

From Genome to Host

Bioinformatics Insights into Enterococcus faecalis

The analysis included identifying Enterococcus faecalis from a DNA fragment, annotating genes and proteins, analyzing metabolic pathways, protein structures, overexpression vectors, domains and interactions, studying pathogenic mechanisms, human immune responses, phylogeny, gene regulation and genomic islands, transmission, and proposing treatment strategies.

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Introduction

Organism information

E. faecalis is a gram positive, coccus shaped lactic acid bacteria, which thrives in the nosocomial environment and is linked to various infections such as UTIs, endocarditis and bacteraemia. [1]

E. faecalis displays multiple traits that increase its virulence.

- **Adherence to the host tissue** - 4 specific types
- **Capsular polysaccharides** - evade phagocytosis
- **Sex pheromones** - conjugative plasmid system
- **Etracellular superoxide** - disruptive oxygen radical
- **Hydrolytic enzymes** - Gelatinase and Hyaluronidase
- **Cytolysin** - forms pores in the cytoplasmic membrane of target cells
- **Biofilm formation** - aids survival in harsh conditions

Glutamine-fructose-6-phosphate transaminase (isomerizing)

The protein Glutamine-fructose-6-phosphate transaminase (isomerizing) Figure 1, is encoded by ORF14 and takes part in carbohydrate metabolism as an enzyme.

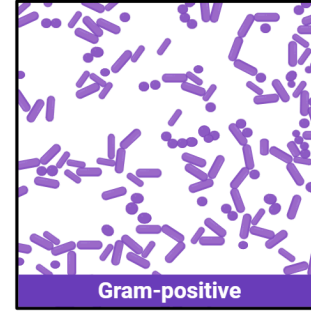
Role in virulence

E. faecalis can mitigate the host immune response by downregulating its carbohydrate metabolism, which also helps in facilitating its own survival in macrophages. [2] As Glutamine-fructose-6-phosphate transaminase (isomerizing) is a carbohydrate derivative and has a role in biosynthetic processes it is also affected by this downregulation. [3]

Figure 1: Experimental structure of Glutamine-fructose-6-phosphate transaminase protein from PBD



Figure 2: Predicted structure of Phosphoglucosamine mutase from AlphaFold



Pathogenicity islands

Pathogenic islands: IslandViewer 4 analysis of *E. faecalis* ATCC 29212, against reference genome of *E. faecalis* V583, detected genomic islands elsewhere, but none within the 84–104 kb fragment, confirming no virulence or resistance genes. [4]

Genes: Contains 15 annotated “housekeeping” and metabolic genes linked to cell-wall biosynthesis, amino-sugar metabolism, energy conversion, stress regulation, nutrient transport, and signaling control. [5]

GC Content: The 20 kb fragment shows a 38.1% GC content, matching *E. faecalis*'s genome average (~37.5%), indicating native chromosomal origin without horizontal gene transfer evidence.

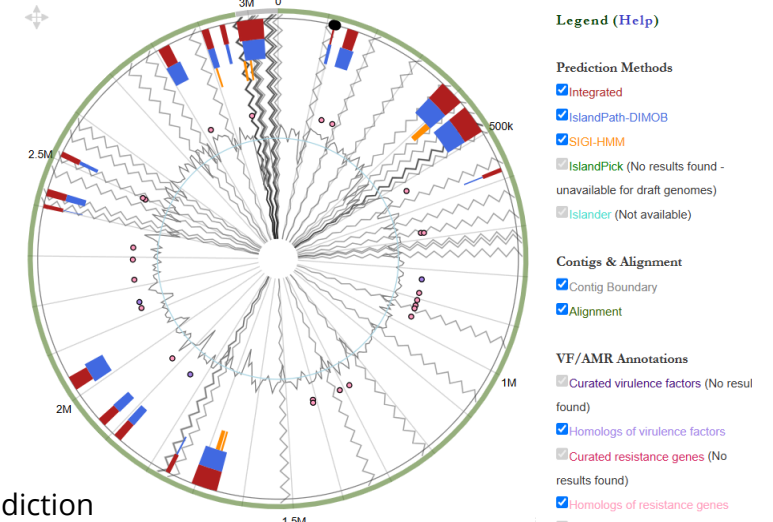
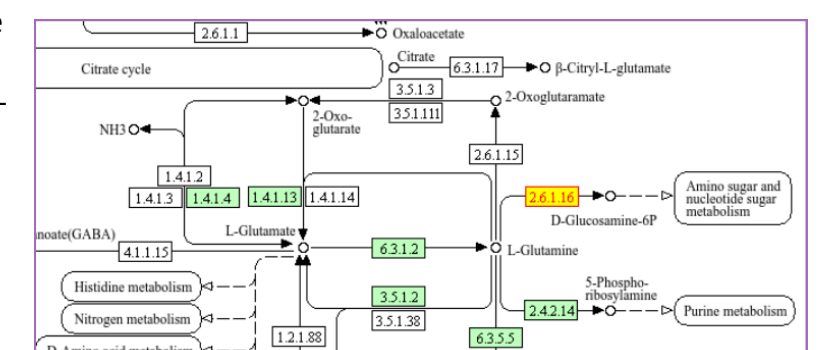


Figure 3: GI prediction

Metabolic Pathways

KEGG orthology: mapping links six genes to metabolic and regulatory pathways, showing evolutionary conservation. Most enzymes participate in shared bacterial processes like carbohydrate, nitrogen, and tRNA metabolism, while *cdaA*'s c-di-AMP signaling is uniquely restricted to Gram-positive bacteria. [3]

Figure 4: The enzyme catalyzes the first step in hexosamine metabolism, converting fructose-6-phosphate into glucosamine-6-phosphate using glutamine as a nitrogen source leading to Amino sugar and Nucleotide sugar biosynthesis.



Enzymes: *glmS* and *glmM* for peptidoglycan biosynthesis, *glnA* and *nifJ* for nitrogen and energy metabolism, *cdaA* for signaling, and *miaA* and *hflX* for translation stability—indicating conserved core metabolic functions. [3]

Methods

GenBank annotation – Verified gene functions.

KEGG mapping – Linked genes to pathways.

IslandViewer 4 – Detected genomic islands.

GC content – Calculated nucleotide composition.

Orthology analysis – Compared evolutionary conservation.

Enzyme classification – Assigned EC/KO roles.

Comparative genomics – Aligned with reference genome.

Benchling - Overexpression vector and related analysis

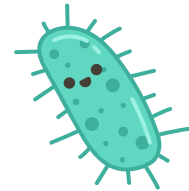
ORFfinder - Can find ORFs in a DNA sequence

UniProt - Information on proteins

InterPro - Information of protein domains and functions

BLAST (NCBI) – Identified homology and annotations of DNA and protein sequences.

Literature study – Reviewed *E. faecalis* research.



Overexpression vector

Experimental design

The glutamine fructose 6P transaminase, encoded by ORF14 in the target sequence was amplified in silico via PCR and cloned into and overexpression vector.

The GOI extends in the original sequence from bp 3396 to bp 1588, on the negative strand. Two primers were designed to flank the GOI on either side:

Forward primer: EcoRI restriction site
Backward primer: BamHI restriction site

In silico PCR was performed to obtain the cloned GOI. The pET vector was chosen and the final construct (figure 6) was successfully assembled through a digestion and ligation protocol, using BamHI and EcoRI as restriction sites.

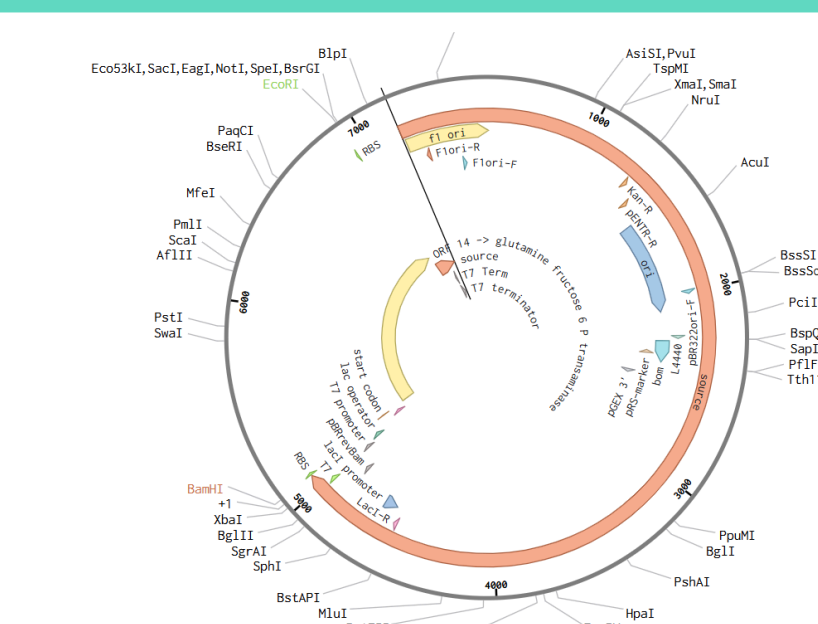


Figure 6: Final construct. Yellow arrow indicates ligated GOI

Relevance

Glutamine 6P transaminase is involved in carbohydrate metabolism. The overexpression leads to a decreased virulence, as the pathogens ability to downregulate its metabolism and avoid host detection is hindered. [2]

Transmission and infection

Cause of infection

The overgrowth of the bacterium in the gut increases the probability of it translocating to the bloodstream and to the other body parts. [15]

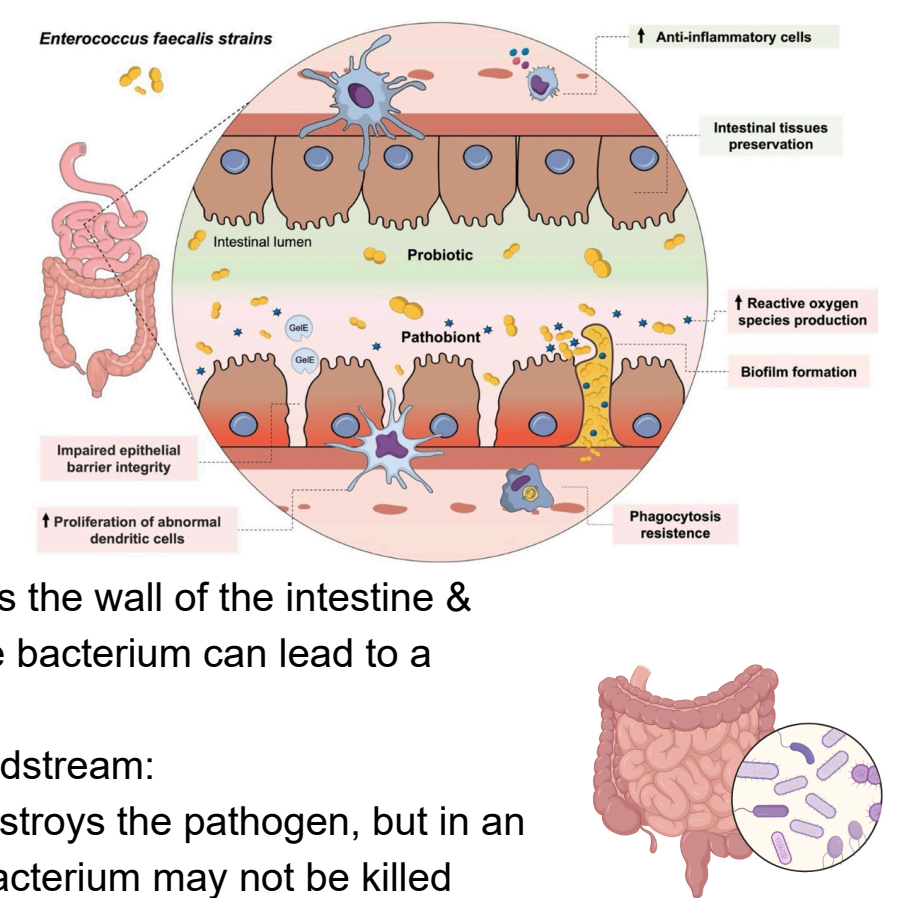
Transmission

The transmission of the bacterium can happen through both direct contact and indirect contact. [16]

Figure 5: Dual characteristics of *E. faecalis* depending on environment

Mechanisms of Translocation

- **Engulfing** of the pathogen by macrophages:
 - Once engulfed, the bacterium is transported across the wall of the intestine & into the underlying lymph system. Failure to kill the bacterium can lead to a systemic spread.
- **Diffusion** of the Enterococci from the gut into the bloodstream:
 - In a healthy individual, the innate system easily destroys the pathogen, but in an individual with a weakened immune system, the bacterium may not be killed which can lead to contamination of other body parts and to an infection. [15]



Host Immunity

Transmission Pathways

Spreads mainly via the fecal–oral route and persists on surfaces due to environmental resilience. In hospitals, biofilm formation enables transmission through contaminated equipment and hands, while endogenous strains cause infections when host defenses weaken. [6][7][8][9][10]

Immune response

Host recognition of *E. faecalis* via TLR2 triggers inflammation and neutrophil recruitment, yet the bacterium's stress-response and biofilm genes promote persistence. It resists clearance, alters cytokine signaling, and causes chronic or site-dependent immune modulation. [11][12][13][14]

Possible treatments

Preventative measures

- Proper hygiene in hospitals
- Consuming the prescribed amount of antibiotics for the prescribed duration to reduce the chances of bacterium mutating and developing resistance to the particular antibiotic
- Hosting educational campaigns to raise public knowledge.

Possible therapies

- Combining two beta-lactam antibiotics, such as cephalosporins, which work synergistically & bind with penicillin-binding proteins, inhibiting cell wall synthesis, and hence, destroying the cell. [17]
- Combining the cell wall-active agents, such as amoxicillin, with aminoglycosides such as gentamicin. [17][18]
- Ozonated Aloe Vera diluted in 70% ethanol and chlorophorm showed moderate results for treating root canal infections caused by the bacteria. [19]

Conclusion

Even though the bacterium is intrinsically resistant to the individual antibiotics, it can be destroyed by combining the antibiotics together. However, due to the plasticity of the Enterococci's genome, a definitive cure has not been found and likely will not be found which makes preventative measures a high priority. [20]

