jor.20943

Lawrence JR, Swerhone GDW, Kuhlicke U, Neu TR. 2007. In situ evidence for microdomains in the polymer matrix of bacterial microcolonies. Canadian Journal of Microbiology 53: 450-458. DOI: 10.1139/w06-146

Lu TK, Collins JJ. 2007. Dispersing biofilms with engineered enzymatic bacteriophage. Proceedings of the National Academy of Sciences 104: 11197-11202. DOI: 10.1073/pnas.0704624104

Martí M, Trotonda MaP, Tormo-Más Man, Vergara-Irigaray M, Cheung AL, Lasa Ii, Penadés JR. 2010. Extracellular proteases inhibit protein-dependent biofilm formation in Staphylococcus aureus. Microbes and Infection 12: 55-64. DOI: 10.1016/j.micinf.2009.10.005

Pestrak MJ, Baker P, Dellos-Nolan S, Hill PJ, Passos da Silva D, Silver H, et al., Howell. 2019. Treatment with the Pseudomonas aeruginosa Glycoside Hydrolase PslG Combats Wound Infection by Improving Antibiotic Efficacy and Host Innate Immune Activity. Antimicrobial Agents and Chemotherapy 63: 10.1128/aac.00234-19. DOI: 10.1128/aac.00234-19

Petrova OE, Sauer K. 2016. Escaping the biofilm in more than one way: desorption, detachment or dispersion. Current Opinion in Microbiology 30: 67-78. DOI: 10.1016/j.mib.2016.01.004

Redman WK, Welch GS, Rumbaugh KP. 2020. Differential Efficacy of Glycoside Hydrolases to Disperse Biofilms. Frontiers in Cellular and Infection Microbiology 10: 10.3389/fcimb.2020.00379. DOI: 10.3389/fcimb.2020.00379

Reichhardt C. 2023. The Pseudomonas aeruginosa Biofilm Matrix Protein CdrA Has Similarities to Other Fibrillar Adhesin Proteins. Journal of Bacteriology 205: 10.1128/jb.00019-23. DOI: 10.1128/jb.00019-23

Stiefel P, Mauerhofer S, Schneider J, Maniura-Weber K, Rosenberg U, Ren Q. 2016. Enzymes Enhance Biofilm Removal Efficiency of Cleaners. Antimicrobial Agents and Chemotherapy 60: 3647-3652. DOI: 10.1128/aac.00400-16

Funding:

The research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM104420. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author Contributions: Jeremy R. Ellis: conceptualization, data curation, investigation, methodology, validation, writing - original draft, writing - review editing. Paul A. Rowley: formal analysis, funding acquisition, writing - review editing.

Reviewed By: Whitni Redman Whitni Redman

History: Received January 5, 2024 **Revision Received** January 25, 2024 **Accepted** March 12, 2024 **Published Online** March 25, 2024 **Indexed** April 8, 2024

Citation: Ellis, JR; Rowley, PA (2024). An apparent lack of synergy between degradative enzymes against *Staphylococcus aureus* biofilms. microPublication Biology. 10.17912/micropub.biology.001119