Characterization of methane-oxidizing bacteria isolated from soil

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BACKGROUND

Methane is an important greenhouse gas with a global warming potential significantly higher than carbon dioxide. A large proportion of methane released into the atmosphere is biologically oxidized by methane-oxidizing bacteria (methanotrophs), which play a key role in regulating methane emissions in natural environments [1, 2]. Methanotrophs utilize methane as their sole carbon and energy source through methane oxidation pathways involving key enzymes such as particulate methane monooxygenase. These microorganisms are widely distributed in soils and aquatic environments and contribute significantly to the global methane cycle [1, 3].

Recent advances in whole-genome sequencing have enabled detailed characterization of methanotrophs, allowing researchers to identify genes involved in methane metabolism and explore additional genomic features such as biosynthetic gene clusters that may contribute to ecological interactions [4].

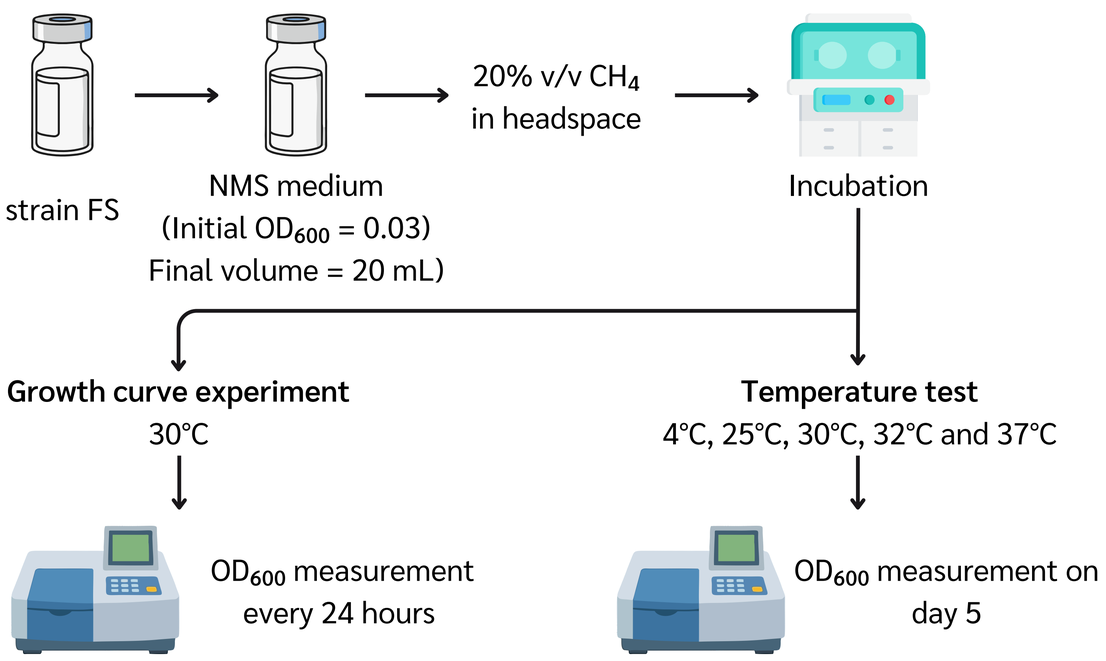
In this study, a methane-oxidizing bacterial strain (strain FS) isolated from soil was characterized using physiological experiments and genome-based analyses to investigate its metabolic potential and genomic features.

OBJECTIVES

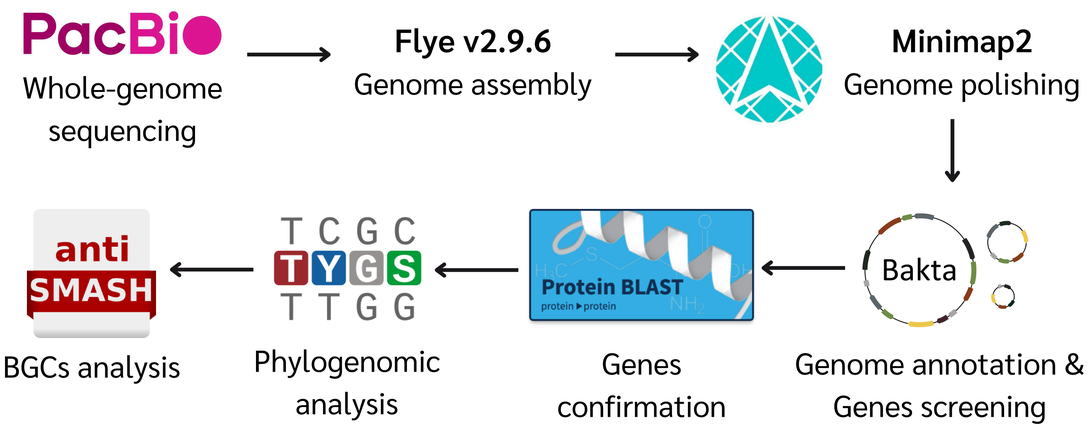
- To investigate physiological characteristics of methane-oxidizing bacterium strain FS isolated from soil
- To analyze genomic features related to methane metabolism and taxonomic classification using whole-genome sequencing.

APPROACHES

- **Physiological characterization**



- **Genome analysis**



RESULTS

- **Physiological characterization**

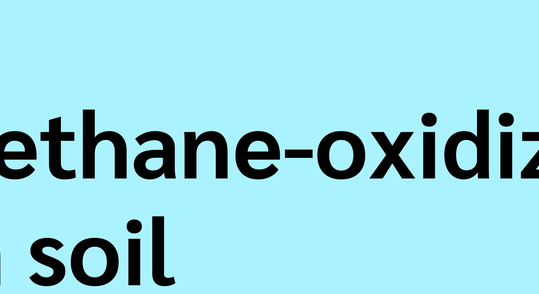


Figure 1 Growth curve of strain FS cultivated under methane-supplemented conditions

From figure 1, strain FS showed a typical bacterial growth pattern with a lag phase during the first 24 hours, followed by an exponential phase between 24–96 hours and a stationary phase after 120 hours. The maximum OD reached approximately 0.17–0.18. Data are presented as mean ± SD from four replicates (n = 4).

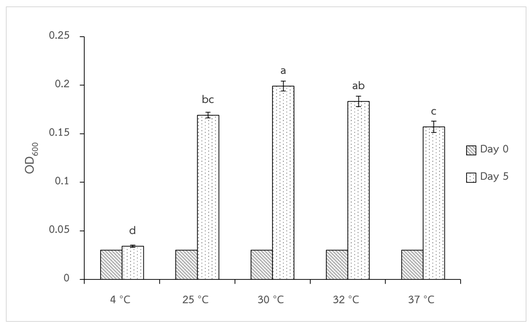


Figure 2 Effect of temperature on the growth of strain FS

From figure 2, growth of strain FS was observed at all tested temperatures, with the highest growth at 30 °C. Growth at 30 °C and 32 °C showed no significant difference, while growth at 4 °C was significantly lower than other temperatures. Statistical analysis was performed using one-way ANOVA ($p < 0.05$), and data are presented as mean ± SD from four replicates (n = 4).

- **Genome-based analysis**

Parameter	Strain FS
Genome size	4,649,771 (~4.65 Mb)
GC content	53.57%
Number of contigs	2
N50	4,628,691 (~4.63 Mb)

Table 1 Genome assembly statistics of strain FS

Region	Type	Similarity
1.1	ranthipeptide	-
1.2	NRPS	Low
1.3	RiPP-like	-
1.4	terpene	-
1.5	terpene	-
1.6	hserlactone	-
1.7	arylpolyene	Low
1.8	NRP-metallophore, NRPS	Low
1.9	betalactone	Low
1.10	terpene	-
1.11	darobactin	-

Table 2 Predicted biosynthetic gene clusters identified in the genome of strain FS

Gene	Strain FS	Locus tag	Length (aa)	Identity (%)	Closest reference
pmoA	Methane monooxygenase subunit A	HIOMAM_00054	250	95.60	*Methylobacter luteus*
pmoB	Methane monooxygenase subunit B	HIOMAM_00055	412	95.39	*Methylobacter marinus*
pmoC	Methane monooxygenase subunit C	HIOMAM_00056	257	90.66	*Methylomonas* sp.
mxaD	MDH accessory protein	HIOMAM_02223	188	90.43	Methylococcaceae bacterium
mxaS	MDH-associated protein	HIOMAM_03503	279	86.74	*Methylomicrobium* sp. Wu6
mxaK	Electron transfer protein	HIOMAM_03506	188	88.30	*Methylomicrobium*
hps	3-hexulose-6-phosphate synthase	HIOMAM_00524	395	89.63	*Methylomicrobium agile*

Table 3 Methane metabolism-related genes identified in strain FS

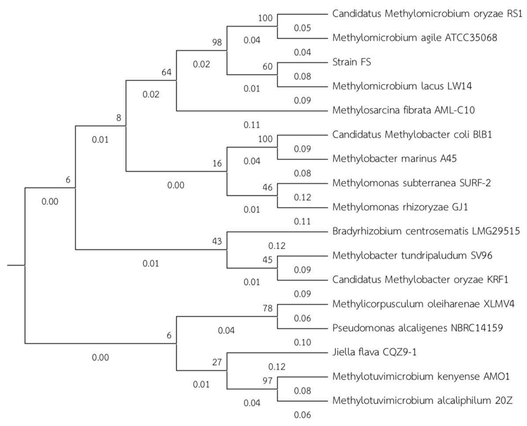


Figure 3 Phylogenomic tree of strain FS based on whole-genome sequences

From figure 3, phylogenomic analysis based on whole-genome sequences placed strain FS within the methanotrophic lineage and showed a close relationship with members of the genus *Methylomicrobium*. The tree topology indicates that strain FS forms a distinct branch compared with closely related reference strains, suggesting genetic differentiation within this group.

CONCLUSIONS

Strain FS isolated from soil was characterized as a methane-oxidizing bacterium based on physiological and genomic analyses. The strain showed optimal growth at 30 °C and possessed key methane oxidation genes, including *pmo* and *mxa*, as well as the RuMP pathway. Genome analysis indicated that strain FS is closely related to the genus *Methylomicrobium*, while dDDH results suggest it is genetically distinct from known species within this genus. These findings provide important insights into the metabolic potential and ecological role of this methane-oxidizing bacterium.

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