SOS-inducible biofilms of bacteria treated with antimicrobial agents

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1. Pseudomonas aeruginosa under DNA replication inhibition tends to form biofilms via Arr: Bacteria infecting eukaryotic hosts often encounter therapeutic antimicrobial and DNA damage agents and respond by forming biofilms. While mechanisms of biofilm response are incompletely understood, they seem to involve a bacterial second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) signaling. We hypothesized that DNA replication inhibition induces bacterial biofilm formation via c-di-GMP signaling. Evidently, we found that *Pseudomonas aeruginosa* mounted biofilm response to the subinhibitory DNA replication inhibitors, hydroxyurea and nalidixic acid, but the planktonic proliferation was inhibited. The biofilm response was suppressed either genetically by mutations rendering planktonic resistance or biochemically by reversal of replication inhibition. Biofilms were induced by a mechanism of stimulated adhesion of planktonic filaments having impaired DNA replication as examined with fluorescence microscopy. The induction was suppressed by either inhibitor or mutation of Arr—a c-di-GMP phosphodiesterase. These results suggest that *P. aeruginosa* under DNA replication stress tends to form biofilms via Arr. The profound implication of the SOS response, the planktonic-sessile and bacteria-cancer relationships is discussed.

2. SOS involvement in biofilm formation: Bacterial biofilm formation can be induced by antimicrobial and DNA damage agents. These agents trigger the SOS response, in which SOS sensor RecA stimulates auto-cleavage of repressor LexA. These observations lead to a hypothesis of a connection between the stress-inducible biofilm formation and the RecA-LexA interplay. To test this hypothesis, we conducted three biofilm assays: the standard 96-well assay, the confocal laser scanning microscopy, and the newly developed biofilm-on-paper assay. We found that the biofilm stimulation by a DNA replication inhibitor hydroxyurea was dependent on RecA and appeared repressed by the non-cleavable LexA of *Pseudomonas aeruginosa*. Surprisingly, deletion of *lexA* led to reduction of both normal and stress-inducible biofilm formation, the observations suggesting that the wild-type LexA contributes to biofilm formation. The decreases did not result from poor growth of the mutants. These results suggest SOS involvement in the hydroxyurea-inducible biofilm formation. Besides, with the paper biofilm assay, we found that degradation of biofilm matrix DNA by DNase I appeared to render the biofilms susceptible to the replication inhibitor. The conundrums concerning the roles of LexA in DNA release and biofilm context are discussed.

Keywords *Pseudomonas aeruginosa*; bacterial biofilm; DNA replication inhibitor; SOS response; signal transduction; computer simulation.