Inhibition of high-priority respiratory pathogens is a general feature of the fastidious nasal commensal *Dolosigranulum pigrum*

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Objectives

- In 2019, antimicrobial resistance contributed to almost 5 million deaths globally[1], highlighting a need to find alternatives.
- The nasal commensal *Dolosigranulum pigrum,* present within ~40% of human nasal passages, rarely causes infections, and is negatively associated with several other nasal pathogens, making it a promising potential probiotic[2,3,4].
- Much is unknown about the species' core genomic features as there are only 28 published genomes to date, and it is currently the sole member of the genus[5].
- The mechanism of action preventing pathogenetic colonization is still not well understood[3,4].

Objectives

- To expand the existing database of *D. pigrum* isolates and sequenced genomes
- To develop a quantitative assay to assess the effectiveness of *D. pigrum* strains at antagonizing various respiratory pathogens
- To determine the mechanism of action *D. pigrum* uses to inhibit growth of pathogens
- To determine strains of *D. pigrum* that are well suited for probiotic use by characterizing their growth rate, inhibition strength, and carrying capacity

Conclusions

- *D. pigrum* is capable of directly inhibiting the growth of common nasal pathogens
- There is a high variability of relative growth rate, carrying capacity, and antagonistic strength even in genetically similar *D. pigrum* strains.
- There are no strong correlations between growth rate or carrying capacity and antagonistic strength.
- Some strains of *D. pigrum* possess a high carrying capacity and antagonistic strength and low growth rate, making them prime probiotic candidates.

Methods

- 117 participants from the Washington, DC area had nasal swabs collected and were sequenced to identify microbiome composition.
- Swabs with >105 *D. pigrum* 16S copies/uL were cultured, yielding 41 *D. pigrum* strains from 39 participants and added to an existing strain collection.
- Growth rate and carrying capacity were assessed via optical density measured in a 96-well plate
- To measure antagonistic strength a standard concentration of *D. pigrum* was spot inoculated onto an agar plate and incubated for 22-24 hours. Then, a standardized concentration of pathogen culture is spot inoculated over *D. pigrum* growth and incubated for an additional 16-24 hours. The zone of inhabitation diameter around the *D. pigrum* was then measured.
- All measured attributes were classified into high (>1 SD+mean), low (< mean-1 SD), or medium bins.

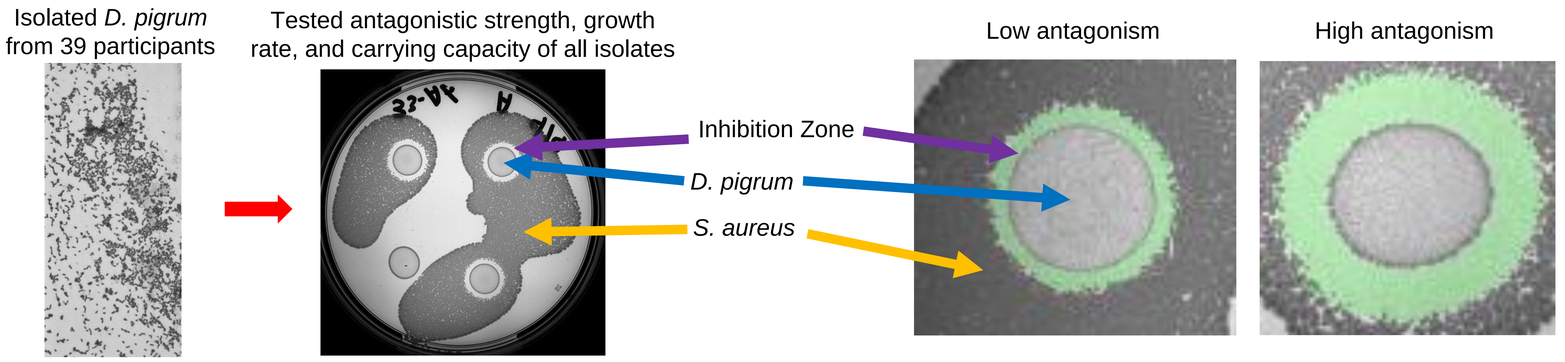


Figure 1: Methods used for assessment of *D. pigrum* antagonistic strength

Results

- Antagonistic strength varied significantly between isolates (Figure 2A). Genetically similar isolates showed high variability in antagonistic strength. Out of 56 isolates tested, 55 displayed at least some antagonism against *S. aureus, A. baumanii, P. aeruginosa,* and *M. catarrhalis* (Figure 2B).
- Doubling time and carrying capacity was highly variable between strains (Figure 3). Genetically similar strains were not necessarily similar in behavior. Some strains, such as that marked in purple, had fast doubling time, high carrying capacity, and high antagonism strength, making them ideal probiotic candidates.
- Antagonism against one pathogen was well correlated with antagonism against other pathogens (Figure 4). Neither carrying capacity nor growth rate was significantly correlated with antagonism against any pathogen.

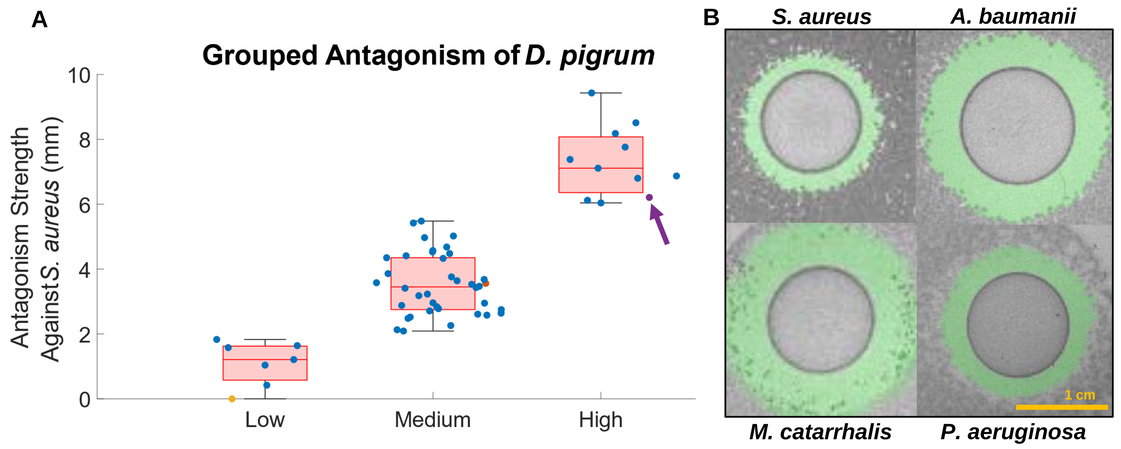
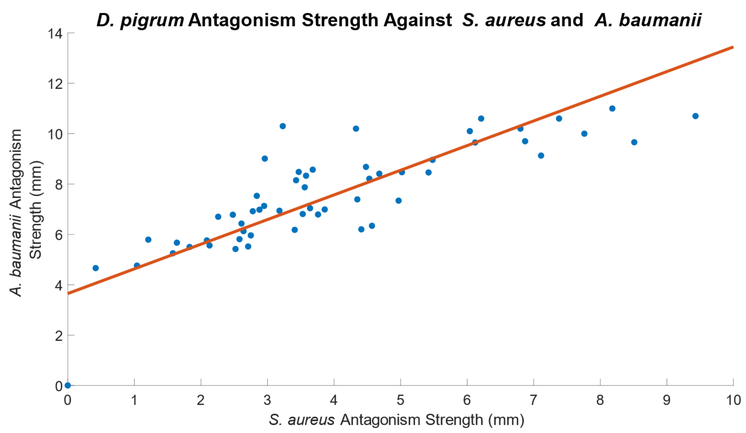


Figure 2: Antagonism of *D. pigrum* against *S. aureus* and other pathogens. Genetically similar isolates are highlighted in red and yellow. Purple arrow highlights a potential probiotic candidate.



Pathogen	S. aureus	A. baumanii	P. aeruginosa	M. catarrhalis
S. aureus	1	.84	.85	.60
A. baumanii	.84	1	.94	.78
P. aeruginosa	.85	.94	1	.68
M. catarrhalis	.60	.78	.68	1

Figure 4: Spearman correlations in antagonistic strength of *D. pigrum* against multiple pathogens.

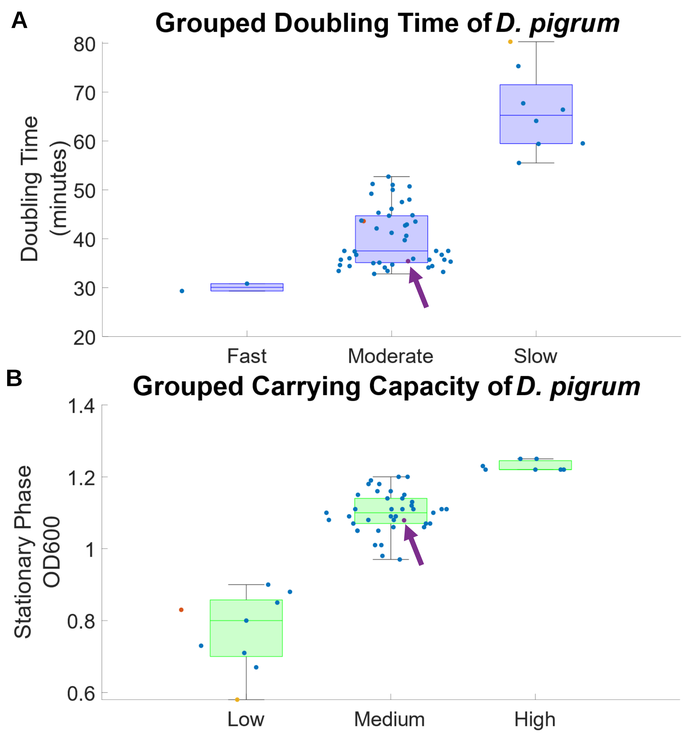


Figure 3: Growth characteristics of *D. pigrum.* Genetically similar isolates are highlighted in red and yellow. Purple arrow highlights a potential probiotic candidate.

Acknowledgements

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