Investigation of Bacterial Microcompartment Assembly in *Fusobacterium nucleatum*

Diane Nguyen[1]*, Sophia Choi[1]*, Dana Franklin[2], Yi-Wei Chen[3], and Hung Ton-That[2,3]

[1]Bruins in Genomics, University of California, Los Angeles, California, USA; [2]Molecular Biology Institute, University of California, Los Angeles, California, USA; [3]Division of Oral & Systemic Health Sciences, School of Dentistry, University of California, Los Angeles, California, USA; *Contributed equally

Abstract

Fusobacterium nucleatum is an oral commensal but associated with adverse pregnancy outcomes. Preliminary studies revealed that *F. nucleatum* utilizes ethanolamine from the placenta as nutrients through an ethanolamine utilization (Eut) system promoting preterm birth. This process involves the formation of bacterial microcompartments (BMCs) to compartmentalize ethanolamine metabolism. However, it is unclear whether the BMCs represent a widespread evolutionary adaptive feature. By analyzing Eut orthologs in approximately 69 *Fusobacterium* genomes from available databases, we found significant variations of Eut determinants among different *Fusobacterium* species and subspecies, but high conservation in the same taxonomy groups. To study BMC assembly, we constructed a recombinant plasmid expressing potential BMC components (EutL/M_1/M_2/N), using crossover PCR with specific primers. The generated plasmid were transformed into *Escherichia coli* DH5α. Once confirmed by DNA sequencing, this plasmid will benefit future studies that examine BMC formation in a heterologous system to determine the essential BMC determinants in *F. nucleatum*.

Background

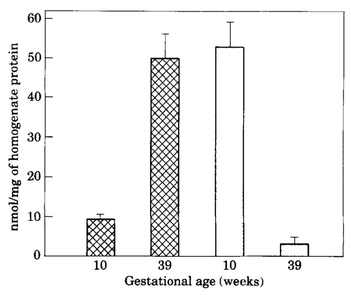
Fusobacterium nucleatum

- Gram-negative anaerobic opportunistic pathogen primarily found in the oral cavity of humans.
- Utilizes ethanolamine as a vital source for carbon and nitrogen.
- One of the most prevalent species implicated in adverse pregnancy outcomes (APOs).

Brennan & Garrett (2019)

Ethanolamine Utilization and APOs: As an Oral Species, Why is *F. nucleatum* Attracted to the Placenta?

- During pregnancy, it was found that a significantly higher level of ethanolamine was detected at week 39, in comparison to other substances or amino acids.

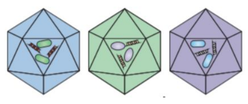


Then, is ethanolamine utilization an important factor leading to APOs?

Sooranna, Burston,, Ramsay, & Steer. (1994)

eut Genes and Bacterial Microcompartments (BMCs)

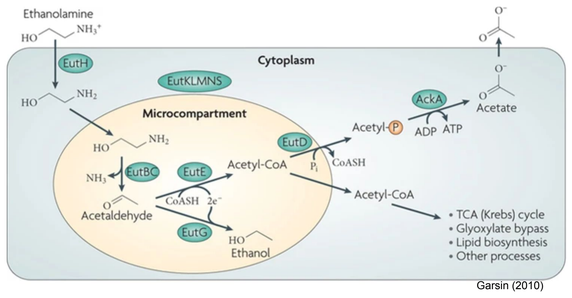
- Genes coding for an ethanolamine utilization (eut) system are clustered in the genome of *F. nucleatum* and other bacteria.
- Ethanolamine utilization in *F. nucleatum* involves the formation of BMCs, which are compartmentalized organelles that enhance enzymatic efficiency and protect bacterial cells from toxic intermediates.



Kerfeld. *et al.* (2018)

Why is this Significant in Our Study?

- In enterococci, the genes *eutK, eutL, eutM*, *eutN*, and *eutS* code for the structural proteins of BMCs.



Garsin (2010)

- In *F. nucleatum*, *eutK* is absent and it is not clear if *eutS* is active, nor is the function of several hypothetical proteins such as *2810* and *2820* whose orthologs are not found in some of the other species.
- Although ethanolamine utilization is associated with the bacterial pathogenesis, not much is known about the process of BMC assembly or whether BMCs are a widespread evolutionary adaptive feature.

Genomic Comparison of *Fusobacterium* Species

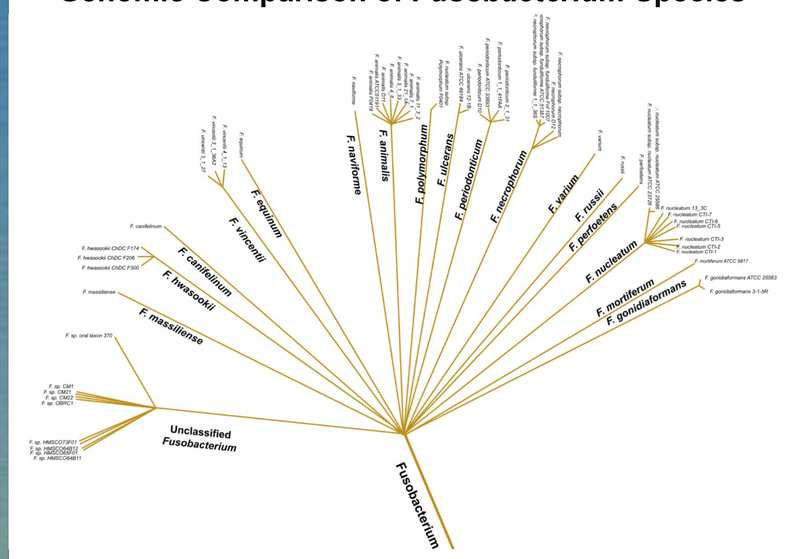


Fig. 1 Phylogenetic Tree of *Fusobacterium* species. The phylogenetic tree modified from NCBI Lifemap shows 69 *Fusobacterium* species which were analyzed in this study.

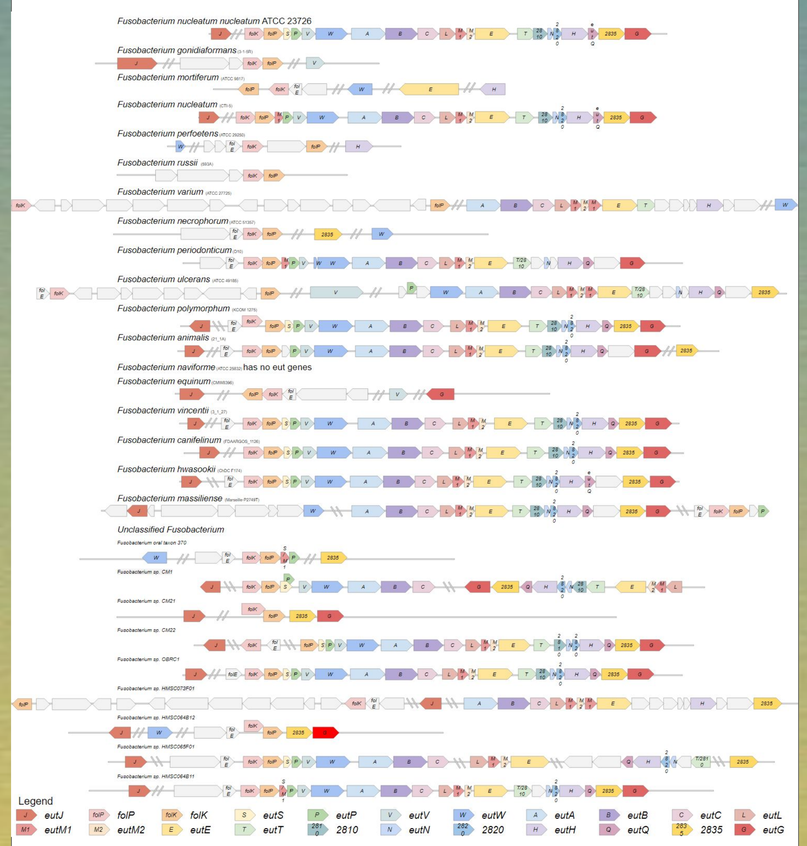
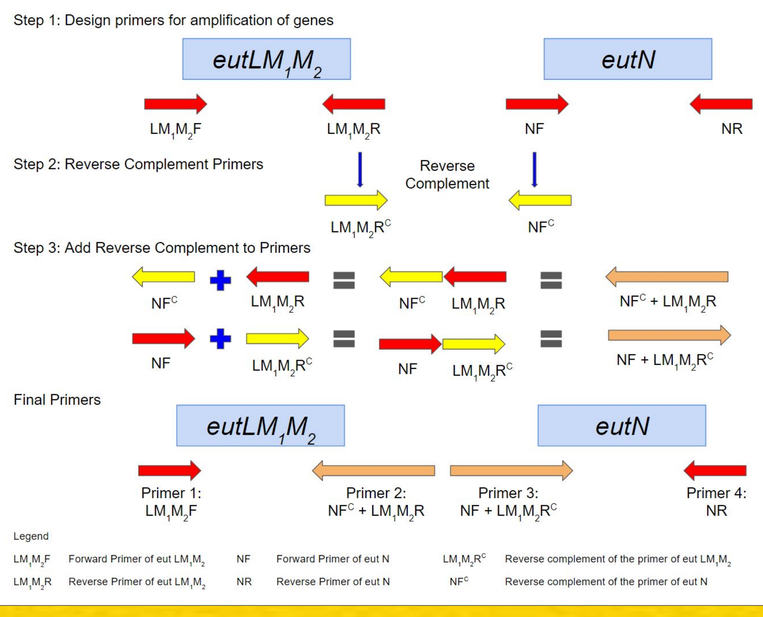


Fig. 2 Conservation of *eut* Genes in Fusobacterial Species. Homology and BLAST analyses reveal the conservation and variation of the eut genes in various *Fusobacterium* species, including *F. nucleatum*, *F. animalis*, *F. polymorphum*, and *F. vincentii.* Similar genes are color-coded.

Engineering of Recombinant Plasmid Expressing *eutLM₁M₂N* in *Escherichia coli* DH5α

Primer Design



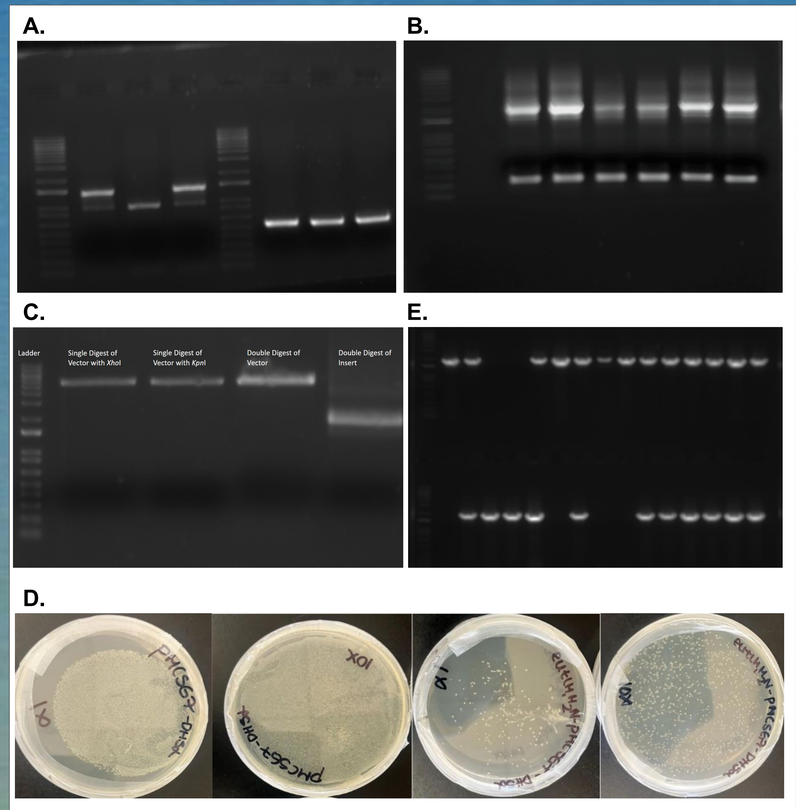


Fig. 3 Construction of an *eutLM₁M₂* Recombinant Plasmid. (A) Shown are PCR products of *eutLM₁M₂* and *eutN*. **(B)** Crossover PCR generated the *eutLM₁M₂N* product. **(C)** The presence of insert DNA in the cloned plasmid (pMCSG7 backbone) was verified by digestion. **(D)** The generated plasmid was transformed into *E. coli* DH5α and selected agar plates containing carbenicillin. **(E)** Shown are colony-PCR products following transformation for confirmation.

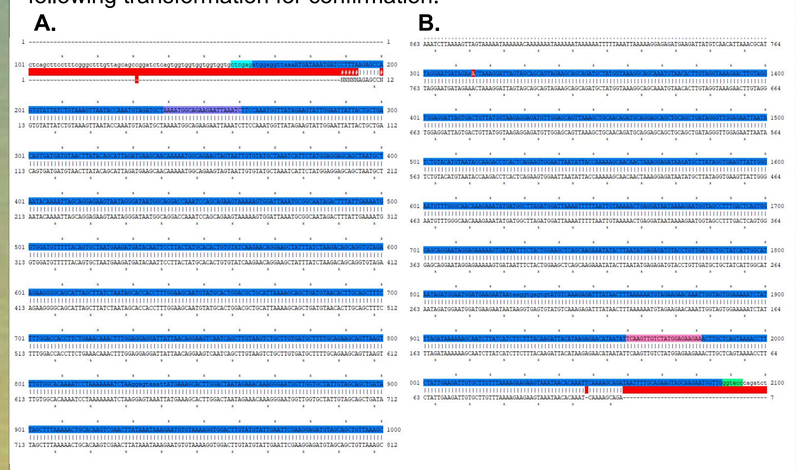


Fig. 4 Confirmation of the Recombinant Plasmid expressing *eutLM₁M₂N* by DNA sequencing. Plasmid DNA was subjected to DNA sequencing with specific primers targeting to **(A)** *eutL*, and **(B)** *eutN* in the insert *eutLM₁M₂N*.

Conclusions/Discussions

- Within the *Fusobacterium* species, we found vast variations in the *eut* genes.
  - Possibly different mechanisms were evolved in metabolizing ethanolamine as a carbon and nitrogen source.
  - The results generated can lead to future research that examines potential variation of ethanolamine utilization mechanisms.
- We were able to construct a recombinant plasmid expressing EutLM₁M₂N.
  - Can be beneficial to the future studies dissecting the mechanisms of BMC assembly.

Acknowledgements

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