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Introduction



by Mavra Nasir, Quantitative Biomedical Science (QBS)

Pseudomonas aeruginosa is an opportunistic pathogen.

P. aeruginosa is often difficult to treat due to its ability to produce biofilms, in which cells are surrounded by a self-produced extracellular matrix of polysaccharides, lipids, and proteins.

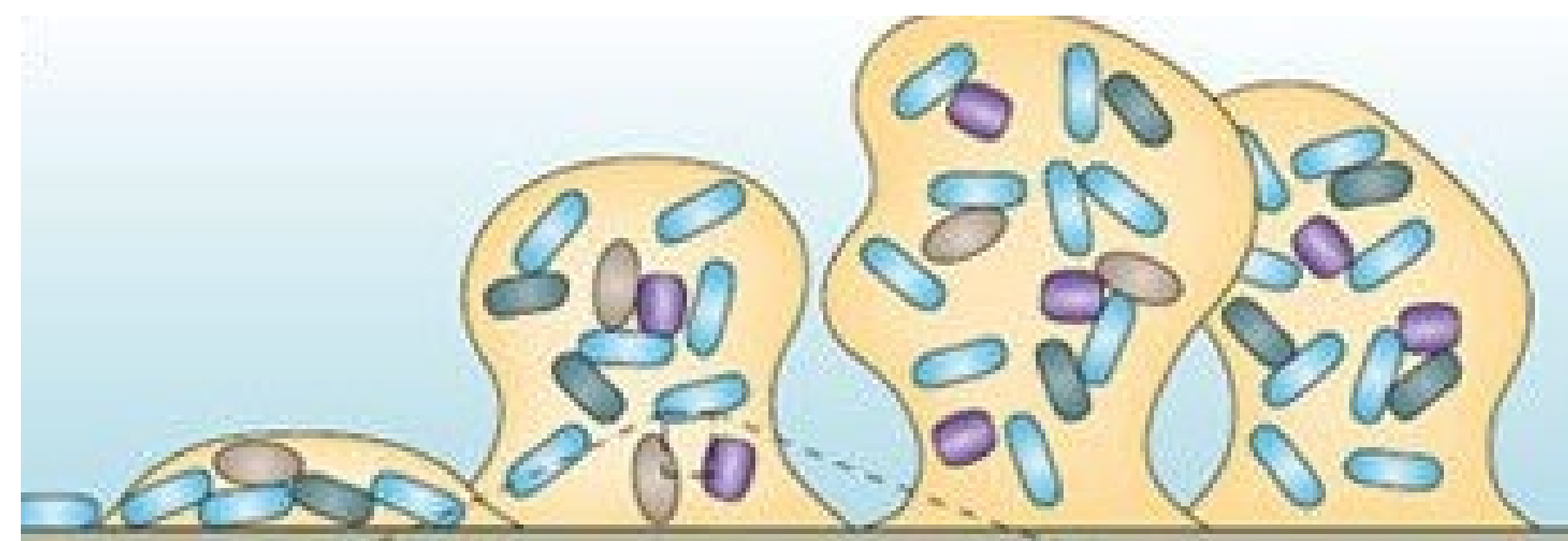


Figure edited from Flemming, H. C., & Wingender, J. (2010). The biofilm matrix. *Nature reviews microbiology*, 8(9), 623.

These biofilms offer a barrier to protect the bacteria from natural host defenses and treatments such as antibiotics.

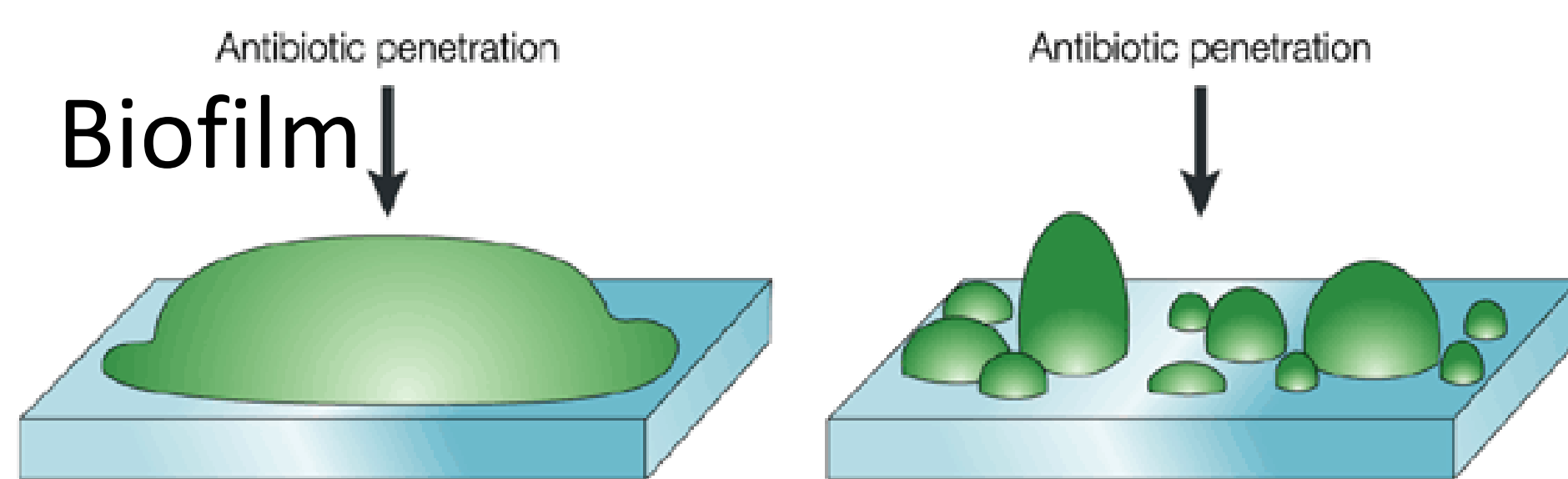


Figure edited from Davies, D. (2003). Understanding biofilm resistance to antibacterial agents. *Nature reviews Drug discovery*, 2(2), 114.

Methods

Visual Assay

Once colonies with the transposon insertion were grown, colony morphology could be observed to assess the amount of biofilm formation.



Non-wrinkled control strain: PA14



Moderately wrinkled control: PA14_DamrZ

Wrinkling is directly associated with increased biofilm production. The smooth strain, PA14, is smooth in order to be able to observe an increase of wrinkling in transposon-inserted colonies. If there is a change in wrinkling, selected mutants move on to be sequenced.

Transposon Mutagenesis

By mating *E. coli* with a moderately wrinkled *P. aeruginosa*, strain DamrZ, a transposon was inserted. Using selective media with IRG, an antibiotic that transposon carried resistance to, and gentamycin, an antibiotic lethal to *E. coli*, *P. aeruginosa* colonies with the transposon insertion was selectively grown.

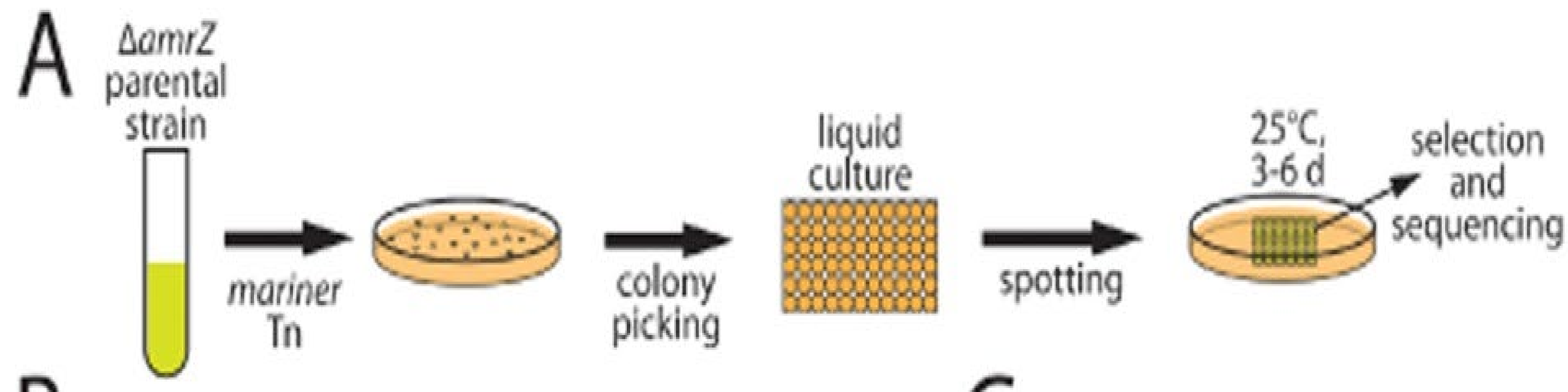
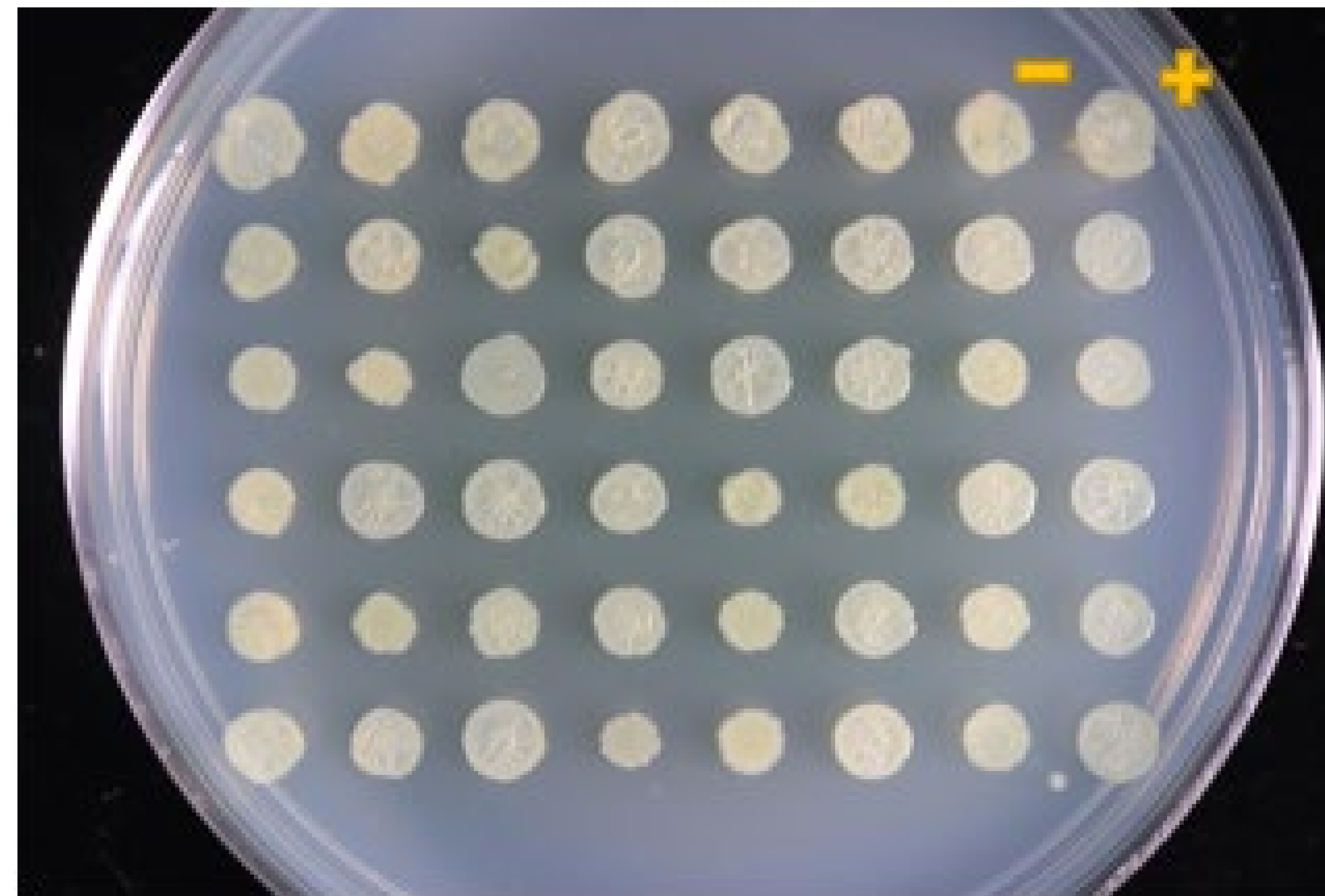


Figure edited from] Cabeen, M. T., Leiman, S. A., & Losick, R. (2016). Colony-morphology screening uncovers a role for the *Pseudomonas aeruginosa* nitrogen-related phosphotransferase system in biofilm formation. *Molecular microbiology*, 99(3), 557-570.



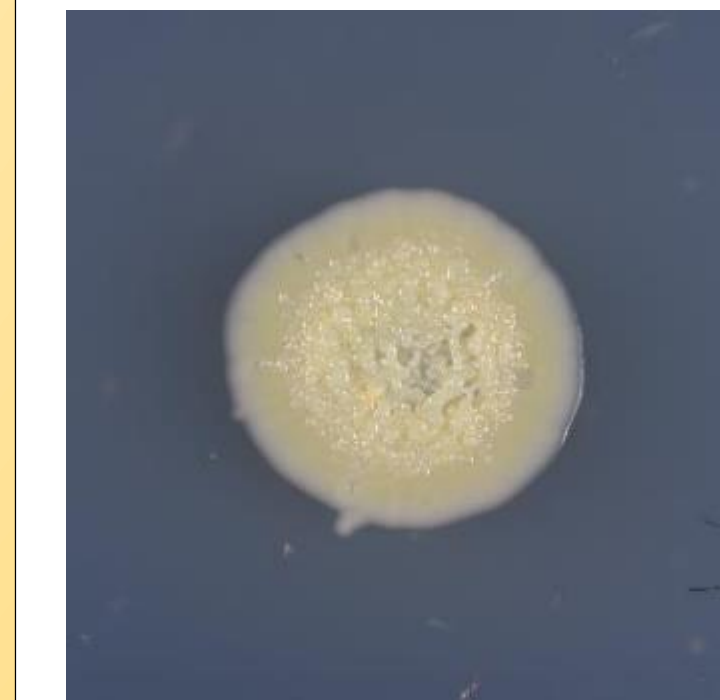
Visual assay for wrinkling in PA mutants

- Smooth control, PA14
- + Moderately wrinkled control, PA14_DamrZ

Sequencing

Following the visual assay for the transposon-inserted mutants, mutants are purified using PCR to find the location of the transposon insertion.

Results



CSS 149

PA_30280, trxB1
thioredoxin reductase

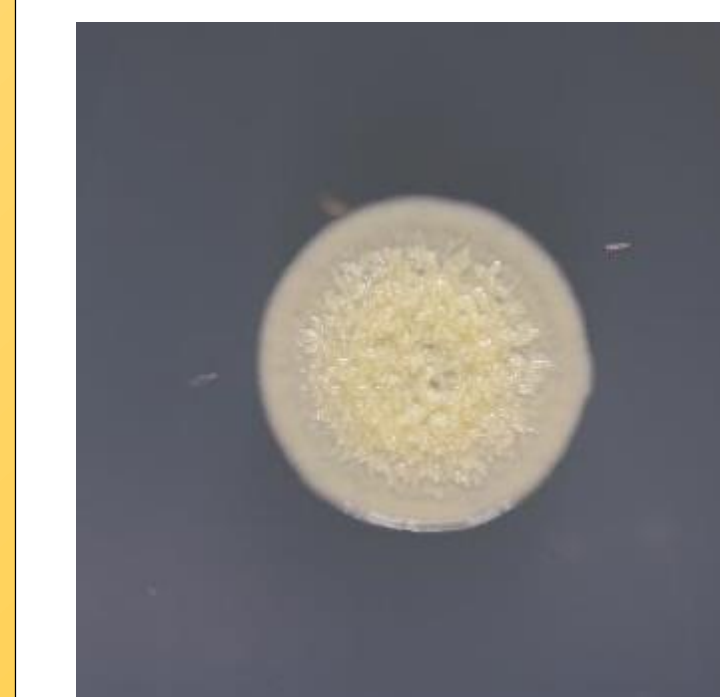
Thioredoxin reductase plays a role in DNA synthesis and defense against oxidative stress



CSS 136

PA14_71530 PurU2

Formyltetrahydrofolate Deformylase
Activated by host responses in infections



CSS 150

Upstream PA14_21970=PA3249
(transcriptional regulator)

No known interaction with biofilm production

intergenic region 21960 and 21970

Conclusion:

Mutants 136, 149, and 150 show phenotypic changes consistent with biofilm production increase which can be associated with genes not previously known to have a role in biofilm formation.

Future Directions

In the future, we will work to quantify the amount of biofilm being produced by using a Congo Red dye assay and by measuring cyclic diGMP levels in the cell.

Acknowledgements

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