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Article

Predicted AS3MT Proteins Methylate Arsenic and Support Two Major Phylogenetic AS3MT Groups

Jesper Torbøl Pedersen,[§] Jessica De Loma,[§] Michael Levi, Michael Palmgren,* and Karin Broberg*

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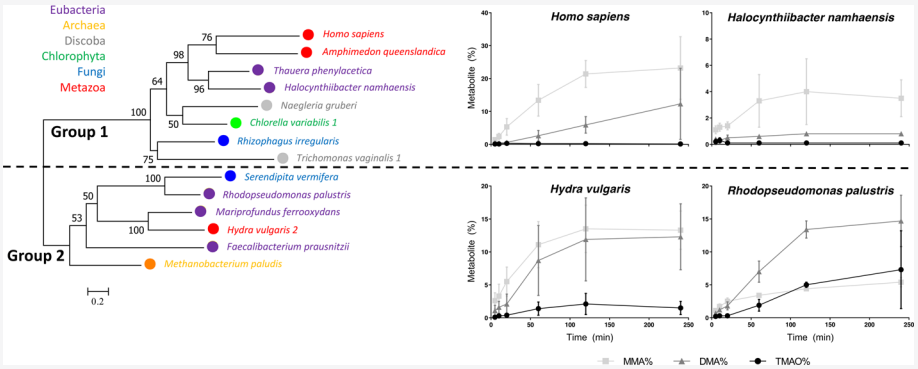

ABSTRACT: Inorganic arsenic is one of the most toxic and carcinogenic substances in the environment, but many organisms, including humans, methylate inorganic arsenic to mono-, di-, and trimethylated arsenic metabolites, which the organism can excrete. In humans and other eukaryotic organisms, the arsenite methyltransferase (AS3MT) protein methylates arsenite. AS3MT sequences from eukaryotic organisms group phylogenetically with predicted eubacterial AS3MT sequences, which has led to the suggestion that AS3MT was acquired from eubacteria by multiple events of horizontal gene transfer. In this study, we evaluated whether 55 (out of which 47 were predicted based on protein sequence similarity) sequences encoding putative AS3MT orthologues in 47 species from different kingdoms can indeed methylate arsenic. Fifty-three of the proteins showed arsenic methylating capacity. For example, the predicted AS3MT of the human gut bacterium *Faecalibacterium prausnitzii* methylated arsenic efficiently. We performed a kinetic analysis of 14 AS3MT proteins representing two phylogenetically distinct clades (Group 1 and 2) that each contain both eubacterial and eukaryotic sequences. We found that animal and bacterial AS3MTs in Group 1 rarely produce trimethylated arsenic, whereas *Hydra vulgaris* and the bacterium *Rhodopseudomonas palustris* in Group 2 produce trimethylated arsenic metabolites. These findings suggest that animals during evolution have acquired different arsenic methylating phenotypes from different bacteria. Further, it shows that humans carry two bacterial systems for arsenic methylation: one bacterium-derived AS3MT from Group 1 incorporated in the human genome and one from Group 2 in *F. prausnitzii* present in the gut microbiome.

INTRODUCTION

Elevated concentrations of inorganic arsenic occur in the groundwater and soil in many parts of the world. Arsenic is one of the most potent toxicants. In humans, it is a potent carcinogen and has also been associated with severe non-malignant diseases, such as lung and cardiovascular diseases[1−4] and child morbidity and mortality.[5−7]

Most mammals metabolize inorganic arsenic via methylation to methylarsonic acid (MMA) and dimethylarsinic acid (DMA),[8] and there are different models proposed for the mechanism of arsenic methylation.[9−11] The main enzyme catalyzing the methylation is arsenite methyltransferase, called AS3MT in animals[12−14] and ArsM in microorganisms, the latter of which is part of the arsenic resistance ars operon.[15] The main function of AS3MT and ArsM is to methylate inorganic arsenite. In humans, there is also a unique AS3MT^{d2d3} isoform that probably lacks methyltransferase activity and may have some role in the development of schizophrenia.[16] Some bacteria and fungi can methylate DMA further to volatile trimethylarsine (TMA),[17] which can be further oxidized to soluble trimethylarsine oxide (TMAO).

Arsenic methylation is advantageous to organisms; for example, *As3mt* knockout mice retain much higher concentrations of arsenic in different tissues than wild-type mice do.[18] In humans, methylation of arsenic reduces the retention of arsenic in the body,[19,20] supporting the idea that methylation

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Table 1. Initial Screen of Methylation Activity Among the 55 AS3MT Proteins[a]

group	species	protein ID	molecular weight (kDa)	% iAs	% MMA	% DMA	% TMAO
1	***Homo sapiens****	EAW49666.1	41.7	67	20	13	0
1	*Pan troglodytes 1*	XP-009457416.2	41.9	32	24	43	1
1	*Pan troglodytes 2*	XP-508007.3	42.6	58	22	20	0
1	***Amphimedon queenslandica***	XP-019859500.1	41.4	77.5	13.5	9	0
1	*Ectocarpus siliculosus*	CBJ30211.1	47.6	99	1	0	0
1	***Halocynthiibacter namhaensis***	WP-052245118.1	39.4	65	24	11	0
1	*Nematostella vectensis*	XP-001635188.1	41.3	4	1	94	1
1	*Phaeodactylum tricornutum*	XP-002183586.1	45.3	94	2	4	0
1	*Xenopus tropicalis**	NP-001135714.1	42.7	65	25	10	0
1	*Zooshikella ganghwensis*	WP-027709893.1	39.9	93	6	1	0
1	*Danio rerio**	NP-001034928.1	41.5	60	31	9	0
1	*Macaca fascicularis*	XP-005566383.1	41.7	65	29	6	0
1	*Mus musculus**	NP-065602.2	41.8	10	17	72.5	0.5
1	***Thauera phenylacetica***	WP-004380359.1	40.2	77	8	15	0
1	***Naegleria gruberi***	XP-002673101.1	45.5	48	11	40	1
1	*Acanthamoeba castellanii*	XP-004346011.1	39.7	98	0	0	2
1	*Chlamydomonas reinhardtii**	PNW76465.1	44.8	63	3	25	9
1	*Candidatus Methanoplasma termitum*	WP-048113480.1	40.8	89	7	4	0
1	***Chlorella variabilis 1***	XP-005845903.1	44.2	73	18	9	0
1	*Chlorella variabilis 2*	XP-005847544.1	38.5	100	0	0	0
1	*Ostreococcus tauri*	XP-022840421.1	43.6	58	27	15	0
1	*Spizellomyces punctatus*	XP-016608596.1	38.1	85	10	4	1
1	*Klebsormidium nitens*	GAQ93433.1	44.5	98	2	0	0
1	***Rhizophagus irregularis****	PKK65787.1	40.4	21	26	52	1
1	*Cyanidioschyzon merolae 1**	XP-005539091.1	43.8	34	10	55	1
1	*Cyanidioschyzon merolae 2*	XP-005535535.1	41.0	98	0	2	0
1	*Galdieria sulphuraria*	XP-005706047.1	39.9	88.5	3	8.5	0
1	*Geoalkalibacter ferrihydriticus*	WP-040100226.1	38.5	62	22	16	0
1	*Methyloterricola oryzae*	WP-045226361.1	38.2	71	3	19	7
1	*Nannochloropsis gaditana 1*	XP-005856121.1	38.3	84	11	5	0
1	*Nannochloropsis gaditana 2*	EWM25846.1	43.5	82	16	2	0
1	*Paludibacterium yongneupense*	WP-028536552.1	37.9	94	4	2	0
1	*Stylonychia lemnae*	CDW79757.1	39.0	85	8	7	0
1	***Trichomonas vaginalis 1***	XP-001307696.1	38.7	33	14	52	1
1	*Trichomonas vaginalis 2*	XP-001327848.1	38.7	18	8	73	1
1	*Desulfitobacterium hafniense 1*	WP-015943273.1	29.1	98	0	2	0
1	*Desulfitobacterium hafniense 2*	WP-005812925.1	38.7	93	5	2	0
2	*Aspergillus nidulans*	XP-662229.1	29.9	94	2	3	1
2	*Byssochlamys spectabilis*	GAD92999.1	29.5	95	1.5	2.5	1
2	*Desulfohalobium retbaense*	ACV68241.1	27.8	98	0	2	0
2	*Halapricum salinum***	WP-049994191.1	26.7				
2	*Haloterrigena limicola*	WP-008014917.1	29.2	98	2	0	0
2	*Hydra vulgaris 1*	XP-002165192.1	28.7	98	0.5	1	0.5
2	***Hydra vulgaris 2***	XP-012554525.1	28.7	65.5	8	15.5	11
2	*Sedimenticola selenatireducens 1*	WP-029132664.1	39.8	94.5	3.5	2	0
2	*Sedimenticola selenatireducens 2*	WP-029134203.1	28.1	94	4	2	0
2	***Methanobacterium paludis***	WP-013826852.1	27.3	91	1.5	4.5	3
2	*Methanocella conradii*	WP-014406891.1	28.7	95	2	2	1
2	***Faecalibacterium prausnitzii***	VDR33833.1	29.2	92	3.5	4.5	0
2	*Mariprofundus ferrooxydans*	WP-018293684.1	28.7	88	6	6	0
2	***Rhodopseudomonas palustris****	WP-011159102.1	29.6	19	2	9	70
2	*Cutaneotrichosporon oleaginosum*	XP-018276890.1	29.7	78	2	11	9
2	*Metarhizium robertsii*	XP-007826674.1	34.4	82	5	13	0
2	*Pleomorphomonas koreensis*	WP-156899591.1	28.6	50	8.5	35	6.5
2	***Serendipita vermifera***	KIM29307.1	32.2	87	2	7	4

[a]Proteins marked in bold were further evaluated by kinetic analysis. Proteins marked with an asterisk have previously been shown to have arsenic methylating activity (not predicted); protein with two asterisks was not expressed.

of arsenic to DMA in mammals (and further to TMA in bacteria) acts as a detoxification mechanism. However, the first methylated product, MMA, especially in its trivalent form, is considered to be more toxic and carcinogenic than arsenite.[21]

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In addition, it has been shown that the gut microbiota of mammals is important for arsenic tolerance,[22] but so far, it has not been investigated whether the bacteria that give arsenic tolerance contain active AS3MT proteins.

Recent phylogenetic analyses of predicted AS3MT orthologues provide a novel perspective on the development of tolerance to arsenic.[23,24] These analyses placed different animal and fungal AS3MT homologues in different prokaryotic clades with strong statistical support, consistent with horizontal gene transfer (HGT) of AS3MT from multiple prokaryotic phyla. Two major phylogenetic groups were identified with sequences from humans and other animals in the first group (here on referred to as Group 1) and the animal *Hydra vulgaris* in the second group (referred to as Group 2). The importance of HGT for adaptation of eubacteria and archaea is well established, but adaptation of resistance to a toxic environment by gene transfer from prokaryotes to eukaryotes was unexpected.

The aim of this study was to test whether AS3MT homologues in phylogenetically distinct organisms are true AS3MT orthologues by characterizing the biochemical properties of individually expressed proteins. Our results confirm this assumption and provide further evidence that HGT from eubacteria to eukaryotes is likely to have happened on more than one occasion.

MATERIALS AND METHODS

Phylogenetic Analysis. Detailed phylogenetic analysis is described in Palmgren et al.[23,25] Sequences with significant similarity (expect value of $<e^{-50}$) to the well-characterized *Homo sapiens* AS3MT were identified in the NCBI protein database using the Basic Local Alignment Search Tool (BLAST) program (http://blast.ncbi.nlm.nih.gov/). In the case of fungi and other nonanimal eukaryotes, sequences with significant similarity (expect value of $<e^{-50}$) to AS3MT were selected. Among bacterial sequences, a subset was selected that showed the highest level of sequence similarity to AS3MT. Since predicted proteins were based on similarity to the human AS3MT, we proceeded to call all predicted proteins from different species included in this study following the human protein nomenclature. Protein alignment was performed with MUSCLE.[26] The evolutionary history was inferred assuming an LG + INVGAMMA model,[27] as identified by ProtTest.[28] Phylogenetic analyses were subsequently conducted using Bayesian inference and maximum likelihood methods. Bayesian inference was performed with MrBayes 3.2.6[29] and maximum likelihood analyses with RAxML 8.2.12.[30] The MrBayes analysis was performed using the following settings: eight chains of Markov chain Monte Carlo iterations and a heated parameter of 0.05 with trees sampled every 1000 generations. In the RAxML analyses, clade robustness was assessed with 1000 rapid bootstrap inferences followed by thorough analysis of maximum likelihood to obtain statistical support for the placement of nodes. Both the MrBayes and the RAxML analyses were run on the CIPRES Science Gateway[31] in the Extreme Science and Engineering Discovery Environment (XSEDE).

Reagents. All reagents were from Merck (Germany) unless otherwise specified.

Gene Synthesis and Cloning. Fifty-five *AS3MT* genes representing all branches and kingdoms of the phylogenetic tree for AS3MT[23] were chosen for expression and activity analysis (Table 1). The large majority of these proteins are based on protein sequence similarity predicted methyltransferases (n = 47), whereas AS3MT of *H. sapiens*,[13,14] the frog *Xenopus tropicalis*,[32] the mouse *Mus musculus*,[33] the fish *Danio rerio*,[34] the green alga *Chlamydomonas reinhardtii*,[35] the red alga *Cyanidioschyzon merolae*,[36] the fungus *Rhizophagus irregularis*,[37] and the alpha proteobacterium *Rhodopseudomonas palustris*[38] have been characterized as having arsenic methylating capacity before (n = 8). Predicted full-length genes, codon optimized for *Escherichia coli* expression, were synthesized by General Biosystem Inc. (USA) and inserted into pET-28+ using *NcoI* and *XhoI* sites to insert six histidines (His_6) at the C-terminal end.

Protein Expression and Partial Purification. The genes were expressed in *E. coli* BL21(DE3). Cells transformed with *AS3MT* expression plasmids were precultured overnight at 37 °C in 5 mL of Luria Broth (LB) media containing 50 μg/L kanamycin. Cells were transferred to 500 mL of LB media containing 50 μg/L kanamycin in 2 L flasks for 3−4 h. At OD ≈ 0.8, 150 μM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added and incubated for 4 h. Cells were harvested by centrifugation at 4500g for 10 min at 4 °C and resuspended in buffer A: 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) pH 7.5, 150 mM KCl, 10% glycerol, 5 mM imidazole, and 10 mM 2-mercaptoethanol. Cells were lysed with one pass through a French press at 30 kpsi, and 0.4 mM phenylmethane sulfonyl fluoride (final concentration) was added immediately after to avoid protein degradation. Cell lysate was centrifuged at 100 000g for 1 h at 4 °C to remove unbroken cells and membranes. The supernatant was incubated with 2 mL of Ni^{2+}−TED agarose resin for 2 h at 4 °C with slow agitation. The resin was washed with 10 column volumes (CV) of buffer A followed by AS3MT elution using 10 CV of buffer A supplemented with 200 mM imidazole. The elute was concentrated to approximately 3 mL using a 10 kDa spin column, and the protein concentrations were determined using Bradford reagent and bovine serum albumin as a standard. Expression and purity were evaluated using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), prepared according to standard techniques.

Arsenic Methylation Assay. The arsenic methylation assay was done in the same way for all proteins. In the initial screening of all 55 proteins, a 2 μM concentration of each protein was added to the reaction mix which contained 50 mM MOPS pH 7, 150 mM KCl, 5 mM glutathione (GSH), 1 mM *S*-adenosyl methionine (SAM), 1 mM tris(2-carboxyethyl)phosphine (TCEP), and 10 μM sodium arsenite. Samples were then incubated for 4 h at 37 °C while shaking in 5 mL Eppendorf tubes with screw-on caps and a 2 cm diameter nitrocellulose MF membrane (Millipore, USA) previously soaked in 150 μL of 0.3% H_2O_2 to trap TMA by oxidizing this volatile arsenic species into the soluble TMAO.[38] The reactions were stopped by adding 6% volume/volume (v/v) of H_2O_2 in order to oxidize arsenic and make it unavailable for the methylating protein.[38] Samples were then diluted 20 times in water and filtered through 0.20 μm syringe filters (Sarstedt, Germany) for arsenic metabolite determination. The nitrocellulose membranes used to trap TMA were digested in 200 μL of 70% nitric acid at 70 °C for 20 min, diluted to 1 mL in water, and filtered through 0.20 μm syringe filters for arsenic metabolite determination.

For further kinetic characterization, 14 AS3MT proteins were selected from species (animals, other eukaryotes, and bacteria) in Group 1 and 2. For each of these 14 AS3MTs, 3 independent expression and purification batches were carried out. The proteins were assayed as for the initial screening of the 55 proteins with the exception that samples were incubated for 5, 10, 20, 60, 120, and 240 min before stopping the reaction.

Arsenic Metabolite Determination. Standard solutions of the four arsenic species sodium (meta)arsenite [As(III), Sigma-Aldrich, USA], sodium hydrogen arsenate heptahydrate [As(V), Sigma-Aldrich], disodium methyl arsonate (MMA, Sigma-Aldrich), and sodium dimethylarsinate trihydrate (DMA, Merck) were prepared in water. Standard solutions of trimethylarsinoxide (TMAO, Toronto Research Chemicals, Canada) were prepared in water and in 20% nitric acid. Pyridine (Fisher Scientific, USA), methanol (VWR international, USA), nitric acid (VWR), and ammonium carbonate (Fisher) were used as mobile phases for high-performance liquid chromatography (HPLC). The NIST urine SRM 2669 [National Institute of Standards and Technology (NIST), USA] was used as a reference material to ensure the accuracy of the arsenic speciation, and recoveries of arsenic metabolites are presented in Supplemental Table S1.

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Five arsenic species [TMAO, As(III), DMA, MMA, and As(V)] in order of detection] were separated and determined using an Agilent 1260 Infinity II LC system (Agilent Technologies, USA) coupled to a 7700x inductively coupled plasma mass spectrometer (ICPMS; Agilent) as described in Supplemental Table S2. Four arsenic species [As(III), DMA, MMA, and As(V)] were separated in a Hamilton PRP-X110 anion exchange column (Hamilton, USA) with operating conditions described in Supplemental Table S3 (method 1), while the cation exchange column EC 250/4 Nucleosil 100-5 SA (Skandinaviska Genetec AB, Sweden) was used to separate TMAO (Supplemental Table S3, methods 2A and 2B). Two different HPLC methods were used to determine TMAO. Initially, method 2A was used but resulted in distorted TMAO peaks caused by the high acid content (20% nitric acid) in the samples. On the basis of the results obtained by method 2A, selected samples (TMAO producers, *H. sapiens*, and *R. irregularis*) were reanalysed using method 2B, resulting in better TMAO peak shape and more accurate quantification since both calibration standards and samples were dissolved in 20% nitric acid. Chromatogram examples are shown in Supplemental Figures S1 and S2.

Arsenic methylation capacity was calculated as the relative concentrations (%) of the different arsenic metabolites. For TMAO, the concentration trapped in the nitrocellulose membrane was added to the TMAO concentration in the reaction mix suspension. This considers potential dripping from the membrane when TMAO becomes soluble in contact with H_2O_2.

RESULTS

The 55 AS3MT proteins from 47 species selected in the present study clustered in two major phylogenetic groups (Figure 1: Group 1 and Group 2). The clustering in two major groups was very similar to our previous study encompassing 150 predicted AS3MT sequences from 134 species[23] with one exception (WP_015943273.1; *Desulfitobacterium hafniense*_1). We were able to express 54 out of the 55 His_6-tagged *AS3MT*-like genes in *E. coli*, and most AS3MT homologues were highly expressed and could be partially purified in a single step employing Ni^{2+}−NTA chromatography: only the predicted AS3MT from *Halapricum salinum* could not be identified via SDS-PAGE. In some cases, the partially purified preparation included two or more major polypeptides, which could be an effect of alternative transcripts or the result of protein instability (Figure 2A). The AS3MT homologues showed variation in the number of amino acid residues in their sequences, and in general, the length of the protein corresponded to the phylogenetic grouping: Group 1 encompassed proteins with higher molecular weight (in average 41.9 kDa) compared with Group 2 (29.9 kDa) (Table 1; Figure 2A).

We then evaluated if the differences in apparent molecular weight also corresponded to functional differences by biochemical analysis of the arsenic methylating capacity. As seen in Table 1, only one of the expressed proteins did not show any methylated products (*Chlorella variabilis* 2) whereas all other proteins were able to methylate arsenic. This demonstrates that the predicted AS3MT proteins selected in this study are indeed true arsenic methyltransferases. The fraction of methylated metabolites after incubating for 4 h was low for some of the AS3MT proteins: <5% of the arsenic was methylated in 8/53 proteins and <10% in 19/53.

To investigate whether proteins belonging to each phylogenetic group have distinct methylation patterns, we further investigated the biochemical properties of 14 proteins that represent the two phylogenetic groups (a phylogenetic tree for the 14 species is shown in Figure 2B). All 14 proteins

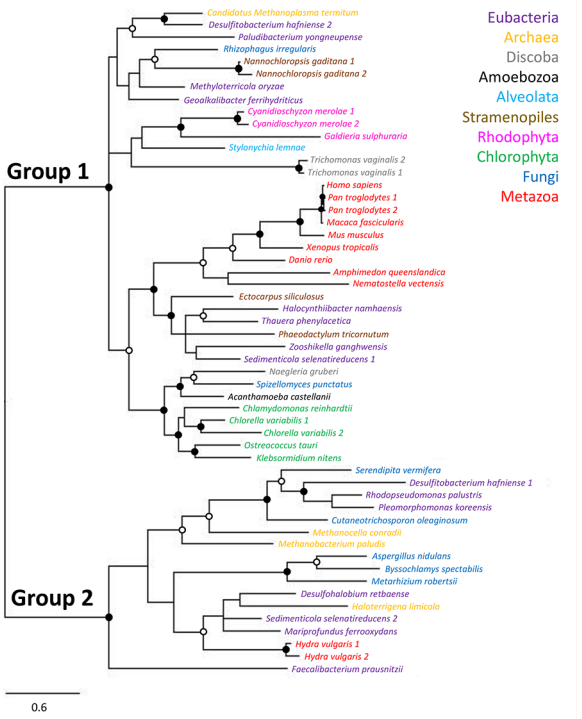

Figure 1. Phylogenetic tree of 54 (*H. salinum* was excluded as it was not expressed) AS3MT-like proteins reveals two major branches: Group 1 and Group 2. Tree is the result of a Bayesian inference analysis carried out using the program MrBayes. Black dots at nodes indicate maximum statistical support (cumulative probability $P = 1$) in the Bayesian inference analysis. White dots at nodes indicate a support value of $P > 0.9$. Bayesian interference analysis was run for 1 000 000 generations, and average standard deviation of split frequencies between the resulting trees was 0.008654. Accession numbers for sequences are given in Table 1. Color codes are given to the right of the figure. Scale bar: 0.6 amino acid substitutions per site.

methylated arsenic although with less efficiency compared to the initial screening (Figure 3). The methylation rate varied markedly between the 14 proteins from 14 different organisms: while the proteins from *Trichomonas vaginalis* and *H. sapiens* methylated approximately 30% of the substrate within 240 min, only 5% was methylated by the AS3MT from *Halocythiibacter namhaensis* (Figure 3). Such quantitative differences should be interpreted with care as assay conditions were identical and not optimized for each protein. However, qualitative differences in product formation were observed. Thus, proteins belonging to Group 1 only showed MMA and DMA methylation with preference for MMA. In Group 2, more proteins were able to produce TMAO and DMA was the predominant metabolite (Figure 3).

DISCUSSION

In this study, we show that predicted AS3MT proteins, from different parts of the tree of life, are indeed true arsenic methyltransferases: out of 54 expressed AS3MT proteins, 53 turned out to have arsenic methylating capacity in vitro. All proteins selected have previously been shown to carry the signature motifs characteristic of SAM-dependent methyltransferases, but AS3MTs in Group 1 and 2 show different molecular weights. The difference in molecular weight is largely due to the varying C-terminal of the protein, a domain with hitherto no known function.[39] Despite the difference in

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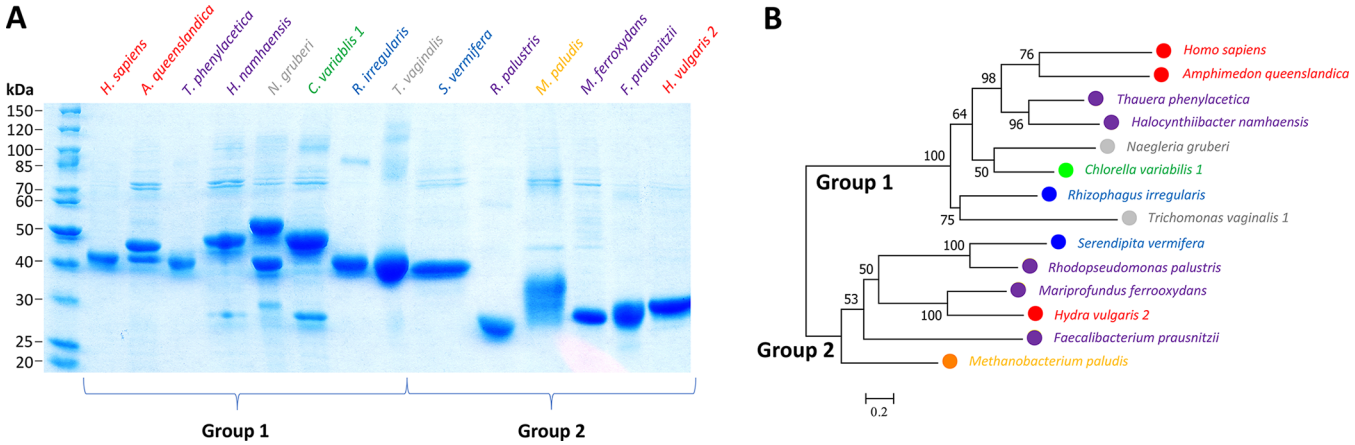

Figure 2. Proteins included in the kinetic characterization analysis. (A) Sodium dodecyl sulfate polyacrylamide gel electrophoresis showing the purity of the 14 His-tagged AS3MTs expressed as recombinant proteins in *E. coli* and subsequently purified by Ni-TED affinity chromatography. (B) Phylogenetic tree of the AS3MTs resulting from a maximum likelihood analysis using RAxML. Best tree (likelihood −9 278.332761) after 1000 bootstrap rounds is shown, as described in the Materials and Methods. Bootstrap values are given at nodes. Accession numbers for sequences are given in Table 1. Each species in the tree is marked with a dot colored according to the taxonomic supergroup to which it belongs. Scale bar: 0.2 amino acid substitutions per site.

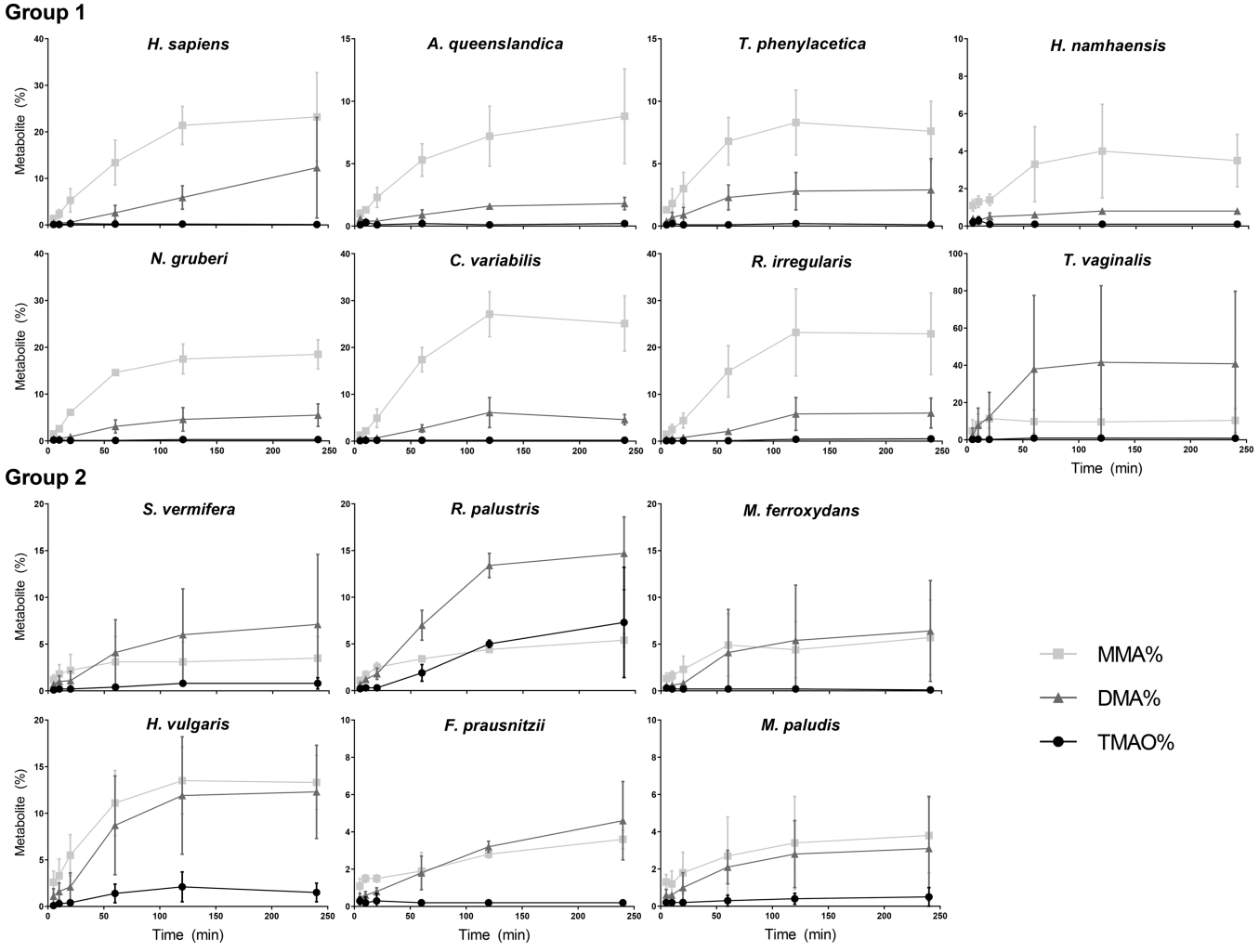

Figure 3. Arsenic methylation over time for 14 selected AS3MT proteins grouped by phylogenetic Group 1 and 2. Note that the range for the *Y* axis varies depending on the protein. Results are presented as mean and standard deviation of three independent replicates.

molecular weight, proteins in both groups methylate arsenic but some differences could be identified. Similar to bacteria in Group 1, the AS3MT protein from *H. sapiens* produced MMA and DMA but no TMAO, whereas the AS3MT from *H.*

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vulgaris in Group 2 produced TMAO, as other bacteria in the same clade, MMA, and DMA. These results indicate functional differences between the groups and support the phylogenetic observation of HGT of AS3MT from multiple prokaryotic phyla to eukaryotes.[23,24]

Interestingly, we found that both predicted AS3MT isoforms from chimpanzee (*Pan troglodytes*) were capable of methylating arsenic. An earlier study reported that this species, based on analysis of two chimpanzee individuals, lacks the ability to methylate inorganic arsenic.[40] However, in the reports by Palmgren et al.,[23] we could not find any genetic explanation for this lack of arsenic methylation capacity. Our finding of arsenic methylation by a predicted AS3MT in the chimpanzee genome suggests that AS3MT-mediated arsenic tolerance of chimpanzee in some cases may be compromised, e.g., by down-regulation, maybe by epigenetic mechanisms, of *AS3MT* expression in this species.

The predicted AS3MT of *F. prausnitzii* has a particular interest. Coryell et al.[22] recently showed that in germ-free mice that received microbiome from different human donors, the microbiome and its composition matter for both the uptake of arsenic in the gut and the tolerance to arsenic toxicity. Further, using an *As3mt*-knockout mouse model, they found that both AS3MT encoded by the mouse genome and an intact gut microbiome are necessary for full protection against arsenic toxicity. This protection was hypothesized to be accounted for by arsenic-metabolizing bacteria in the gut (such from those carrying the Ars operon, containing the *arsM* gene),[15] but this was not clarified in the study. Coryell and co-workers[22] also selected the very common gut bacterium *F. prausnitzii* and showed that this single bacterium could confer resistance to arsenic toxicity. We can here for the first time show that this bacterium actually methylates arsenic, an activity that is likely to contribute to its protection to arsenic. Further, it shows that humans carry two bacterial systems for arsenic methylation: one bacterium-derived AS3MT incorporated in the human genome from Group 1 and one in *F. prausnitzii* from Group 2 present in the gut microbiome. One hypothesis is that AS3MT in human gut bacteria confers a similar arsenic tolerance as that shown in mice. If this is true also for humans, it would be interesting to investigate the gut microbiota in populations susceptible or tolerant to arsenic.[14]

Strengths of the present study are the large number of predicted AS3MT proteins tested and the combination of phylogenetic analysis with phenotypic characterization. Taken together, the data demonstrates that at least two phylogenetically and biochemically divergent AS3MTs have been acquired in separate events from eubacteria to animal genomes and that humans have two AS3MT-based systems for protection against arsenic among which only one is part of the human genome. One originates from an ancient event of HGT from a prokaryote to a eukaryotic ancestor, while the other is part of the genome of a free-living prokaryote.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.0c00375.

Recoveries of arsenic species in NIST urine SRM 2669 level II; ICP-MS equipment and operating conditions; HPLC equipment and operating conditions; chromatograms of arsenic speciation of selected organisms; chromatograms of arsenic speciation of selected organisms (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Michael Palmgren − *Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen DK-1871, Denmark*; Email: palmgren@plen.ku.dk

Karin Broberg − *Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen DK-1871, Denmark; Institute of Environmental Medicine, Karolinska Institutet, Stockholm 171 77, Sweden*; orcid.org/0000-0002-5862-468X; Email: karin.broberg@ki.se

Authors

Jesper Torbøl Pedersen − *Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen DK-1871, Denmark; Institute of Environmental Medicine, Karolinska Institutet, Stockholm 171 77, Sweden*

Jessica De Loma − *Institute of Environmental Medicine, Karolinska Institutet, Stockholm 171 77, Sweden*; orcid.org/0000-0002-2924-854X

Michael Levi − *Institute of Environmental Medicine, Karolinska Institutet, Stockholm 171 77, Sweden*; orcid.org/0000-0001-6419-1975

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.chemrestox.0c00375

Author Contributions

§J.T.P. and J.D.L.: These authors contributed equally

Notes

The authors declare no competing financial interest.

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