Population dynamics during swarming of *Pseudomonas aeruginosa*

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Swarming is a group motility behavior exhibited by bacteria that coordinate to spread over surfaces. Swarms of the bacterium *Pseudomonas aeruginosa* often develop tendril patterns and tendril development requires production of the surfactant rhamnolipid. We recently showed that harder surfaces limit induction of quorum sensing genes including those responsible for rhamnolipid synthesis, but it is not yet clear why similar populations of cells should behave differently on hard surfaces compared with soft (agar) surfaces. Here we explore the population dynamics during *P. aeruginosa* swarming. We find that the population of *P. aeruginosa* does not immediately increase as the swarm expands. We also detail three stages of population development during swarming.

Swarming is a type of surface motility exhibited by *Pseudomonas aeruginosa* and other bacteria where groups of organisms coordinate to expand as an entire population over new surfaces. Robust swarming is dependent upon growth of a minimum population that is induced to produce rhamnolipid via the rhl quorum sensing system of *P. aeruginosa*. We recently showed that *P. aeruginosa* surface growth and quorum sensing vary with changes to the surface environment. *P. aeruginosa* quorum sensing and rhamnolipid production are impaired when growing on harder semi-solid agar surfaces compared with growth on soft surfaces that yields robust rhamnolipid production and swarming. It is not yet understood why quorum sensing genes near the advancing swarm edge were less induced on hard agar; even addition of excess acyl homoserine lactone did not improve swarming. We have further investigated the differentiation between populations of cells growing on these two types of surfaces. Here we show that swarm zone expansion is not synchronous with population growth under conditions of optimal swarming.

Given the increased surface coverage that occurs for tendril-forming swarms, we theorized that this spreading should provide a growth advantage. Swarming was investigated on both soft and hard agar plates containing FAB-glucose. Plate assays were inoculated with 1 μL culture containing 2.0 ± 0.1 x 10^7 cfu (pregrown planktonically on FAB-glucose) and incubated at 30°C for 39 h. At designated intervals, swarms were harvested using sterile spatulas and collected in 10 mL sterile buffer. These samples were serially diluted and 10 μL of each dilution was spotted on LB plates (in triplicate) for five replicates (i.e., n = 15) and incubated at 28°C for ~24 h.

We observed that swarming expansion was not synonymous with increases in bacterial cell population. Swarms that develop on soft vs. hard agar can be differentiated into three distinct stages of behavior (Fig. 1). During Stage I (approximately the first 20 h of our experiment) there are no differences between soft and hard agar swarms. During Stage II (hours 20–30) we see expansion of soft agar swarms (Fig. 1B) but no expansion of hard agar swarms (Fig. 1E). Curiously, the total bacterial population is not different on soft and hard agar at Stage II—both swarm types have increased nearly two orders of magnitude from Stage I. During Stage III (greater than 30 h) we see a continued expansion of swarm area.

**Key words:** rhamnolipid, motility, bacterial growth

Submitted: 07/04/11
Accepted: 07/04/11
DOI: 10.4161/cib.4.6.17109

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Addendum to: Kamatkar NG, Shrout JD. Surface hardness impairment of quorum sensing and swarming for *Pseudomonas aeruginosa*, PLoS ONE 2011; 6:20888; PMID:21687741; DOI:10.1371/journal.pone.0020888

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of Xavier et al. who showed a significant growth advantage by bacteria swarming on soft agar over non-swarming bacteria on hard agar or a rhamnolipid-deficient mutant. Stages I–III are readily distinguished by plotting the change in area and fluorescence intensity per unit time (i.e., the first derivative of the area and total fluorescence with time).

The findings we classify as Stage II, where no growth advantage is apparent between these swarm conditions were not expected. We were initially surprised that Stage II lasted for such a long duration, namely hours in our experiments. Close inspection of the cell counts, however, suggests that bacterial growth during Stages II and III of swarming is comparatively slow to the transition between Stage I to Stage II. Thus, as soft agar swarm populations produce ample rhamnolipid to aid swarming, swarm spreading becomes rapid compared with cell growth.

It is still unclear why quorum sensing and rhamnolipid production is more inhibited on hard agar despite similar populations of cells. This result, however, suggests that bacterial growth is not inhibited under poor swarming conditions. It will be useful to probe these populations further to understand the physiology of bacteria growing on optimal swarming conditions. Previous study suggests these cells are sensing a better nutrient environment and are more induced for quorum sensing.7,9

Acknowledgments
This work was supported by the Indiana Clinical and Translational Science Institute (NIH # UL1RR025761) to J.D.S. and the University of Notre Dame College of Science (Summer Undergraduate Research Fellowship) to M.J.S.
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Figure 2. Fluorescent imaging analysis of P. aeruginosa swarming over time on (A–C) soft (0.45%) and (D–F) hard (0.6%) agar. Representative fluorescent images from (A and D) Stage I (~0–20 h), (B and E) Stage II (20–30 h), and (C and F) Stage III (>30 h). Fluorescence intensity from all images is pseudo-colored to display highest cell density in white. (G) Rate of change (i.e., first derivative) in the swarm area and population with time.