Isolation and Identification of *Microvirga thermotolerans* HR1, a Novel Thermo-Tolerant Bacterium, and Comparative Genomics among *Microvirga* Species

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Supplementary Materials:



Figure S1. Two-dimensional TLC of polar lipids of strain HR1. Chloroform/methanol/water (65:25:4, v/v/v) was used in the first direction, followed by chloroform/aceticacid/methanol/water (80:18:12:5, v/v/v/v) in the second direction. The plate was sprayed with 5% ethanolic molybdophosphoric acid. The color agent of (**a**), (**b**), (**c**) was ninhydrine, molybdenum blue, and molybdophosphate, respectively. Diphosphatidylglycerol (DPG); phosphatidylethanolamine (PE); phosphatidylglycerol (PG); phosphatidylcholine (PC); phospholipids (PL); aminolipid (AL).



Figure S2. Phylogenetic tree based on the *rpoB* sequence. This tree was constructed by the neighborjoining method, and showed the phylogenetic relationship between strain HR1 and closely related species.



Figure S3. Phylogenetic tree based on the *gyrB* sequence. This tree was constructed by the neighborjoining method, and showed the phylogenetic relationship between strain HR1 and closely related species.



Figure S4. Phylogenetic tree based on the *recA* sequence. This tree was constructed by the neighborjoining method, and showed the phylogenetic relationship between strain HR1 and closely related species.



Figure S5. (a) Circular genome map and circular representation of the *Microvirga thermotolerans* HR1 3.82 Mb chromosome. (b) Scatter plot illustrating whole-genome sizes, average percent GC content, and numbers of predicted genes and encoded putative proteins.



Figure S6. Mathematical modeling and linear comparison of *Microvirga*. (a) Mathematical modeling of the pan genome and core genome of *Microvirga*. (b) Linear comparison of the complete genomes in the *Microvirga* strains.

	Microvirga Vignae BR3299	Microvirga Guangxiensis CGMCC1.7666		
Protein ID	annotation	Protein ID	annotation	
KLK89555.1	nitrogen fixation protein NifW	SCX86875.1	NifU homolog involved in Fe-S cluster formation	
KLK90284.1	nitrogen fixation protein NifX	SCZ11145.1 I	Radical SAM superfamily enzyme, MoaA/NifB/PqqE/SkfB family	
KLK90285.1 nit	rogenase iron-molybdenum cofactor biosynthesis protein Nif	NSCZ11151.1	NifU homolog involved in Fe-S cluster formation	
KLK90286.1 nitrogenase iron-molybdenum cofactor biosynthesis protein NifE			nitrogen regulatory protein P-II family	
KLK90561.1	iron-molybdenum cofactor biosynthesis protein NifQ	SCY82617.1	NtrC family, nitrogen regulation sensor histidine kinase GlnL	
KLK93004.1	nifZ protein	SCY82600.1	NtrC family, nitrogen regulation response regulator GlnG	
KLK93006.1	nitrogen fixation protein NifB	SCY82575.1	NtrC family, nitrogen regulation sensor histidine kinase NtrY	
KLK93698.1	nitrogen fixation protein NifU	SCY82559.1	NtrC family, nitrogen regulation response regulator NtrX	
KLK93996.1	nitrogen regulatory protein	SCY79163.1	nitrogen regulatory protein P-II family	
KLK91767.1	nitrogen fixation protein FixI	SCY72062.1	Nitrogen fixation protein FixH	
KLK91765.1	nitrogen fixation protein FixL	SCY71901.1	Nitrogen fixation regulation protein	
KLK90288.1	nitrogenase molybdenum-iron protein subunit alpha	SCY57825.1	PTS system, nitrogen regulatory IIA component	
KLK90287.1	nitrogenase molybdenum-iron protein subunit beta	SCY48327.1	PTS IIA-like nitrogen-regulatory protein PtsN	
KLK89635.1	nodulation protein NodU			
KLK89637.1	nodulation protein NodJ			
KLK89639.1	nodulation protein NodZ			
KLK89789.1	Nodulation protein W			
KLK91190.1	nodulation protein NolV			
KLK91191.1	nodulation protein NolT			
KLK91850.1	Nodulation protein W			
KLK92820.1	nodulation protein NolW			
	Microvirga Lutononidis WSM3557		Microvirga Sp. KLBC 81	
Protein ID	annotation	Protein ID	annotation	
EIM24386.1	NifQ	PVE24750.1	NifU family protein	
EIM27506.1	iron-sulfur cluster biosynthesis protein, NifU-like protein	PVE21492.1	nitrogen fixation protein NifQ	
EIM29761.1	putative TIM-barrel protein, nifR3 family	PVE21501.1	nitrogenase iron-molybdenum cofactor	
EIM30571.1	iron-sulfur cluster biosynthesis protein, NifU-like protein	PVE21502.1	nitrogenase iron-molybdenum cofactor	
EIM30714.1	nitrogenase cofactor biosynthesis protein NifB	PVE21503.1	nitrogen fixation protein NifX	
EIM30716.1	NifZ domain-containing protein	PVE21504.1	NifX-associated nitrogen fixation prot	
EIM30732.1	nitrogen fixation protein NifX	PVE21183.1	nitrogen fixation protein NifZ	

Table S1. The genes coding protein relative to nitrogen fixation and nodules formation in Microvirga genomes.

Table S1. Cont.

EIM	30733.1	nitrogenase molybdenum-iron cofactor biosynthesis protein NifN PVE20977.1		cysteine desulfurase NifS
EIM	30734.1	nitrogenase molybdenum-iron cofactor biosynthesis protein NifE	E PVE21515.1	nitrogenase molybdenum-iron protein
EIM	31020.1	Nitrogen fixation protein NifW	PVE21500.1	nitrogenase molybdenum-iron protein
EIM	30737.1	nitrogenase iron protein	PVE22099.1	nitrogen fixation protein FixH
EIM	30736.1	nitrogenase molybdenum-iron protein alpha chain	PVE21676.1	nodulation protein NOLX
EIM	30735.1	nitrogenase molybdenum-iron protein beta chain	PVE21685.1	nodulation protein NolW
EIM	30717.1	putative nitrogen fixation protein FixT	PVE20930.1	nodulation protein NolU
EIM	29760.1	signal transduction histidine kinase, nitrogen specific	PVE20939.1	nodulation protein
EIM	29759.1	nitrogen regulation protein NR(I)	PVE20899.1	nodulation protein NodU
EIM	29496.1	nitrogen regulatory protein PII	PVE20900.1	nodulation factor ABC transporter
EIM	29287.1	PTS IIA-like nitrogen-regulatory protein PtsN	PVE20901.1	nodulation protein NodJ
EIM	29168.1	nitrogen regulatory protein PII	PVE20902.1	nodulation protein
EIM	30624.1	ATP-binding ABC transporter family nodulation protein NodI	PVE20904.1	nodulation protein NodZ
EIM	30627.1	Nodulation protein A (NodA)	PVE20614.1	nodulation protein NodF