Investigation of



Bacterial Microcompartment Assembly in Fusobacterium nucleatum

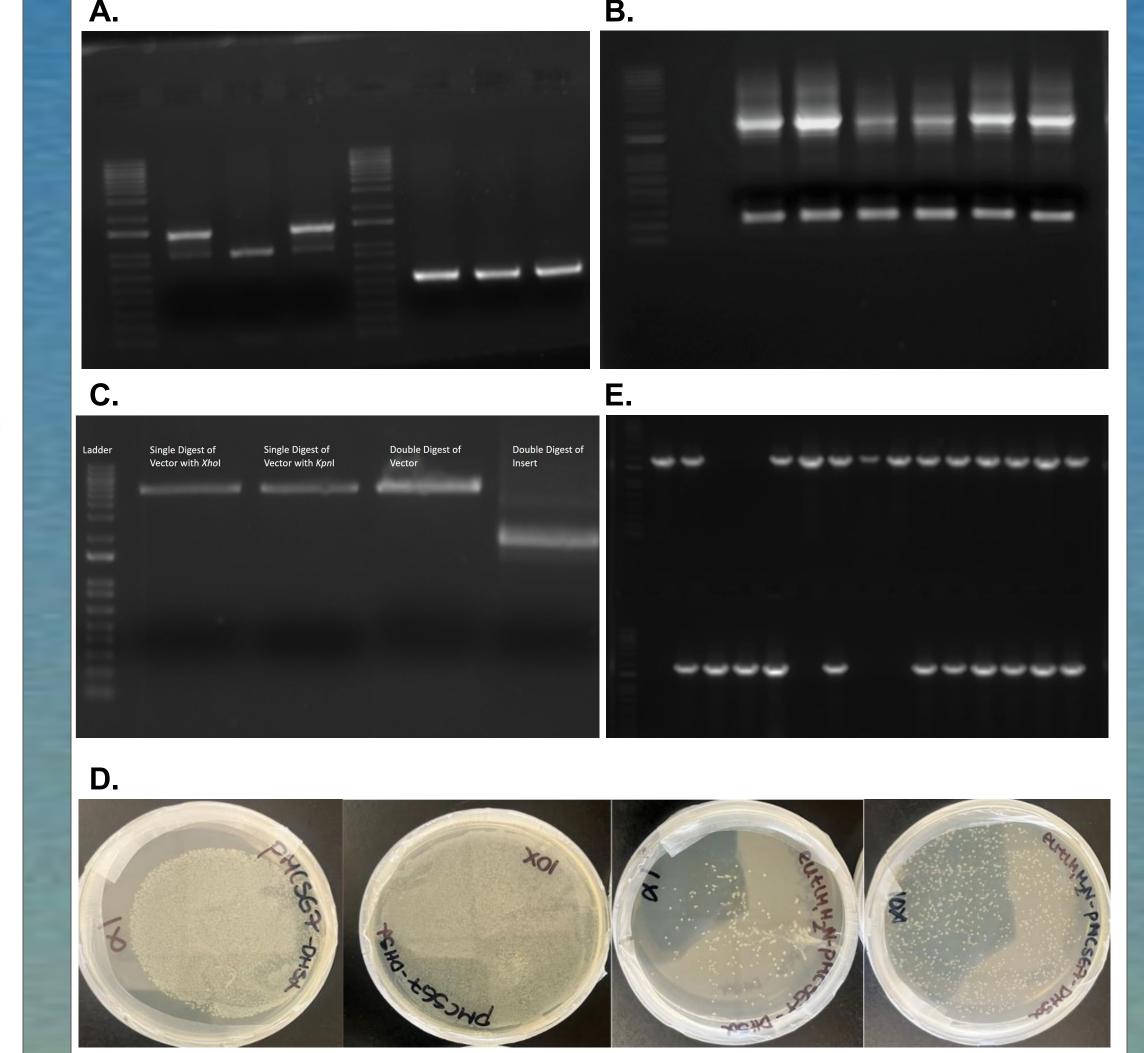
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Abstract

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

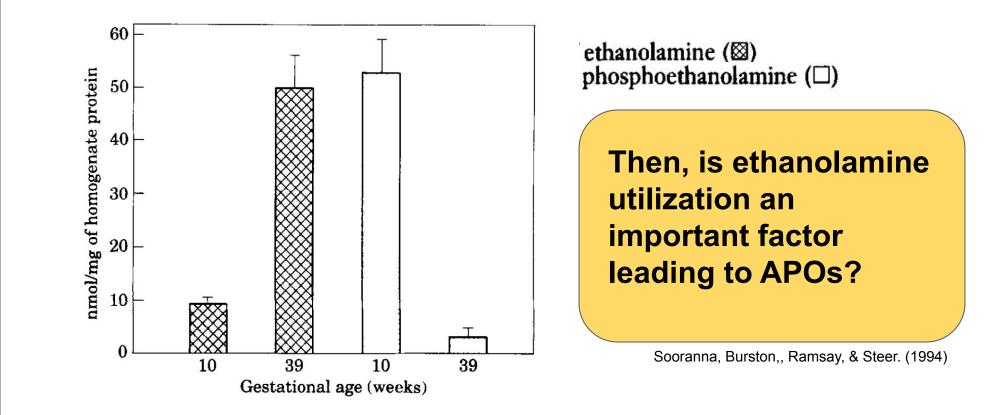
Fusobacterium nucleatum

- 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.



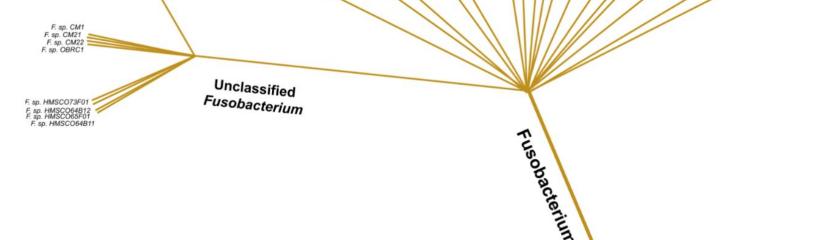


Fig. 1 Phylogenetic Tree of Fusobacterium species. The phylogenetic tree modified from NCBI Lifemap shows 69 Fusobacterium species which were analyzed in this study.

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| | J folk tolP ff V | | |
| | Fusobacterium mortiferum (ATCC 9817) | | |
| | | | |
| | Fusobacterium nucleatum (cTLS) | | |
| | J folk fold M P V W A B C L M M 2 E T 2835 G | | |
| | Fusobacterium perfoetens (ATCC 29250) | | |
| | Fusobacterium russii (1589A) | | |
| | folk folP | | |
| | Fusobacterium varium (ATCC 27725) | | |
| folk | | | |
| | Fusobacterium necrophorum (ATCC 51357) | | |
| | foir foir 2835 W | | |
| | Fusobacterium periodonticum (D10) | | |
| | | | |
| | Fusobacterium ulcerans (ATCC 49185) | | |
| fol folk | | | |
| | Fusobacterium polymorphum (KCOM 1275) | | |
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| | Fusobacterium animalis (21_14) | | |
| | $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$ | | |
| | Fusobacterium naviforme (ATCC 28832) has no eut genes | | |
| | Fusobacterium equinum(cMW8398) | | |
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| | Fusobacterium vincentii (3_1,27) J J E folk folk folk S P V W A B C L M M E T 10 N 2 H Q 2835 G | | |
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| | Fusobacterium canifelinum (FDAARGOS_1126) | | |
| | Fusobacterium hwasookii (choc F174) | | |
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| | Fusobacterium massiliense (Marselle-P2749T) | | |
| ~ | | | |
| | Unclassified Fusobacterium | | |
| | Fusobacterium oral taxon 370 S | | |
| · | | | |
| | Fusobacterium sp. CM1 | | |
| | | | |
| | Fusobacterium sp. CM21 folk | | |
| | 1 for 2835 G | | |

Fig. 3 Construction of an eutLM₁M₂ Recombinant Plasmid. (A) Shown are PCR products of $eutLM_1M_2$ and $eutN_1$ (B) Crossover PCR generated the *eutLM* $_1M_2N$ 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.

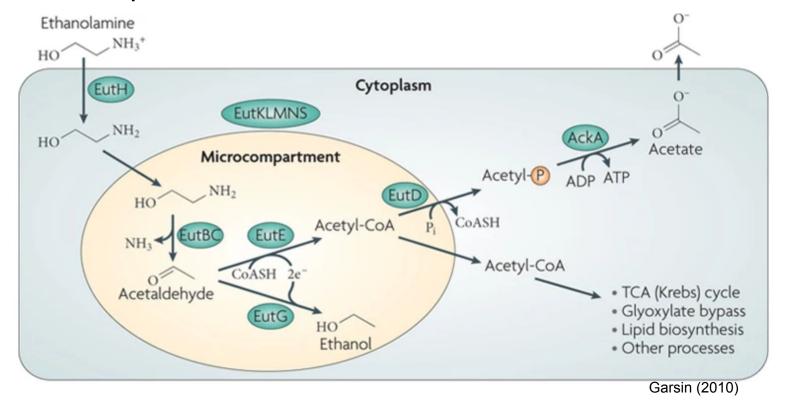
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eut Genes and Bacterial Microcompartments (BMCs)

- 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.

Why is this Significant in Our Study?

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



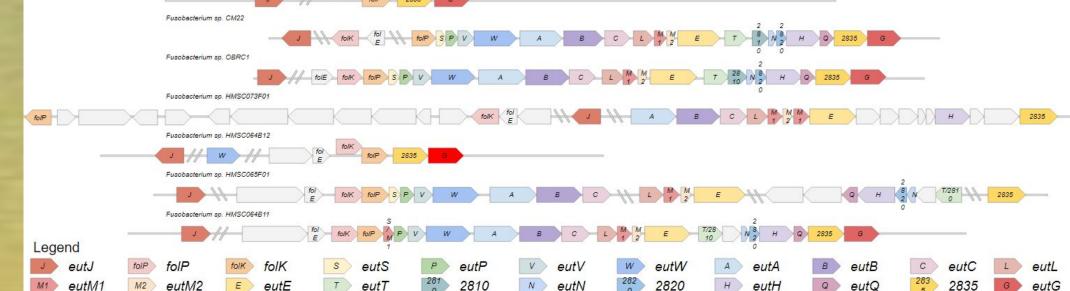
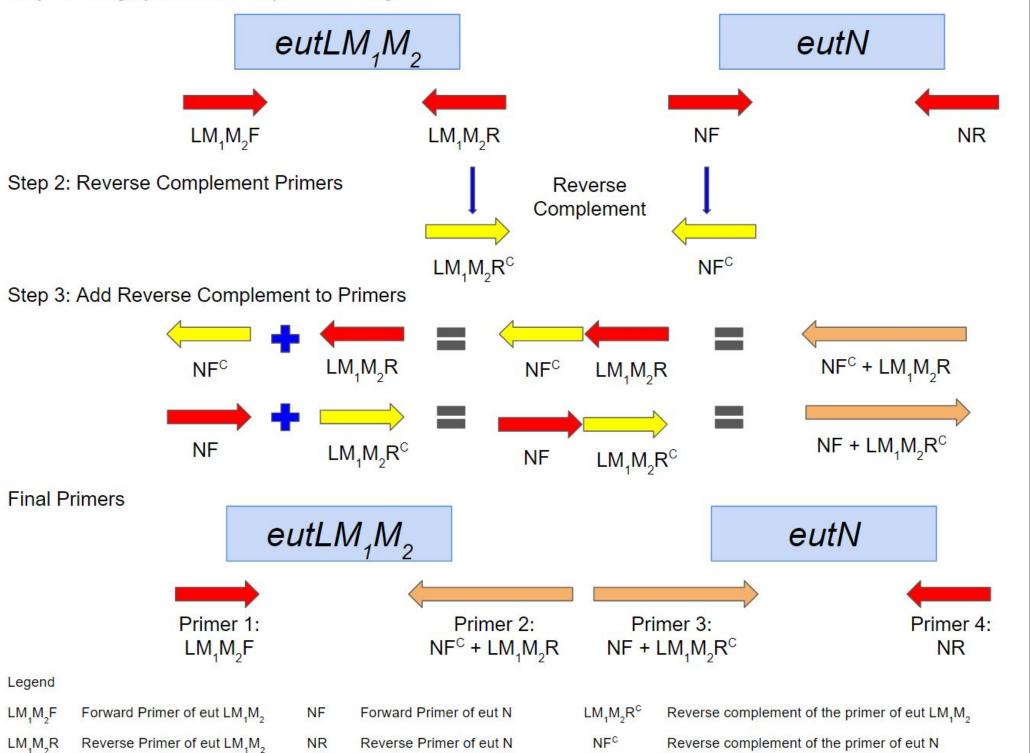


Fig. 2 Conservation of eut Genes in Fusobacterial Species. Homology and BLAST analyses reveal the conservation and variation of the eut genes in various Fusobacterium species, including F. nucleatum, F. animalis, F. polymorphum, and F. vincentii. Similar genes are color-coded.

Engineering of Recombinant Plasmid Expressing *eutLM*₁*M*₂*N* in *Escherichia coli* DH5α

Primer Design

Step 1: Design primers for amplification of genes



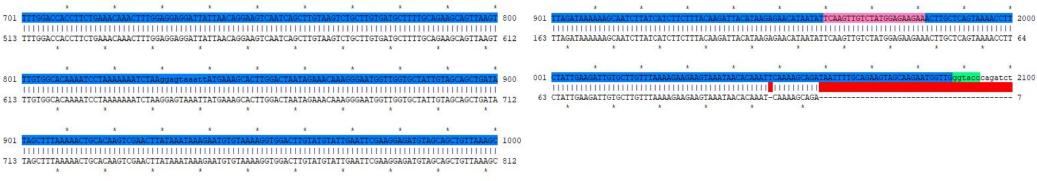


Fig. 4 Confirmation of the Recombinant Plasmid expressing eutLM₁M₂N by DNA sequencing. Plasmid DNA was subjected to DNA sequencing with specific primers targeting to (A) eutL, and (B) eutN in the insert $eutLM_1M_2N$.

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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• 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.

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