MICROBIOLOGY

Extracellular DNA Required for Bacterial Biofilm Formation

Cynthia B. Whitchurch,[1] Tim Tolker-Nielsen,[2] Paula C. Ragas,[2] John S. Mattick[1]*

Bacterial biofilms are structured communities of cells enclosed in self-produced hydrated polymeric matrix adherent to an inert or living surface (*1*). Formation of these sessile communities and their inherent resistance to antibiotics and host immune attack are at the root of many persistent and chronic bacterial infections (*1*), including those caused by *Pseudomonas aeruginosa*, which has been intensively studied as a model for biofilm formation (*2*, *3*). The matrix, which holds bacterial biofilms together, is a complex mixture of macromolecules including exopolysaccharides, proteins, and DNA (*4*). The latter has been presumed to be derived from lysed cells and has not been thought to represent an important component of biofilm structure. However, it has been known for many years that some bacteria, including *P. aeruginosa*, produce substantial quantities of extracellular DNA through a mechanism that is thought to be independent of cellular lysis and that appears to involve the release of small vesicles from the outer membrane (*5*, *6*).

During studies of alginate biosynthesis in *P. aeruginosa*, we discovered that the majority of the extracellular material that reacted in the carbazole colorimetric assay was not exopolysaccharide but DNA [as determined by its peak absorbance at 260 nm, by electrophoretic display, and by its deoxyribonuclease (DNase) but not ribonuclease sensitivity] and therefore hypothesized that this DNA may play a functional role in *P. aeruginosa* biofilms. Using a tube ring assay (*2*), we found that addition of DNase I to the culture medium strongly inhibited biofilm formation (Web fig. 1A) (*7*), although not bacterial growth per se.

We then investigated the effect of DNase I on biofilm formation in more detail using a flow-chamber system (*8*). Four flow-chamber channels were inoculated with green fluorescent protein (GFP)–tagged *P. aeruginosa* PAO1, and two channels each were irrigated with minimal medium with or without DNase I. The presence of DNase I in the medium prevented biofilm formation. The channels irrigated with medium without DNase I were extensively colonized after 3 days, whereas the channels irrigated with DNase I–containing medium were essentially without cells, or contained few attached cells, after the same period (Web fig. 1B).

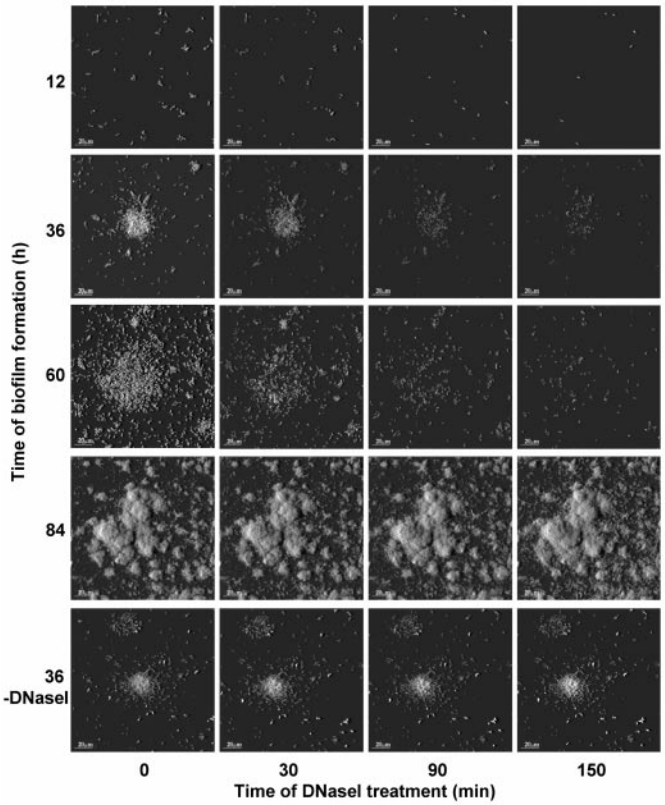

Fig. 1. Effect of DNase I on established biofilms. Five flow-chamber channels were inoculated with GFP-tagged *P. aeruginosa* PAO1 (*7*) and irrigated with minimal medium without DNase I to allow the establishment of *P. aeruginosa* biofilms of varying age. After 12, 36, 60, and 84 hours, the medium was shifted to minimal medium supplemented with DNase I, and the fate of the biofilms was followed by recording confocal laser scanning micrographs (*8*) at various times after the shift. The bottom panels show a control experiment with a shift to medium without DNase I, demonstrating that the laser recordings, or the medium shift per se, did not lead to biofilm dissolution.

We also investigated whether DNase I could dissolve established biofilms. To this end, we inoculated five flow-chamber channels and irrigated them with minimal medium without DNase I to allow the establishment of *P. aeruginosa* biofilms of varying age. At various times, the medium was shifted to medium supplemented with DNase I, and the fate of the biofilms was followed by confocal laser scanning microscopy (Fig. 1). The biofilms that were 12, 36, and 60 hours old when the DNase I treatments were initiated became dissolved, whereas the biofilm that was 84 hours old at the time of DNase I exposure was only affected to a minor degree. This suggests that the matrix in mature biofilms may be strengthened by other substances or that mature biofilms may produce sufficient proteolytic exoenzymes to locally inactivate the DNase I (*7*).

These results indicate that extracellular DNA is required for the initial establishment of *P. aeruginosa* biofilms and perhaps biofilms formed by other bacteria that specifically release DNA. The source of this DNA is unclear, but it is presumably derived from membrane vesicles rather than cell lysis as we saw no evidence of the latter during biofilm formation.

Much of the tissue damage associated with *P. aeruginosa* infections of the cystic fibrosis (CF) lung epithelia is due to inflammatory responses of the host immune system, which may include responses to bacterial DNA (*9*). The current treatment regime for CF patients includes inhalation of nebulized recombinant human DNase I as a therapy to reduce the viscosity of purulent sputum. Our findings suggest that DNase I treatment might be beneficial as a early prophylactic measure to prevent the establishment of chronic *P. aeruginosa* infection of the CF lung by inhibiting biofilm formation. They also suggest that DNase I may be useful in preventing bacterial biofilms in other contexts.

References and Notes

1. J. W. Costerton, P. S. Stewart, E. P. Greenberg, *Science* **284**, 1318 (1999).
2. G. A. O'Toole, R. Kolter, *Mol. Microbiol.* **30**, 295 (1998).
3. P. K. Singh *et al.*, *Nature* **407**, 762 (2000).
4. I. W. Sutherland, *Trends Microbiol.* **9**, 222 (2001).
5. Y. Muto, S. Goto, *Microbiol. Immunol.* **30**, 621 (1986).
6. J. L. Kadurugamuwa, T. J. Beveridge, *J. Bacteriol.* **177**, 3998 (1995).
7. Supplementary material is available on *Science* Online at www.sciencemag.org/cgi/content/full/295/5559/1487/DC1.
8. T. Tolker-Nielsen *et al.*, *J. Bacteriol.* **182**, 6482 (2000).
9. G. B. Lipford, K. Heeg, H. Wagner, *Trends Microbiol.* **6**, 496 (1998).

[1]ARC Special Research Centre for Functional and Applied Genomics, Institute for Molecular Bioscience, University of Queensland, Brisbane 4072, QLD, Australia. [2]Molecular Microbial Ecology Group, BioCentrum-DTU, Technical University of Denmark, DK-2800, Lyngby, Denmark.

*To whom correspondence should be addressed. E-mail: j.mattick@imb.uq.edu.au