Purine and Carbohydrate Availability Drive Enterococcus faecalis Fitness During Wound and Urinary Tract Infections

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BACKGROUND

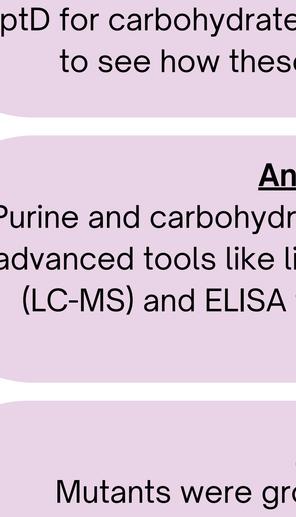
Wound infections represent a major global health challenge, affecting approximately 11 million people annually and costing healthcare systems over $20 billion. Chronic wounds, such as diabetic ulcers, are especially prone to bacterial colonization. Among these, Enterococcus *faecalis* is a persistent and resilient pathogen. Its ability to thrive in diverse environments, resist antibiotics, and form biofilms complicates treatment and prolongs infection. Despite its prevalence in both acute and chronic wounds, the metabolic mechanisms driving E. *faecalis* survival and virulence in these settings remain poorly understood. This study investigates the critical role of purine biosynthesis and carbohydrate transport in E. *faecalis* fitness and persistence during infections, offering new insights into potential therapeutic targets in the treatment and prevention of infection of wounds such as surgical sites, diabetic wounds, and chronic ulcers.

METHODS

Samples taken from mice infected with *E. Faecalis*

Mouse wounded and infected with *E. faecalis* — Samples taken — Samples taken

t=0h — **t=8h** (8hpi) — **t=72h** (3dpi)



Transposon Sequencing (Tn-seq): Screened ~15,000 mutants for fitness genes.

RNA Sequencing (RNA-seq): Analyzed gene expression changes in E. *faecalis* during infection.

Testing Mutant Strains: Mutant strains missing key genes (purEK for purine synthesis, mptD for carbohydrate transport) were tested in wound infections to see how these deletions affected bacterial survival.

Analyzing Nutrient Levels: Purine and carbohydrate levels in wounds were measured using advanced tools like liquid chromatography-mass spectrometry (LC-MS) and ELISA to understand nutrient availability during infection.

Growth Experiments: Mutants were grown in purine- or carbohydrate-limited environments to confirm the importance of these nutrients for bacterial fitness.

RESULTS AND ANALYSIS

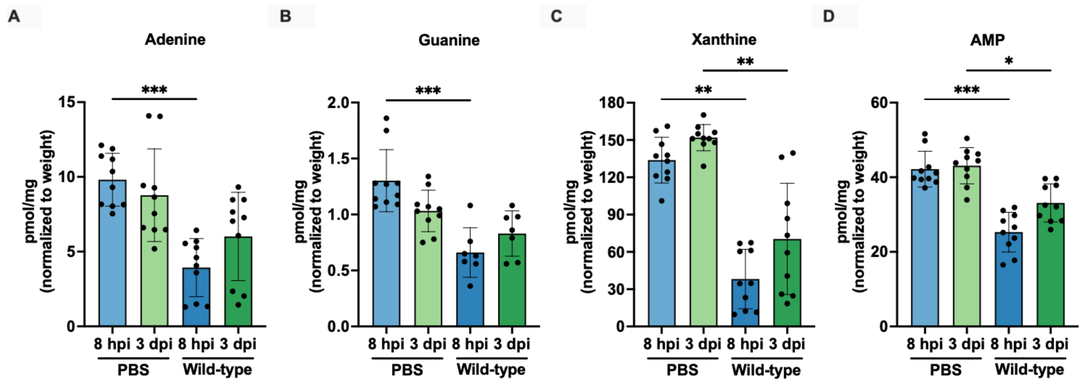


Figure 1 "data represents one mouse, and error bars represent SD from the mean; N = 2, n = 5 mice per group per experiment. Statistical analysis was performed using the Mann-Whitney U test; *P < 0.05, **P < 0.01, ***P < 0.001."

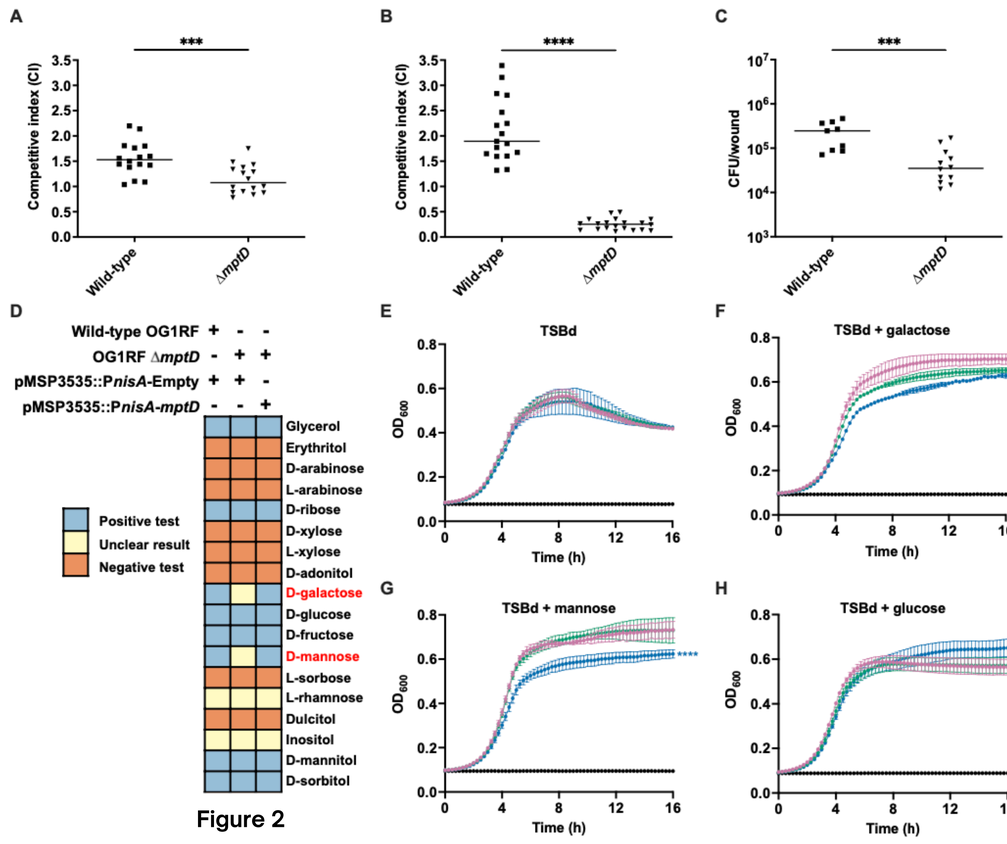


Figure 2

Figure 1. De novo purine biosynthesis is critical for E. *faecalis* during early infection when purines in the wound environment are scarce. Significant reductions in adenine (60%), guanine (49%), and AMP (40%) at 8 hpi.

Figure 2. E. faecalis de novo purine biosynthesis contributes to E. faecalis replication during acute infection as well as to persistence in wounds.

Figure 2A. Tn-seq data revealed pur operon mutants were underrepresented

Figure 2B. Significant fitness reduction in ΔmptD mutants.

Figure 2C. Complementation restored growth, confirming the necessity of purine biosynthesis.

Figure 2D. Results shown are a subset of the 50 carbohydrates and corresponding results .

Figures 2E-H. Growth kinetics of wild-type OG1RF pMPSP3535::PnisA-Empty, OG1RF ΔmptD pMSP3535::PnisA-Empty, and OG1RF ΔmptD pMSP3535::PnisA-mptD in TSBd media supplemented (**E**) without additional carbohydrates and with 1% (wt/vol) (**F**) galactose, (**G**) mannose, and (**H**) glucose over 16 h.

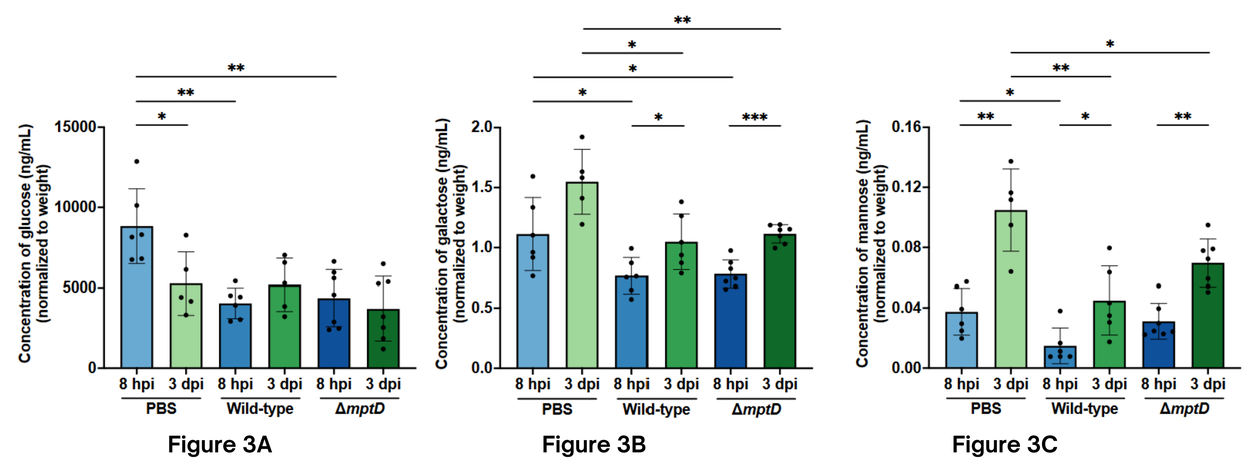


Figure 3A — Figure 3B — Figure 3C

Each data point represents measurement from one mouse; error bars represent SD from the mean; N = 1, n = 6–7 mice. Statistical analysis was performed using the Mann-Whitney U test; *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 3A-C. Carbohydrate availability changes as E. faecalis wound infection progresses. Mice were wounded and inoculated with sterile PBS, wild-type OG1RF, or OG1RF ΔmptD. Wounds were harvested at 8 hpi and 3 dpi and subjected to (**A**) glucose, (**B**) galactose, and (**C**) mannose quantification.

CONCLUSION:

This study reveals that metabolic pathways for purine biosynthesis and carbohydrate transport are indispensable for E. *faecalis* fitness in infection settings. Purine Biosynthesis: is critical for replication during early infection when purines are scarce. MptABCD PTS is shown to be essential for persistence in dynamic wound environments where carbohydrates are limited.

Potential Therapeutic Implications include targeting purine biosynthesis for inhibit acute bacterial growth and inhibiting MptABCD transport also offers a strategy to prevent bacterial persistence. These pathways represent promising therapeutic targets for managing chronic infections caused by E. *faecalis*.

REFERENCES:
Tan CAZ, Chong KKL, Yeong DYX, Ng CHM, Ismail MH, Yap ZH, et al. Purine and carbohydrate availability drive Enterococcus *faecalis* fitness during wound and urinary tract infections. mBio [Internet]. 2023 Dec 11;15(1). Available from: https://doi.org/10.1128/mbio.02384-23