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[P005] Poster

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[P03-367] Analysis of Membrane vesicle formation factors in *Paracoccus denitrificans*

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Keywords：membrane vesicles formation、quorum sensing、transposon mutants

[Purpose]
Many bacteria exist in complex communities and interact with one another in diverse environments. Among the factors that play important roles in these interactions, membrane vesicles (MVs) have attracted considerable attention. MVs are spherical membrane bound structures with diameters of approximately 20~400 nm that are released from bacterial membranes and are produced by a wide range of bacterial species. MVs function as carriers that deliver diverse biomolecules, including nucleic acids and signaling molecules involved in cell-cell communication, to other cells. We previously demonstrated that the denitrifying model bacterium *Paracoccus denitrificans* regulates biofilm formation through the MV mediated transfer of signaling molecules. Although MVs have been demonstrated to play diverse biological roles, the molecular mechanisms underlying their formation remain incompletely understood. One reason for this limitation is the lack of systems that enable comprehensive screening of genes involved in MV formation. Here, we established a high-throughput screening system in *P. denitrificans* to comprehensively identify genes involved in MV formation.
[Method]
In this study, we focused on the enrichment of intercellular communication signaling molecules within MVs and established a system that enables indirect and high throughput evaluation of MV production. First, a library of random transposon (Tn) mutants was generated using an MV overproducing strain of *P. denitrificans* as the parental strain.For strains selected through this screening, the genomic regions containing Tn insertions were determined by sequencing, allowing the identification of genes potentially involved in MV formation. Furthermore, the functions of these genes were subsequently analyzed.
[Results]
Using the screening system we established, a total of 10,098 Tn mutants were examined. Among these mutants, six strains exhibited approximately a 1.5 fold increase in MV production compared with the parental strain, whereas MV production was reduced to less than half in 57 strains.
[Consideration]
The genes identified through this screening included not only components related to membrane structure but also those associated with two component regulatory systems and substrate transport. These findings suggest that multiple processes may contribute to MV formation.
[Conclusion]
In this study, we established a system that enables the evaluation of MV production and successfully performed a screening to identify factors involved in MV formation. The genes obtained through this screening suggest that the processes leading to membrane vesicle formation may be diverse and multifaceted.