Pangenomic Analysis of *Methylomonas* Reveals Its Potential as a Bioresource for Agricultural Methane Mitigation

Chanakan Khamchet[1], **Sahassawat Chailungka**[1], **Senoch Seanpong**[2] **and Nuttapol Noirungsee**[1*]

[1] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.
[2] Institute of Plant Science and Microbiology, Department of Biology, University of Hamburg, Hamburg, Germany
[*] Corresponding author: nuttapol.n@cmu.ac.th

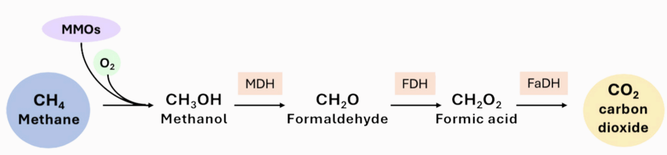
BACKGROUND

Methane emissions from agricultural activities, particularly rice paddies, contribute significantly to global greenhouse gas levels. The oxidation of methane by methanotrophic bacteria plays a crucial role in mitigating these emissions. Among these bacteria, ***Methylomonas*** spp., a group of type I methane-oxidizing bacteria (MOB) within the Gammaproteobacteria (Ogiso, T., et al., 2012), have been identified as potential biological resources for reducing methane emissions in agricultural settings.

Despite their known role in methane oxidation, the genomic diversity and functional potential of *Methylomonas* spp. remain underexplored. Previous studies suggest that *Methylomonas* species inhabit a variety of environments, including aquatic systems, soil, and plant-associated habitats such as rhizospheres and endospheres (Liu, J., et al., 2020). The efficient rhizosphere colonization of these rhizobacteria is a prerequisite for exerting their plant beneficial functions (Liu, et al., 2024). This raises the possibility that certain strains, such as *Methylomonas* sp. TEB, could function as endophytic bioresources that not only contribute to methane oxidation but also enhance plant growth.

Furthermore, including heavy metal contamination, pose significant challenges to microbial survival and agricultural productivity. The identification of heavy metal resistance genes within *Methylomonas* sp. TEB suggests that this strain may have additional adaptive advantages, making it a promising candidate for agricultural applications.

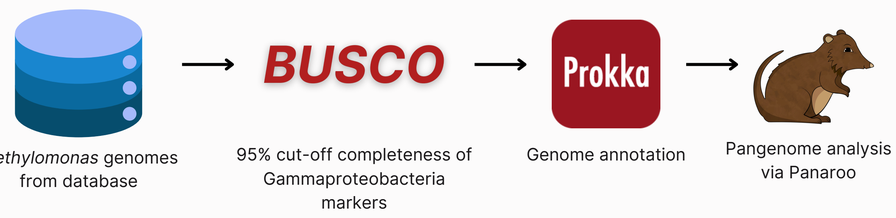
Given these factors, a comprehensive pangenomic analysis of *Methylomonas* is necessary to determine its potential as a bioresource for plant-microbe interactions and agricultural methane mitigation. Understanding the genomic basis of its endophytic capabilities and metal resistance mechanisms will provide insights into its ecological role and potential biotechnological applications.



General pathway of methane oxidation by aerobic methanotrophs. MMOs, methane monooxygenases; MDH, methanol dehydrogenase; FDH, formaldehyde dehydrogenase; FaDH, formate dehydrogenase. (Adapted from Semrau et al., 2018)

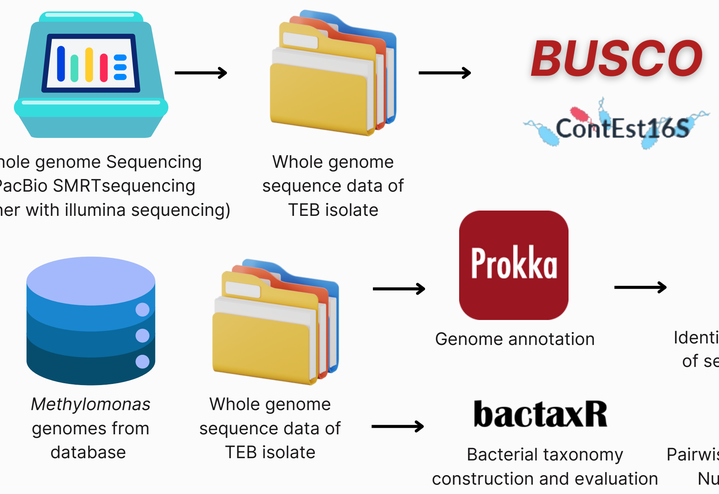
METHODOLOGY

Methylomonas be an endophytic bioresource.



Methylomonas genomes from database → 95% cut-off completeness of Gammaproteobacteria markers → Genome annotation → Pangenome analysis via Panaroo

Whole Genome Sequencing and Genome analysis.



Whole genome Sequencing (PacBio SMRTsequencing together with illumina sequencing) → Whole genome sequence data of TEB isolate → BUSCO, ContEst16S

Methylomonas genomes from database; Whole genome sequence data of TEB isolate → Prokka → Genome annotation → anti SMASH: Identifying biosynthetic loci of secondary metabolites

bactaxR: Bacterial taxonomy construction and evaluation

FastANI: Pairwise comparison of Average Nucleotide Identity (ANI)

CONCLUSION

Analysis of the pangenome reveals that the genus *Methylomonas* sp. represents a novel biological resource with potential applications in agriculture. Moreover, *Methylomonas* sp. TEB has been identified as a promising endophytic biological resource for enhancing biomass production and mitigating methane emissions. Genomic analysis of *Methylomonas* sp. TEB has also identified genes associated with heavy metal resistance, and physiological testing further demonstrates its ability to tolerate cadmium.

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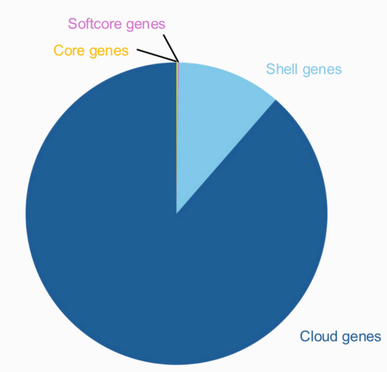
OBJECTIVES

- To study the pangenome of *Methylomonas* to determine whether it can serve as an endophytic bioresource for agricultural applications in reducing methane emissions
- To study the pangenome of *Methylomonas* to evaluate its metal tolerance and ability to tolerate cadmium

RESULTS

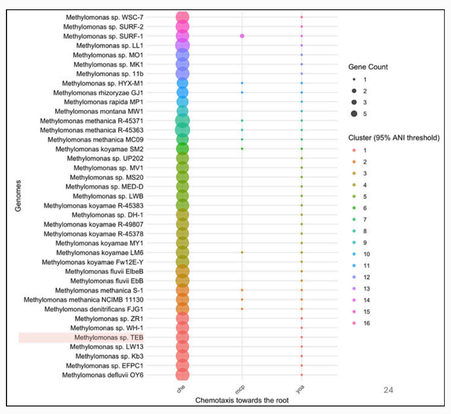
Methylomonas pangenome

Gene type	Number
Core genes (99%<=strain<=100%)	68
Soft core genes (95%<=strain<=99%)	72
Shell genes (15%<=strain<=95%)	6552
Cloud genes (0%<=strain<=15%)	51956

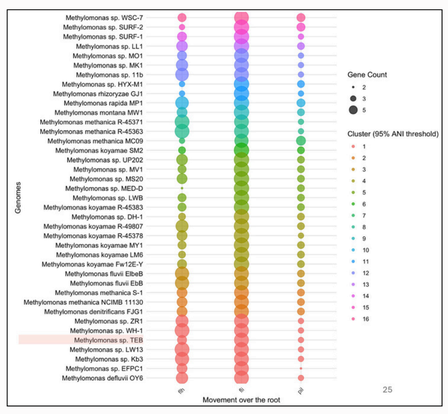


The pie chart of genes in the *Methylomonas* pangenome constructed with 41 genomes

Chemotaxis towards the root



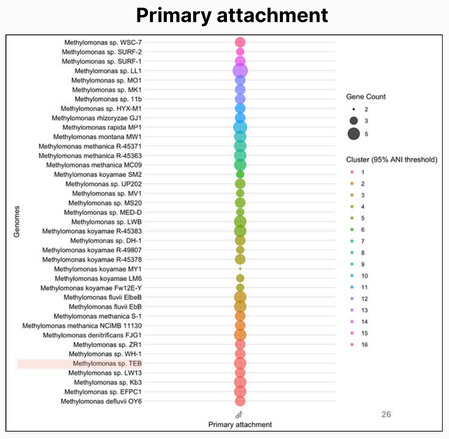
Movement over the root



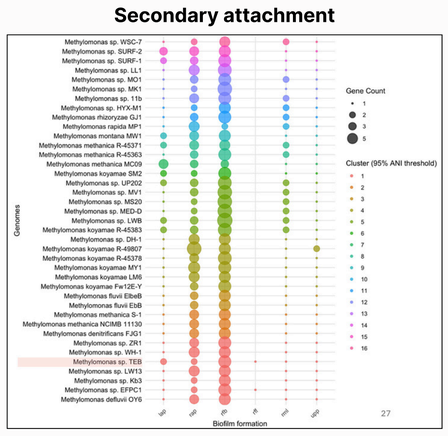
Results of the pangenome analysis related to
Chemotaxis towards the root
che - chemotactic responses
mcp, yoa - chemoreceptor

Movement over the root such as
flh, fli - flagella
pil - pilli

Primary attachment



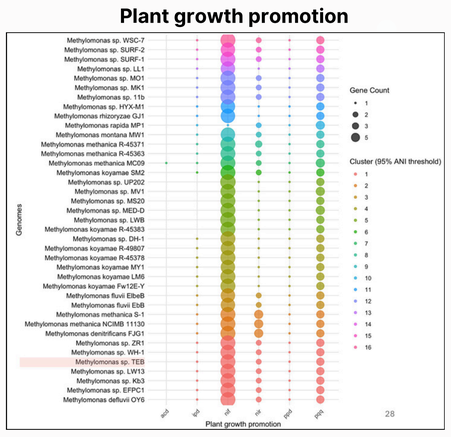
Secondary attachment



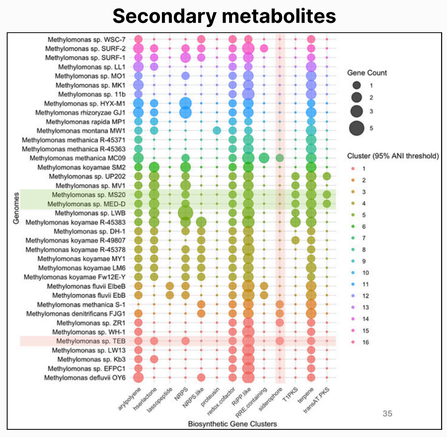
Results of the pangenome analysis related to
Primary attachment
opr - Major outer membrane protein

Secondary attachment
upp - Unipolar polysaccharide
lap, rap - Extracellular proteins
rfb, rff, rml - lipopolysaccharides

Plant growth promotion



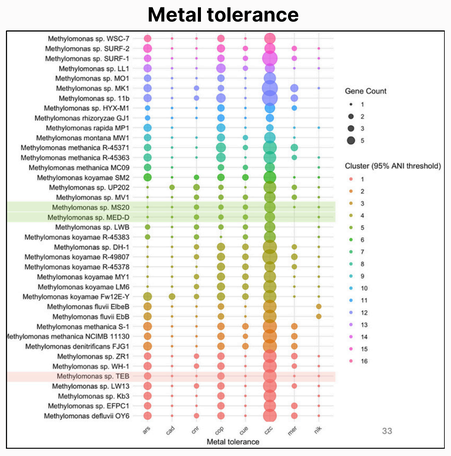
Secondary metabolites

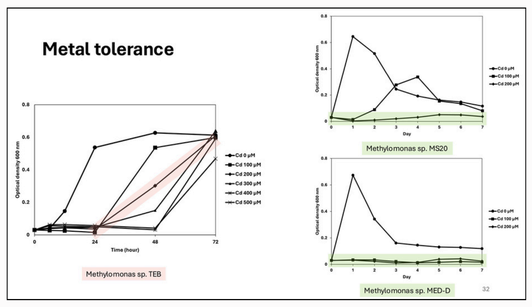


Results of the pangenome analysis related to
Plantgrowth promotion
acd - ACC deaminase
ipd, ppd - Auxin synthesis
nif - Nitrogen fixation
nir - Nitric oxide synthesis
pqq - Phosphate solubilization

Secondary metabolites
Siderophores - iron chelator

Metal tolerance





Results of the pangenome analysis related to
Metal tolerance
ars : arsenic
cad : cadmium
czc : cadmium, cobalt, zinc
cnr : nickel, cobalt
cop, cue : copper
mer : mercury
nik : nickel

From the graph, found that only *Methylomonas* sp. TEB exhibited high tolerance to cadmium. it can be observed that at a concentration of 200 micromolar (highlighted), *Methylomonas* sp.TEB was still able to grow, whereas *Methylomonas* sp. MS20 and *Methylomonas* sp. MED-D showed no growth. At this concentration, cadmium begins to precipitate, forming a visible white precipitate.