

Biodegradation

Online Resource 2

Biodegradation of trimethylamine by a halotolerant strain of *Paracoccus* sp. PS1 and in silico analysis of trimethylamine degrading enzymes.

Madhupa Seth^{a1}, Priyajit Mondal^{a2}, Dhritishree Ghosh^{a3}, Raju Biswas^b, Sumit Chatterjee^c, Subhra Kanti Mukhopadhyay^{a*}

Affiliations

^aDepartment of Microbiology, The University of Burdwan, Burdwan-713104, Purba Bardhaman, West Bengal, India.

Email id and ORCID:

¹sethmadhupa@gmail.com; ORCID ID- 0000-0003-1736-5869

²priyajitmondal1994@gmail.com; ORCID ID- 0000-0002-0903-4050

³dhritishreeghosh@gmail.com; ORCID ID- 0000-0001-8650-2906

^bMicrobiology Laboratory, Department of Botany, Institute of Science, Visva-Bharati (A Central University), Santiniketan, West Bengal, 731235, India. Email id: rajubiswas.26041995@gmail.com. ORCID ID- 0000-0002-6131-7015

^cDepartment of Microbiology, Bose Institute, EN 80, Sector V, Bidhan Nagar, Kolkata-700091, West Bengal, India. Email id: i.sumit2011@gmail.com. ORCID ID- 0000-0001-7365-3713

***Corresponding author:** Subhra Kanti Mukhopadhyay. Email id: skmukhopadhyay@microbio.buruniv.ac.in; Mobile: +91 7908778733. ORCID ID- 0000-0001-9284-0880

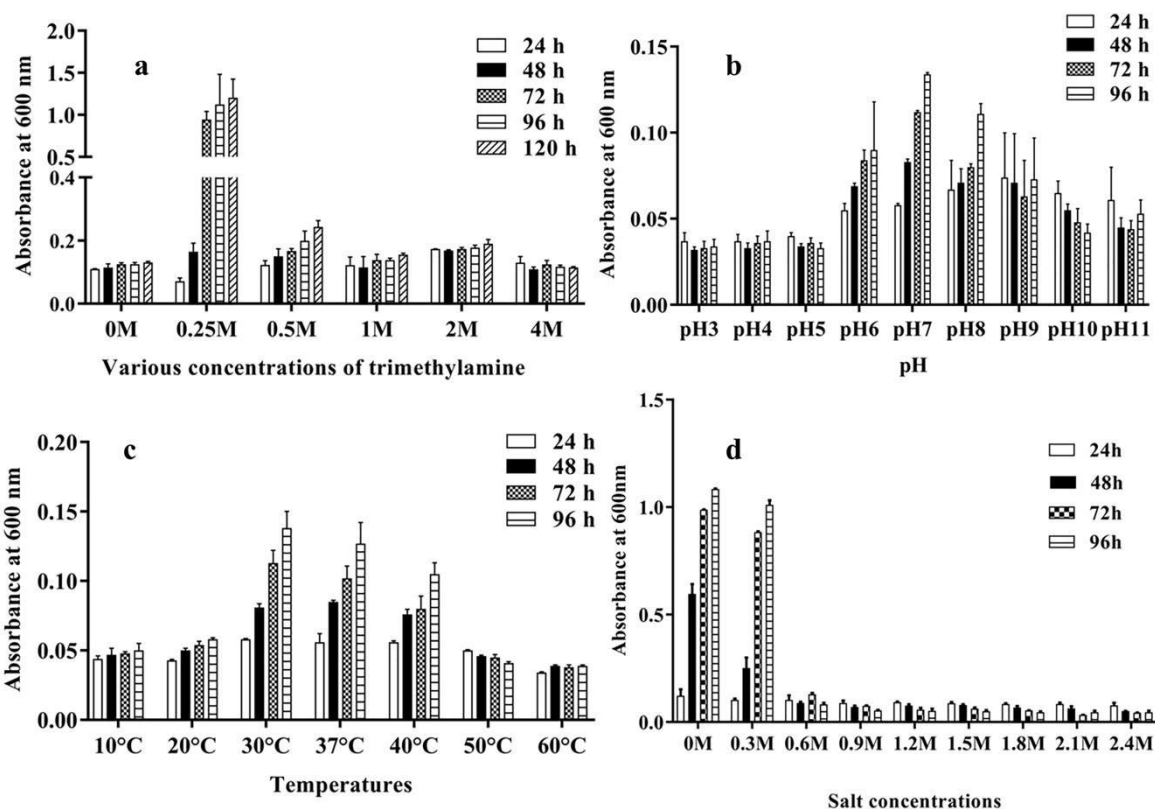
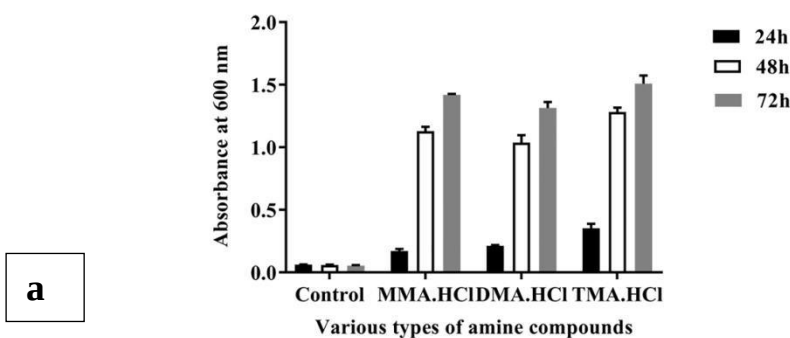


Fig. S1 The various optimum conditions for growth of isolate PS1. **a** TMA utilisation range of isolate PS1. **b** Determination of optimum pH of PS1. **c** The temperature optima of isolate PS1. **d** The halotolerance nature of isolate PS1.



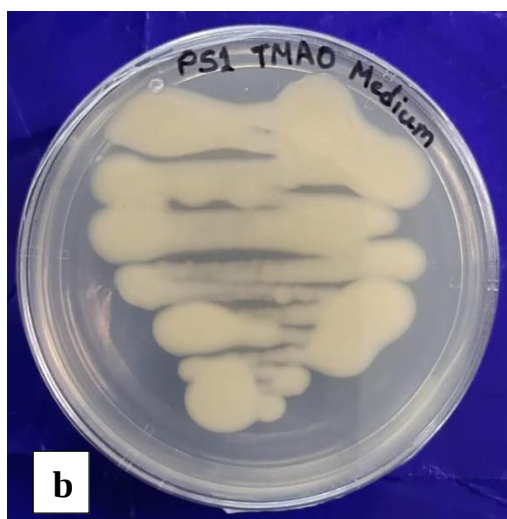


Fig. S2 The use of various amines as carbon and nitrogen source for growth of PS1. **a** This figure shows the ability of isolate PS1 to grow in all three amines produced in the TMA degradation pathway. **b** Growth of isolate PS1 in modified DMB medium supplemented with TMAO as sole carbon and nitrogen source.

Table S1 Table shows the absorbance values at 410 nm of known concentrations of TMA ($\mu\text{g/ml}$).

Concentration of TMA ($\mu\text{g/ml}$)	O.D. at 410nm
0	0 ± 0
5	0.0125 ± 0.000707
10	0.0262 ± 0
15	0.04 ± 0.001414
20	0.054 ± 0.001414
25	0.078 ± 0.004243
30	0.086 ± 0.001414

35	0.091 ± 0
40	0.1085 ± 0.002121
45	0.1345 ± 0
50	0.1605 ± 0.002121

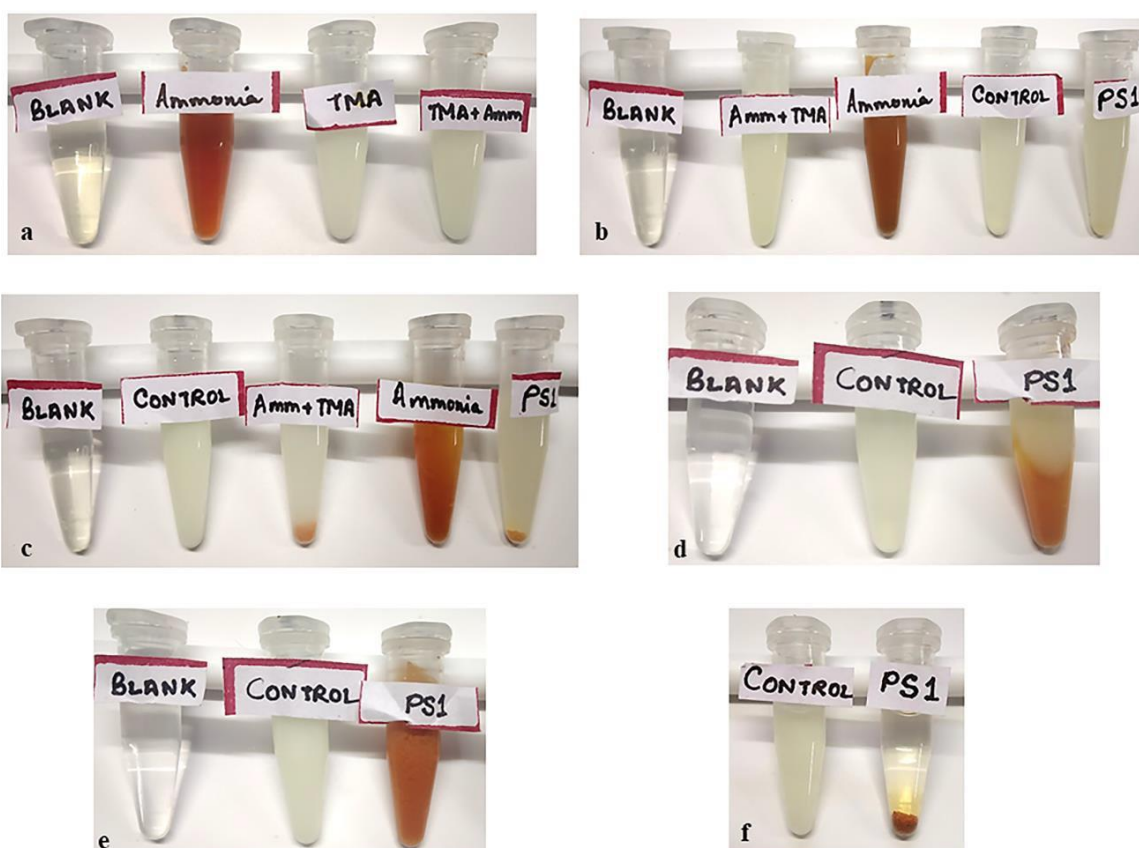


Fig. S3 Nessler assay result. **a** This is the result of all the tubes after addition of few drops of Nessler reagent. The 'blank' tube contains only DM medium without TMA and does not show any white precipitation (pptn), 'ammonia' tube contains only DM + Ammonia medium and produces brown pptn due to no TMA, 'TMA' tube contains 0.4M TMA+ DM which shows white pptn and 'TMA+Ammonia' tube contains 0.4M TMA+ Ammonium salt+ DM medium and shows white pptn as the TMA overpowers the color pptn of ammonia , hence no color of ammonia is detected. **b** 'Control' tube contains uninoculated TMA+ DM medium and 'PS1' tube contains the supernatant after 24hrs growth of PS1 in TMA+ DM medium . **c** The 'ammonia+TMA' tube

here contains high concentration of ammonia and low concentration of TMA, hence the brown pptn is observed and the 'PS1' tube contains sup of PS1 after 72 hrs growth which also shows brown pptn as a result of utilization of TMA for growth by PS1 and increase in released ammonia content respect to the 'control' tube shows white pptn due to no utilisation of TMA, hence no ammonia production. **d** The 'PS1' tube contains sup after 120 hrs growth. **e** 'PS1' tube contains sup collected after 168 hrs of growth. **f** 'PS1' tube contains sup collected after 216 hrs of growth which finally proves that the TMA has been extensively been utilised by PS1 so clearance of white ppt and increase in brown ppt compared to the white pptn in the 'control' tube where TMA has remained unutilised.

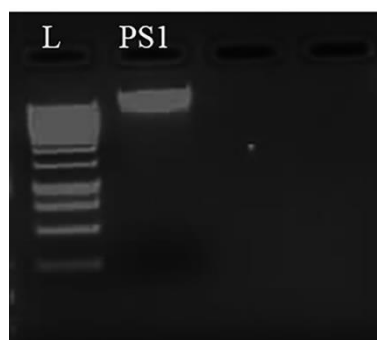


Fig. S4 The DNA band image of PS1 picture taken in gel doc (BIO-RAD Gel DocTM EZ Imager). The lane L is the 500bp ladder used and the lane PS1 is the DNA band for isolate PS1.

Table S2 Assembly statistics of strain PS1 genome sequence.

Contigs (≥ 0 bp)	120
Contigs (≥ 1000 bp)	97
Contigs (≥ 5000 bp)	70
Contigs (≥ 10000 bp)	61
Contigs (≥ 25000 bp)	41
Contigs (≥ 50000 bp)	26
Total length (≥ 0 bp)	4196916
Total length (≥ 1000 bp)	4185118

Total length ($\geq$ 5000 bp)	4127004
Total length ($\geq$ 10000 bp)	4065992
Total length ($\geq$ 25000 bp)	3715797
Total length ($\geq$ 50000 bp)	3134627
Contigs	120
Largest contig	357659
Total length	4196916
GC (%)	68.11
N50	104159
N75	48348
L50	12
L75	27
N's per 100 kbp	0
Complete BUSCO (%)	97.3
Partial BUSCO (%)	0

132
133
134

BLASTP Search Result

Database: prokaryotes

Protein sequence database entries related to query - 500 hits

Show alignment

Entry	K	number	bits	E-val
Top 10 Clear Select operation Exec				
☒ pamn:pAMV1p0112 tmm; trimethylamine monooxygenase	K18277	820	0.0	
☒ pye:A6J80_01575 NAD(P)/FAD-dependent oxidoreductase	K18277	818	0.0	
☒ fap:GR316_12235 NAD(P)-binding domain-containing protein	K18277	814	0.0	
☒ ceh:CEW89_17235 potassium transporter	K18277	813	0.0	
☒ pami:JCM7686_pAMI6p102 trimethylamine monooxygenase	K18277	813	0.0	
☒ rhp:LPB142_05620 potassium transporter	K18277	811	0.0	
☒ red:roselon_03573 small molecule metabolism	K18277	809	0.0	
☒ rbg:BG454_03475 NAD(P)/FAD-dependent oxidoreductase	K18277	801	0.0	
☒ cid:P73_0139 flavin-containing monooxygenase	K18277	801	0.0	
☒ sil:SP01551 tmm; Trimethylamine (TMA) monooxygenase	K18277	798	0.0	

Fig. S5a KEGG BLAST result of Tmm sequence of isolate PS1.

BLASTP Search Result

Database: prokaryotes

Protein sequence database entries related to query - 500 hits

Show alignment

Entry	K	number	bits	E-val
Top 10 Clear Select operation Exec				
☒ rhp:LPB142_05615 aminomethyltransferase	K00605	1287	0.0	
☒ ceh:CEW89_17230 aminomethyltransferase	K00605	1265	0.0	
☒ fap:GR316_12230 DUF1989 domain-containing protein	K00605	1263	0.0	
☒ cid:P73_0140 glycine cleavage system T protein (aminomethylt...	K00605	1245	0.0	
☒ laqu:R2C4_19775 tdm; trimethylamine-oxide aldolase Tdm	K00605	1118	0.0	
☒ pye:A6J80_01580 DUF1989 domain-containing protein	K00605	1117	0.0	
☒ lej:ETW24_17925 tdm; trimethylamine-oxide aldolase	K00605	1115	0.0	
☒ lcae:K3721_16200 tdm; trimethylamine-oxide aldolase Tdm	K00605	1115	0.0	
☒ lev:ETW23_00235 tdm; trimethylamine-oxide aldolase	K00605	1113	0.0	
☒ taw:EI545_08455 DUF1989 domain-containing protein	K00605	1112	0.0	
☐ lmd:METH_18405 aminomethyltransferase	K00605	1111	0.0	

Fig. S5 b KEGG BLAST result of Tdm sequence of isolate PS1.

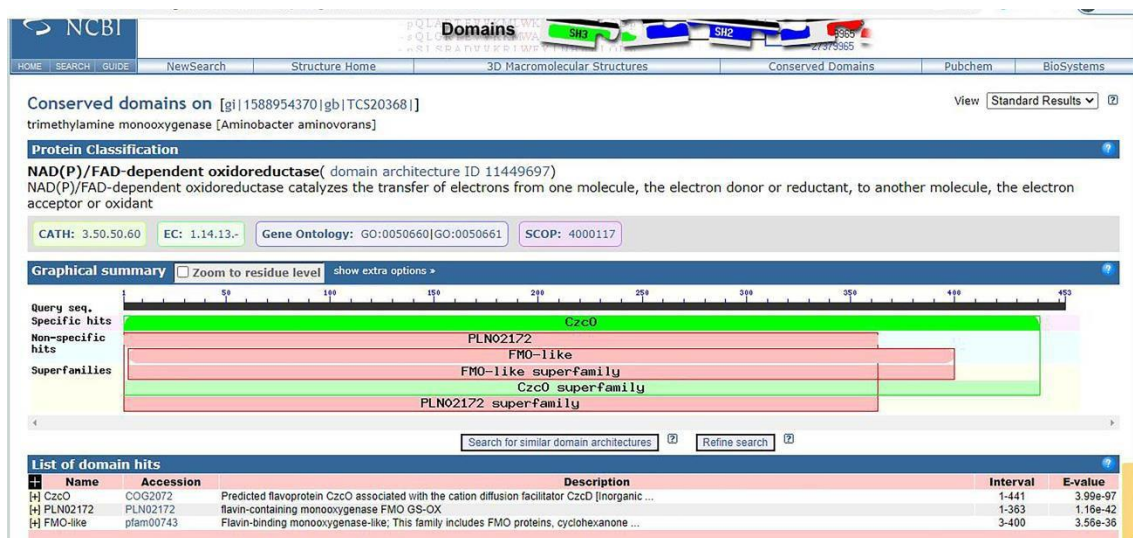


Fig. S6a The reported Tmm sequence of *Aminobacter aminovorans* (Aguirre et al., 2018) contains the conserved domain FMO-like superfamily which is essential in the degradation of trimethylamine.

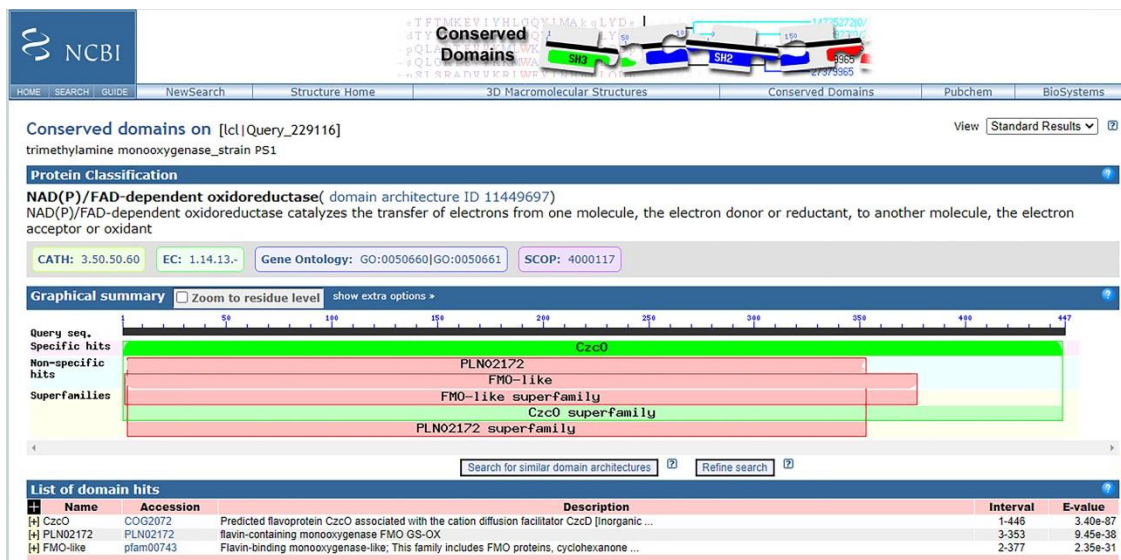


Fig. S6b The Tmm sequence of *Paracoccus* sp. strain PS1 also contains the FMO-like superfamily conserved domain which is essential enzyme in the degradation pathway of trimethylamine.

Table S3 Sequence identity and similarity of *Paracoccus* sp. PS1 Tmm with other reported sequences of the same enzyme from various *Paracoccus* strains and intergenus strains.

Sr.No.	Organism	Accession No.	Sequence identity (%) with Tmm	Sequence similarity (%) with Tmm
1..	<i>Paracoccus</i> sp. J39	WP_028717534.1	100	100
2.	<i>Paracoccus</i> sp. MA	WP_230378712.1	99.1	99.6
3.	<i>Paracoccus binzhouensis</i>	WP_199260516.1	99.1	99.8

4.	<i>Paracoccus</i> sp. BP8	RQP04227.1	98.7	99.3
5.	<i>Paracoccus</i> sp. MKU1	WP_058096661.1	98.2	99.8
6.	<i>Paracoccus siganidrum</i>	WP_119897102.1	97.5	99.1
7.	<i>Paracoccus alcaliphilus</i>	SEN37137.1	89.5	96.4
8.	<i>Paracoccus saliphilus</i>	SIT07820.1	82.6	93.3
9.	<i>Paracoccus laeviglucosivorans</i>	SMO92700.1	80.3	89.7
10.	<i>Ruegeria pomeroyi</i> DSS-3	AAV94838.1	82.6	94.4
11.	<i>Aminobacter aminovorans</i>	TCS20368.1	78.6	93
12.	<i>Paracoccus sanguinis</i>	KGJ21065.1	36.7	66.7

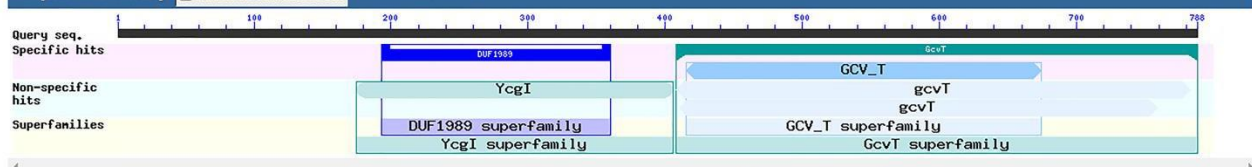
Conserved domains on [gi|2183436447|ref|WP_235790832|]
trimethylamine-oxide aldolase Tdm [Ruegeria pomeroyi]

View **Standard Results**

Protein Classification

aminomethyltransferase family protein(domain architecture ID 18073792)
aminomethyltransferase family protein similar to glycine cleavage system protein T and to Sphingomonas paucimobilis LigM, an O-demethylase that catalyzes a C1-unit transfer from vanillate (VNL) to H4 folate; contains at DUF1989 domain

Graphical summary ☐ Zoom to residue level [show extra options >](#)



[Search for similar domain architectures](#) [Refine search](#)

List of domain hits					
#	Name	Accession	Description	Interval	E-value
[+]	GcvT	COG0404	Glycine cleavage system T protein (aminomethyltransferase) [Amino acid transport and ...	408-788	1.15e-128
[+]	gcvT	PRK00389	glycine cleavage system aminomethyltransferase GcvT;	408-783	2.36e-83
[+]	GCV_T	pfam01571	Aminomethyltransferase folate-binding domain; This is a family of glycine cleavage T-proteins, ...	415-674	2.80e-83
[+]	YcgI	COG3665	Uncharacterized conserved protein YcgI, DUF1989 family [Function unknown];	175-406	1.78e-66
[+]	gcvT	TIGR00528	glycine cleavage system T protein; The glycine cleavage system T protein (GcvT) is also known ...	410-759	3.06e-63
[+]	DUF1989	pfam09347	Domain of unknown function (DUF1989); This family of proteins are functionally uncharacterized.	193-360	1.47e-55

Fig. S7 a. The conserved domains DUF1989 and GCV_T protein of reported TMAO aldolase Tdm) sequences of *Ruegeria pomeroyi* (Lidbury et al., 2015)

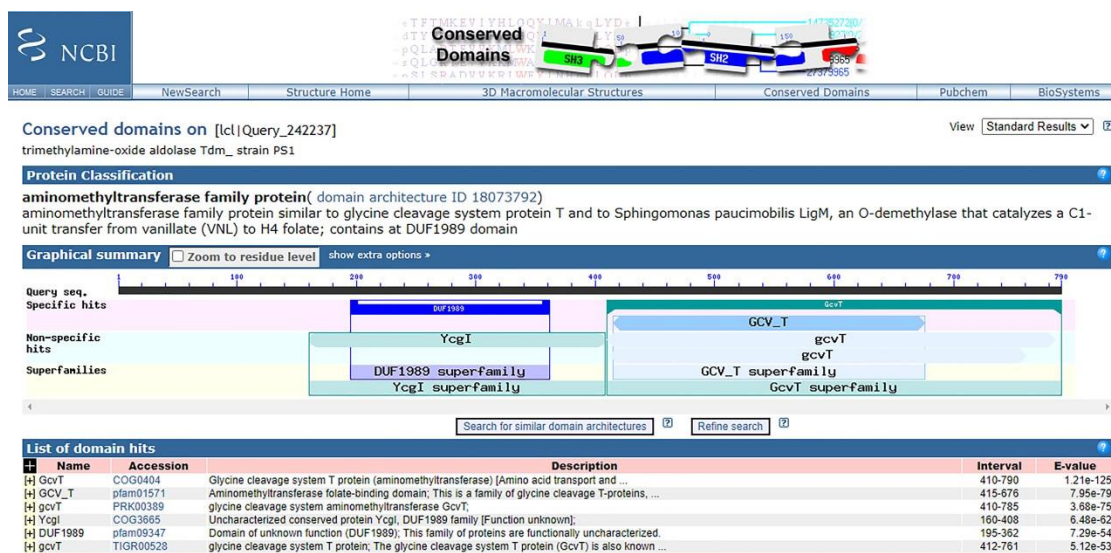


Fig. S7b shows the conserved domains DUF1989 and GCV_T protein of the query sequence (aminomethyltransferase family protein of *Paracoccus* sp. strain PS1).

Table S4 Percentage identity and similarity of Tdm sequence of *Paracoccus* sp. strain PS1 with aminomethyltransferase sequences reported from various *Paracoccus* strains and other Tdm sequences of intergenus strains.

Sr.No.	Organism	Accession No.	Sequence identity (%) with Tdm	Sequence similarity (%) with Tdm
1.	<i>Paracoccus</i> sp. MA	WP_230378713.1	99.2	99.7
2.	<i>Paracoccus</i> sp. BP8	RQP04226.1	98.7	99.7
3.	<i>Paracoccus binzhouensis</i>	WP_199260515.1	97.3	99.4
4.	<i>Paracoccus</i> sp. MKU1	WP_058096662.1	96.1	99.2
5.	<i>Paracoccus siganidrum</i>	WP_119897101.1	95.8	98.9
6.	<i>Paracoccus alcaliphilus</i>	WP_090610859.1	83.2	94.6
7.	<i>Paracoccus alkanivorans</i>	WP_122111064.1	81.8	92.9
8.	<i>Paracoccus onubensis</i>	WP_119746385.1	81.4	92.9
9.	<i>Paracoccus</i> sp. MC1854	WP_182790495.1	81.1	93.2
10.	<i>Paracoccus</i> sp. MC1862	WP_182805778.1	80.8	92.9

11.	<i>Ruegeria pomeroyi</i>	WP_235790832.1	67.1	85.1
12.	<i>Mameliella alba</i>	WP_221240208.1	66.8	85.5
13.	<i>Roseivivax sediminis</i>	WP_149755463.1	66.8	84.5
14.	<i>Tropicibacter</i> sp. LMIT003	WP_121633257.1	66.8	84.3
15.	<i>Leisingera</i> sp. BMJM1	WP_264211506.1	66.6	85.5
16.	<i>Roseovarius mucosus</i>	WP_257898298.1	66.3	85.8
17.	<i>Cereibacter sphaeroides</i>	WP_225297307.1	66.3	85.6
18.	<i>Pararhodobacter marinus</i>	WP_109532851.1	66.1	83.8
19.	<i>Ponticoccus alexandrii</i>	WP_023848942.1	66.0	85.5
20.	<i>Shimia</i> sp. R10_1	WP_209100858.1	65.2	84.5
21.	<i>Marivita</i> sp. S6314	WP_247086485.1	64.0	82.9
22.	<i>Cochlodiniinecator piscidefendens</i>	WP_166417059.1	63.0	82.9
23.	<i>Paracoccus denitrificans</i>	GEK66774.1	22.7	58.3

10 20 30 40 50 60
 MKKRVAIIGA GPSGLAQLRA FQSAAQKGAE IPEIVCFEKQ SNWGGGLWNYT WRTGLDEYGE
 70 80 90 100 110 120
 PVHCSMYRYL WTNGPKEGLE FADYSFEEHF GKQIASYPFR AVMFYDIEGR VVKAGVRDWI
 130 140 150 160 170 180
 RFSTVVRHVE YDEDRGIFTV TAHDLVKDHM YAEFDHVIV ASGHFSTPNV PEYEGFSTFN
 190 200 210 220 230 240
 GRIVHAHDFR DAREFAGKDV LVHGSSSYAE DIGSQCKYK AKSVTSCYRS APHGFKWPDN
 250 260 270 280 290 300
 WQEKPALVRV EGKEAVFKDG SRKEVDAIL CTGYKHYFF LPDDLRLKTA NRLAAADLYK
 310 320 330 340 350 360
 GVVWVHNPRF FYLGWQDQWF TFNMFDAQAW YVRDIVMGRI EVPDQKQVLM ADVREREERE
 370 380 390 400 410 420
 EASDDVKYAI RYQADYVKEL IAETDYPSPD VDGACAFYE WKKHKAEDIM AFRNHSYRSV
 430 440
 ITGTMAPKHH TPNKDALDDS MEAYLMN

10 20 30 40 50 60
 MLQLPPESLF RPPGPPRATHV RRAAGFTLPP GVERYAVPGA GAVAVQIATG DRIEIIINDEG
 70 80 90 100 110 120
 GORCELLAAH ADGRLDPAIL GAAADAPADG LRALLSSDGP GLASLRRRLA ELGIPLAEAR
 130 140 150 160 170 180
 AIGLFGAETR PGETVALTAA APGWLIAAAP GGVHDFEAQD TSTPLTLRIT RANPRRQGHF
 190 200 210 220 230 240
 ALPTPLADPL LDLRVKSATA EAYTVKAGDF IQIIDVDGRQ CTDFQCQDAR KLDRGIVHPL
 250 260 270 280 290 300
 DVTTTRTIQG HAYPHPLHA KYFDHDLTPL VEVVQDTCGR HDAFALACSA KYYDDIGYPG
 310 320 330 340 350 360
 HANCTDNFNA VLAPYGVPEAR AGWMAANFFF NTGIDAHGVM YADEPWSRPG DYVLLRALTD
 370 380 390 400 410 420
 LVCVSSACPD DTTAANGVHL TDHVRSYGA QERFSRATAW RATPDAEPIM TRETAFHQNF
 430 440 450 460 470 480
 AALTRDFVEY RGFNLNSFP ECDPVESYWA CREGVVVMDL SALRKFEVTG PDAEALMNV
 490 500 510 520 530 540
 LTRDVAKLGH GQVVSAMCH PHGGMIDDGT LFRLGHNFR WIGGSDEGVV WIREQAEKLG
 550 560 570 580 590 600
 LNVHRSSTD QHNLAVQGP KSRELHAGIV WTPPHQPALA DLGNFRFTVG RIGGERGVV
 610 620 630 640 650 660
 VVSRTGYTGE LGYEIFCHPR HGDVFEAVW NAGQPLGLKP MGLAGLDLVR IEAGLVFAGY
 670 680 690 700 710 720
 DFSQDTPFE AGVGFTVPLK SKNADFVGRE ALIRRKDHPS RKFGVLEIDG QDAVGHGDCI
 730 740 750 760 770 780
 RIGRAQVGEV CSAMRSPTG QHIALARIDI AHAEPGTAVE VGRLDGHIK IAAKVAAFPH
 790
 YDPKKERPRA

a

b

Fig. S8 Amino acid sequences retrieved from WGS of strain PS1 and analysed by ProtParam tool of Expasy. **a** The amino acid sequence of Tmm of PS1. **b** The amino acid sequence of Tdm of PS1.

Table S5 This table contains the various physiochemical properties of Tdm and Tmm of isolate PS1 depicted by ProtParam tool of Expasy:

Parameters	Trimethylamine monooxygenase (Tmm)	Trimethylamine n-oxide aldolase (Tdm)
Number of amino acids	447	790
pI	5.65	5.86
Aliphatic Index	67.00	81.96
Instability Index	33.26	34.06
Asp+Glu	67	96
Arg+Lys	54	79
MW	51544.14	85725.31
GRAVY	-0.466	-0.115

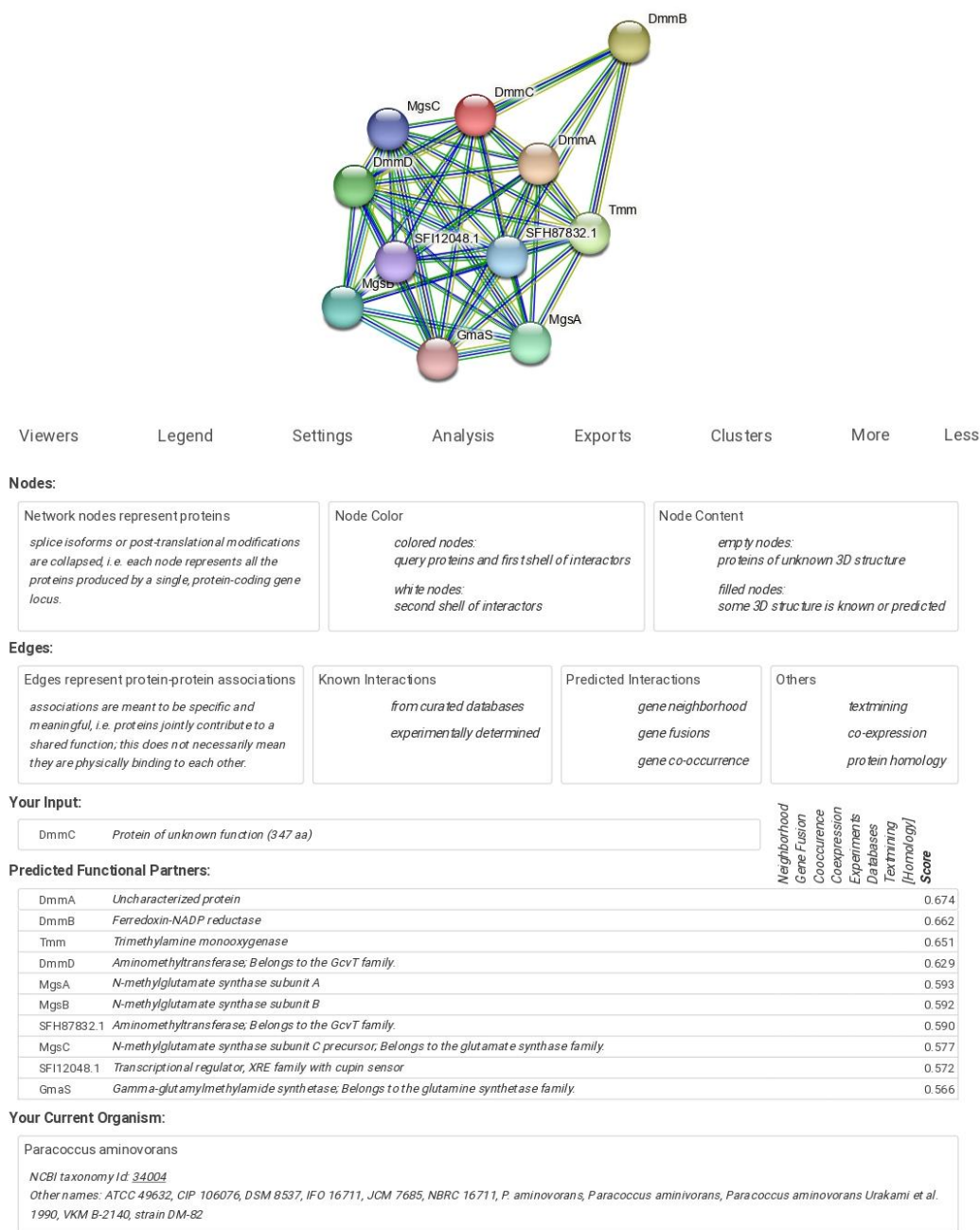


Fig. S9: The protein-protein interactions of proteins involved in TMA degradation pathway by STRING software of *Paracoccus aminovorans*.

Table S6. This table contains the STRING analysis result

Name of the proteins	Identity	protein function in <i>Paracoccus aminovorans</i>	bitscore	E-value	Protein_ID as provided by NCBI after submission	Functional annotated protein name according to PANNZER2 webserver of <i>Paracoccus</i> sp. PS1
Tmm	86.4%	Trimethylamine	831.2	3.1e-242	PRJNA858582:NM6	Trimethylamine

		monooxygenase			80_20190	monooxygenase
Tdm (SFH8783 2.1)	69.5%	Aminomethyltransferase; Belongs to the GcvT family.	1137.1	0.0	PRJNA858582:NM680_20185	Trimethylamine-oxide aldolase Tdm
DmmA	54.7%	Uncharacterized protein	201.1	7e-53	PRJNA858582:NM680_20515	Dimethylamine monooxygenase subunit DmmA family protein
DmmB	62.9%	Ferredoxin-NADP reductase	408.7	3.4e-115	PRJNA858582:NM680_20520	Ferredoxin-NADP reductase
DmmC	77.8%	Protein of unknown function	599.0	2e-172	PRJNA858582:NM680_20525	Dimethylamine monooxygenase subunit DmmC

References

Aguirre A, Bernal P, Maureira D, Ramos N, Vásquez J, Urrutia H, Gentina JC, Aroca G (2018) Biofiltration of trimethylamine in biotrickling filter inoculated with *Aminobacter aminovorans*. Electron J Biotechnol 33:63-67. <https://doi.org/10.1016/j.ejbt.2018.04.004>

Lidbury ID, Murrell JC, Chen Y (2015) Trimethylamine and trimethylamine N-oxide are supplementary energy sources for a marine heterotrophic bacterium: implications for marine carbon and nitrogen cycling. The ISME J 9:760-769. <https://doi.org/10.1038/ismej.2014.149>.

