Libertas Academica
FREEDOM TO RESEARCH

Open Access: Full open access to this and thousands of other papers at http://www.la-press.com.

Microbiology Insights

Evaluative Profiling of Arsenic Sensing and Regulatory Systems in the Human Microbiome Project Genomes

Raphael D. Isokpehi[1], Udensi K. Udensi[2], Shaneka S. Simmons[3,4], Antoinesha L. Hollman[5], Antia E. Cain[6], Samson A. Olofinsae[7], Oluwabukola A. Hassan[7], Zainab A. Kashim[7], Ojochenemi A. Enejoh[7], Deborah E. Fasesan[7] and Oyekanmi Nashiru[7]

[1]Department of Biology, Bethune-Cookman University, Daytona Beach, FL, USA. [2]RCMI Center for Environmental Health, College of Science, Engineering and Technology, Jackson State University, Jackson, MS, USA. [3]Department of Biology, Jackson State University, Jackson, MS, USA. [4]Department of Computer Science, Jackson State University, Jackson, MS, USA. [5]Jarvis Christian College, Hawkins, TX, USA. [6]Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, USA. [7]H3Africa Bioinformatics Network Node, National Biotechnology Development Agency (NABDA), Abuja, Nigeria.

ABSTRACT: The influence of environmental chemicals including arsenic, a type 1 carcinogen, on the composition and function of the human-associated microbiota is of significance in human health and disease. We have developed a suite of bioinformatics and visual analytics methods to evaluate the availability (presence or absence) and abundance of functional annotations in a microbial genome for seven Pfam protein families: As(III)-responsive transcriptional repressor (ArsR), anion-transporting ATPase (ArsA), arsenical pump membrane protein (ArsB), arsenate reductase (ArsC), arsenical resistance operon transacting repressor (ArsD), water/glycerol transport protein (aquaporins), and universal stress protein (USP). These genes encode function for sensing and/or regulating arsenic content in the bacterial cell. The evaluative profiling strategy was applied to 3,274 genomes from which 62 genomes from 18 genera were identified to contain genes for the seven protein families. Our list included 12 genomes in the Human Microbiome Project (HMP) from the following genera: *Citrobacter*, *Escherichia*, *Lactobacillus*, *Providencia*, *Rhodococcus*, and *Staphylococcus*. Gene neighborhood analysis of the arsenic resistance operon in the genome of *Bacteroides thetaiotaomicron* VPI-5482, a human gut symbiont, revealed the adjacent arrangement of genes for arsenite binding/transfer (ArsD) and cytochrome *c* biosynthesis (DsbD_2). Visual analytics facilitated evaluation of protein annotations in 367 genomes in the phylum Bacteroidetes identified multiple genomes in which genes for ArsD and DsbD_2 were adjacently arranged. Cytochrome *c*, produced by a posttranslational process, consists of heme-containing proteins important for cellular energy production and signaling. Further research is desired to elucidate arsenic resistance and arsenic-mediated cellular energy production in the Bacteroidetes.

KEYWORDS: arsenic, arsenate, arsenite, *Bacteroides*, Bacteroidetes, bioinformatics, genomes, gut microbiota, heavy metal transport, Human Microbiome Project, human symbiont, mercuric transport, secondary data analysis, visual analytics

CITATION: Isokpehi et al. Evaluative Profiling of Arsenic Sensing and Regulatory Systems in the Human Microbiome Project Genomes. *Microbiology Insights* 2014:7 25–34 doi:10.4137/MBI.S18076.

RECEIVED: June 24, 2014. **RESUBMITTED:** August 18, 2014. **ACCEPTED FOR PUBLICATION:** August 26, 2014.

ACADEMIC EDITOR: Raul Rivas, Editor in Chief

TYPE: Original Research

FUNDING: This work was funded by Office of the Provost, Bethune-Cookman University, National Institutes of Health (NIH/NIMHD 1P20MD006899, NIH/NIGMS 5T36GM095335, NIH/NIMHD G12MD007581), National Science Foundation (EPS-0903787, DBI-1062057),and U.S. Department of Homeland Security Science and Technology Directorate: 2011-ST-062-000048. The H3Africa Bioinformatics Network (H3ABioNet) is funded by NIH Common Fund Award/NHGRI Grant Number U41HG006941. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the funding agencies.

COMPETING INTERESTS: Authors disclose no potential competing interest.

CORRESPONDENCE: isokpehir@cookman.edu

Introduction

High-throughput technologies for assaying biological macromolecules and metabolites are providing wealth of data on the structure, function, and condition-induced changes within host-associated microbial communities.[1] The influence of environmental chemicals including arsenic, a type 1 carcinogen, on the composition and function of the human-associated microbiota is of significance in human health and disease.[2]

The data from the Human Microbiome Project (HMP) include genome sequences and functional annotations for over 1000 microbial isolates obtained from diverse body sites of healthy adults.[3–5] There is an urgent need for data analytics (modeling and simulation, statistical analysis, and visual analytics) of the wealth of data on the human microbiome for new types of treatment as well as mechanisms of chronic diseases.[6–8] The results from data analytics of microbiome data hold promise to advance knowledge of how the human microbiota at body sites respond to ubiquitous environmental chemicals such as arsenic.

The overall theme of our research is to identify and evaluate microbial gene clusters that are equipped for stress response.[9] In this article, we report the integration of data on the availability and abundance of genes for arsenic stress response in microbial genomes. We reason that the integration of availability (presence or absence) and abundance of genes for functions can be informative on the microbe's potential to perform the functions. In the case of influences of arsenic on the human microbiome, knowledge on the availability and abundance of arsenic stress response genes will guide further research on the pre-systemic metabolism of arsenic by the microbiota at the body site.

Arsenic is a naturally occurring environmental chemical, and drinking water and dietary intake are two main routes through which human beings are exposed to it.[10,11] Pentavalent (arsenate) and trivalent (arsenite) inorganic arsenic species perturbs the normal cell function.[12] The ubiquitous natural occurrence of arsenic means that cells from all domains of life must develop molecular and phenotypic mechanisms to respond to arsenic-induced stress.[13–15] Ingested arsenic is a cause of cancers of the skin, lungs, bladder, and kidneys.[16] Gut microbial metabolism of arsenate produces the more absorbable and toxic arsenite. The genome sequences of single isolates and microbial communities encode mechanisms by which gut microbiota transforms ingested arsenic to more toxic trivalent methylated and thiolated arsenicals prior to their metabolism in human cells.[17,18] Therefore, to make progress on elucidating pre-systemic metabolism of arsenic, it is necessary to identify microbes of the human microbiota with genes for sensing and regulating arsenic.

Exposure of the human microbiota to arsenic presents an unfavorable environment to microbial cells. In microbial genomes, several genes function in the sensing and regulation of inorganic arsenic. We are interested in the genes encoding arsenic resistance operon, the aquaporins, and the universal stress proteins (USPs). These genes encode function for sensing and/or regulating (resistance) arsenic content in the bacterial cell.[19] The best-characterized arsenic genes include As(III)-responsive transcriptional repressor (*arsR*), anion-transporting ATPase (*arsA*), arsenical pump membrane protein (*arsB*), arsenate reductase and related proteins, glutaredoxin family (*arsC*), arsenical resistance operon trans-acting repressor (*arsD*), arylsulfatase family, member H (*arsH*), putative membrane permease (*ArsP*), and As(III)-*S*-adenosylmethionine methyltransferase (*arsM*).[20] The genes for conversion of arsenate to arsenite and arsenite extrusion from the cell are typically organized as operons, such as *arsRBC*, *arsRABC*, and *arsRDABC*, but the genes can also exist alone.[20] Proteins for water and/or glycerol transport across cellular membranes termed aquaporins can also function in arsenic transport.[21] The USP family is a protein family known to enable bacteria, archaea, fungi, viridiplantae, and certain metazoans that respond to stresses.[22–24] The USP family includes proteins that contain 140–160 amino acid (aa) USP domain [PF00582 (or Pfam00582) in the Pfam database].[22–24] The domain architecture of USPs can be (i) one USP domain, (ii) two USP domains in tandem, or (iii) one or two USP domains together with other functional domains including transporters, kinases, permeases, transferases, and bacterial sensor.[24,25] In *Exiguobacterium* sp. PS, a Gram-positive bacteria that lacks arsenic reductase activity, a USP was induced by arsenate stress.[26] The *uspA* of *Escherichia coli* has been evaluated as a sensor to detect chemical toxicants.[27]

The availability of diverse data from HMP allows for secondary data analytics including constructing profiles of functional annotations for genes involved in arsenic sensing and regulation in the HMP genomes collection. Therefore, we report the development of a genome profiling scheme based on the availability of functional annotations for seven Pfam protein families, including known arsenic resistance operon proteins, aquaporins, and USPs. A list of 62 genomes from 18 genera was identified including 12 genomes in the HMP genomes collection. Several noteworthy findings could be a basis for further investigations. For example, in multiple *Bacteroides* genomes, a gene for arsenic binding and transfer (arsD) is adjacent to a gene for cytochrome *c* biogenesis protein. Cytochrome *c*, produced by a posttranslational process, consists of heme-containing proteins important for cellular energy production and signaling.[28,29] Previous reported research on *Bacteroides* and arsenic appears to be limited to phenotypic characterization of susceptibilities to arsenate in which 25% of strains in the *Bacteroides fragilis* group, which included *Bacteroides thetaiotaomicron*, were resistant to 0.01 M arsenate.[30] Further research is desired to elucidate arsenic resistance and arsenic-mediated cellular energy production in the Bacteroidetes.

Methods

Construction of functional annotation profiles for arsenic stress response of microbial genomes. We assembled a list of protein family functions (Pfam) that are known to participate in the metabolism of arsenic and in stress response in bacteria and archaea. The Pfam identifiers, names, and common abbreviation of the proteins are Pfam02374 [anion-transporting ATPase (ArsA)]; Pfam02040 [arsenical pump membrane protein (ArsB)]; Pfam03960 [arsenate reductase and related proteins, glutaredoxin family (ArsC)]; Pfam06953 [arsenical resistance operon trans-acting repressor

(ArsD)]; Pfam01022 [As(III)-responsive transcriptional repressor (ArsR)]; Pfam00230 [major intrinsic protein family (MIP/AQP)]; and Pfam00582 [universal stress protein domain (Usp)]. Genomes with a profile of interest were further grouped into relevance annotation (eg, agriculture, biotechnology, human pathogen, and medical) provided by the Integrated Microbial Genomes (IMG) system. When relevance is not annotated, we tagged the genome as "Not_Reported." A binary matrix that encodes the presence (1) or absence (0) of a relevance annotation for selected genomes was constructed. The binary matrix was visualized with matrix2png.[31]

Integration of availability and abundance of genes for arsenic metabolism and USPs. Genes annotated for presence of the above seven protein families in microbial genomes were initially retrieved from the IMG system (http://img.jgi.doe.gov/) in December 2011 (IMG version 3.5).[32] The datasets were downloaded as Excel spreadsheets and integrated in a visual analytics software (Tableau Desktop Professional, Seattle, WA). The dataset includes several fields including the genome name (Genome) and the gene object identifier (Gene Object ID). The visual analytics tool displayed the availability (presence or absence) and abundance (number of genes annotated with the Pfam function) of each microbial genome. A list of reference genomes sequenced by the HMP was obtained from the HMP catalog (http://www.hmpdacc.org/catalog/).

Because *B. thetaiotaomicron* is a dominant symbiont of the gut of humans and other mammals,[33] we decided to determine the abundance of genes for the arsenic resistance operon (arsRABCD) in the *Bacteroides* genomes in the dataset. A visualization was also generated to provide an overview of the abundance of the arsenic resistance genes in *Bacteroides* genomes.

Functional associations of ArsD encoded transcription units in *B. thetaiotaomicron*. The gene content of the transcription unit with arsenic-associated genes was determined for *B. thetaiotaomicron* using the BioCyc database collection of pathway/genome databases (PGDBs).[34] In BioCyc, a transcription unit is defined as a set of one or more genes that are transcribed to produce a single messenger RNA. Our particular interest is in multigene transcription units. Based on the observed functions in the transcription units, the presence of two Pfam functions in a chromosomal cassette was determined with the Cassette search tool of the IMG system.[32] In the IMG system, a chromosomal cassette is defined as a stretch of protein coding genes with intergenic distance lesser than or equal to 300 base pairs.

Known and predicted functional associations of proteins encoded in a transcription unit were retrieved from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database.[35] With this approach, we expect that the STRING database will provide a score for gene neighborhood evidence for genes in a transcription unit. Other types of evidence that are used in the STRING database to calculate a combined score are gene fusion, co-occurrence, co-expression, experiment, databases, text mining, and homology.[35]

Results

Arsenic stress response profiles for genomes. A seven-digit binary code was assigned to 3,274 genomes (119 archaea, 3033 bacteria, and 122 eukaryota) obtained from the IMG system. The order of the seven Pfam families in the binary code is ArsA, ArsB, ArsC, ArsD, ArsR, Aqp, and Usp (Table 1). As functional annotations of genes could change, we selected only genomes that have the complete profile (111111111). A total of 62 microbial genomes from 18 genera had a binary code in which all the seven Pfam families were present. We further grouped the genomes according to their relevance (eg, agriculture, biotechnology, human pathogen, and medical) to help direct further research. A subset of 57 genomes with the complete profile was mapped to 22 assignments of relevance. Additionally, five genomes did not have an assignment of relevance and their assignment was designated "Not_Reported". A visualization of the matrix of 23-digit binary signatures was constructed for 62 genomes (Fig. 1).

The 12 HMP reference genomes with the complete profile were grouped in the body sites: gastrointestinal tract, skin, and skin wound. The genomes with the complete binary profile from the human gastrointestinal tract were *Citrobacter* sp. 30_2, *E. coli* MS 115-1, *E. coli* MS 198-1, *Lactobacillus fermentum* ATCC 14931, and *Providencia stuartii* ATCC 25827.

Table 1. Position of Pfam protein family annotation in genome binary profile.

Pfam ID	Pfam NAME AND ABBREVIATION	POSITION IN BINARY DIGIT
Pfam02374	Anion-transporting ATPase [ArsA]	1
Pfam02040	Arsenical pump membrane protein [ArsB]	2
Pfam03960	Arsenate reductase and related proteins, glutaredoxin family [ArsC]	3
Pfam06953	Arsenical resistance operon trans-acting repressor, [ArsD]	4
Pfam01022	As(III)-responsive transcriptional repressor [ArsR]	5
Pfam00230	Major intrinsic protein family [MIP/AQP]	6
Pfam00582	Universal stress protein domain [Usp]	7

	Agricultural	Animal_Pathogen	Bioenergy	Bioreactors	Bioremediation	Biotechnological	Biothreat	Cattle_Pathogen	Comparative_analysis	Energy_production	Environmental	Food_industry	Human_Gut_Microbiome_Initiative_HGMI	Human_Microbiome_Project_HMP	Human_Pathogen	Lactic_acid_production	Medical	Not_Reported	Pest_control	Plant_Pathogen	Poultry_Pathogen	Swine_Pathogen	Wastewater_treatment
Bacillus_cereus_95/8201															■		■						
Bacillus_cereus_AH820							■								■		■						
Bacillus_cereus_ATCC_10987							■								■		■						
Bacillus_cereus_H3081.97							■								■		■						
Bacillus_cereus_m1293															■		■						
Bacillus_cereus_R309803															■		■						
Bacillus_cereus_Rock1-15															■		■						
Bacillus_cereus_Rock1-3															■		■						
Bacillus_cereus_Rock3-29															■		■						
Bacillus_cereus_Rock3-42															■		■						
Bacillus_cereus_Rock3-44															■		■						
Bacillus_pseudofirmus_OF4																		■					
Bacillus_selenitireducens_MLS10						■																	
Bacillus_thuringiensis_BMB171																	■						
Burkholderia_phytofirmans_PsJN	■																						
Burkholderia_sp._Ch1-1						■																	
Burkholderia_vietnamiensis_G4	■					■			■											■			
Citrobacter_sp._30_2									■					■			■						
Enterobacter_cloacae_cloacae_ATCC_13047				■													■		■				
Enterobacter_cloacae_SCF1			■																				
Escherichia_coli_FVEC1412		■															■					■	
Escherichia_coli_MS_115-1														■			■						
Escherichia_coli_MS_198-1														■			■						
Escherichia_coli_017:K52:H18_UMN026															■		■						
Escherichia_coli_044:H18_042_(EAEC)																							
Escherichia_coli_TA280		■															■					■	
Exiguobacterium_sp._AT1b																		■					
Klebsiella_pneumoniae_342																		■					
Klebsiella_pneumoniae_pneumoniae_MGH78578		■						■							■		■						
Lactobacillus_fermentum_ATCC_14931														■			■						
Lactobacillus_plantarum_WCFS1						■						■				■							
Listeria_innocua_Clip11262																	■						
Listeria_monocytogenes_08-5578		■						■	■						■		■				■	■	
Listeria_monocytogenes_08-5923		■						■							■		■				■	■	
Listeria_monocytogenes_10403S																		■					
Magnetospirillum_magnetotacticum_MS-1						■																	
Methylobacillus_flagellatus_KT					■	■				■	■												■
Nitrosomonas_sp._AL212						■																	
Providencia_stuartii_ATCC_25827													■	■			■						
Rhodococcus_erythropolis_PR4						■																	
Rhodococcus_erythropolis_SK121														■			■						
Rhodococcus_opacus_B4						■																	
Salmonella_enterica_enterica_sv_Javiana_GA_MM04042433															■		■						
Salmonella_enterica_enterica_sv_Newport_SL317															■		■						
Salmonella_enterica_enterica_sv_Paratyphi_B,_SPB7															■		■						
Salmonella_enterica_enterica_sv_Saint_Paul_SARA29															■		■						
Salmonella_enterica_sv_Newport_SL254															■		■						
Shewanella_putrefaciens_CN-32						■																	
Shewanella_sp._ANA-3					■	■																	
Shewanella_sp._W3-18-1																		■					
Staphylococcus_epidermidis_ATCC_12228																	■						
Staphylococcus_epidermidis_BCM-HMP0060									■					■			■						
Staphylococcus_epidermidis_M23864:W1									■					■			■						
Staphylococcus_epidermidis_M23864:W2(grey)																							
Staphylococcus_epidermidis_RP62A															■		■						
Staphylococcus_epidermidis_SK135														■			■						
Staphylococcus_epidermidis_W23144														■			■						
Staphylococcus_haemolyticus_JCSC1435		■				■									■		■						
Staphylococcus_hominis_SK119														■			■						
Staphylococcus_saprophyticus_saprophyticus_ATCC_15305															■		■						
Yersinia_enterocolitica_enterocolitica_8081															■		■						
Yersinia_intermedia_ATCC_29909															■		■						

Figure 1. Visualization of binary-encoded matrix for relevance of genomes with genes for arsenic operon, aquaporin, and universal stress protein. Data were obtained from the Integrated Microbial Genomes. Black square, relevance annotated for genome; white square, relevance not annotated for genome. When relevance data were not available, we entered a "Not_Reported" for data processing.

Additionally, the selected genomes based on the complete seven-digit profile and isolated from the skin were five strains of *Staphylococcus epidermidis*, *Staphylococcus hominis* SK119, and *Rhodococcus erythropolis* SK121. The locus tags for genes encoding the protein families are presented in Table 2. The locus tags are presented in the sequence of the arsRDABC operon (Table 2). The arrangement of the genes with reference to their function is presented in Table 3. In *Citrobacter* sp. 30_2, two arsenic resistance gene clusters were identified with the same gene order of arsRDABC. Overall, a pattern in Table 3 is that arsA and arsD when present are adjacent for all the genomes from the four Gram-negative bacteria. However, in *L. fermentum* ATCC 14931, the gene cluster order was arsRABDC.

Integration of availability and abundance of genes for arsenic sensing and regulation. The integration of data fields from several data sources was accomplished through visual analytics tasks. Figure 2 is a visualization that integrates the data on binary code; body site; genome; and the availability of Pfam annotations and abundance (number of genes) for 12 reference genomes sequenced by the HMP. Several patterns can be identified from Figure 2. As gastrointestinal pre-systemic metabolism is an essential step of arsenic metabolism in humans, we further investigated the abundance of arsenic

Table 2. Locus tags for genes encoding selected arsenic-associated protein families in five Human Microbiome Project reference genomes.

Pfam FAMILY	*CITROBACTER* SP. 30_2	*ESCHERICHIA COLI* MS 115-1	*ESCHERICHIA COLI* MS 198-1	*LACTOBACILLUS FERMENTUM* ATCC 14931	*PROVIDENCIA STUARTII* ATCC 25827
arsR (Pfam01022)	CSAG_00049	HMPREF9540_00434	HMPREF9552_00168	HMPREF0511_0214	PstuA_020100015920
	CSAG_00058	HMPREF9540_00675	HMPREF9552_02803	HMPREF0511_1131	PstuA_020100016320
	CSAG_00761	HMPREF9540_01104	HMPREF9552_02903	HMPREF0511_1475	PstuA_020100017025
	CSAG_02502	HMPREF9540_04804	HMPREF9552_02908		
	CSAG_04185	HMPREF9540_04813			
	CSAG_04189				
	CSAG_04238				
	CSAG_04297				
arsD (Pfam06953)	CSAG_00050	HMPREF9540_04807	HMPREF9552_02907	HMPREF0511_1134	PstuA_020100016315
	CSAG_00055	HMPREF9540_04812			
	CSAG_04239				
arsA (Pfam02374)	CSAG_00051	HMPREF9540_04808	HMPREF9552_02906	HMPREF0511_1132	PstuA_020100007500
	CSAG_00054	HMPREF9540_04811			PstuA_020100016310
	CSAG_04240				
	CSAG_04243				
arsB (Pfam02040)	CSAG_00052	HMPREF9540_00433	HMPREF9552_02905	HMPREF0511_1133	PstuA_020100016305
	CSAG_04241	HMPREF9540_04810			
arsC (Pfam03960)	CSAG_00053	HMPREF9540_00432	HMPREF9552_01835	HMPREF0511_0280	PstuA_020100013100
	CSAG_02267	HMPREF9540_04809	HMPREF9552_01863	HMPREF0511_0923	PstuA_020100013225
	CSAG_02283	HMPREF9540_05016	HMPREF9552_02904	HMPREF0511_0962	PstuA_020100016300
	CSAG_04242	HMPREF9540_05044			
Aqp (Pfam00230)	CSAG_01847	HMPREF9540_02867	HMPREF9552_00087	HMPREF0511_1378	PstuA_020100002768
	CSAG_01948	HMPREF9540_03890	HMPREF9552_03971		
	CSAG_04569	HMPREF9540_04727			
Usp (Pfam00582)	CSAG_00404	HMPREF9540_00348	HMPREF9552_01620	HMPREF0511_0613	PstuA_020100008480
	CSAG_00475	HMPREF9540_00443	HMPREF9552_02920	HMPREF0511_1339	PstuA_020100010015
	CSAG_01459	HMPREF9540_00492	HMPREF9552_03271	HMPREF0511_1387	PstuA_020100010755
	CSAG_01471	HMPREF9540_01169	HMPREF9552_03967	HMPREF0511_1569	PstuA_020100010765
	CSAG_01741	HMPREF9540_02863	HMPREF9552_04168	HMPREF0511_1702	PstuA_020100011480
	CSAG_03714	HMPREF9540_04247	HMPREF9552_05110		PstuA_020100015380
	CSAG_03977	HMPREF9540_04414	HMPREF9552_05202		PstuA_020100019714
	CSAG_04126				
	CSAG_00328				

resistance genes in 43 *Bacteroides* genomes (Fig. 3). There were several noteworthy findings from the visualization including (i) multiple copies of arsA, arsD, and arsR were observed in the genomes of *Bacteroides intestinalis* 341, DSM 17393, and *B. thetaiotaomicron* VPI-5482; (ii) all the *Bacteroides* genomes did not include the annotation for arsB (Pfam2040, arsB); and (iii) in 21 *Bacteroides* genomes, only one *arsC* gene per genome was annotated. Clearly, the *B. intestinalis* and *B. thetaiotaomicron* strains have multiple arsA, arsD, and arsR, which is indicative of the presence of at least two arsenic resistance operons. The genomic context of the genes in the arsenic operons of *B. thetaiotaomicron* is presented in Figure 4. Further analysis of the Pfam domain composition of the three genes annotated with the Pfam for the arsA gene revealed that two genes (BT_0116 and BT_0802) had only the Pfam02374 annotation, whereas BT_3895 had a protein domain annotation of Pfam02374 (arsA) and Pfam10609 (ParA/MinD ATPase like).

Functional associations of ArsD-encoded transcription units in *B. thetaiotaomicron*. Figure 4 provides an overview of the two transcription units, the predicted function of proteins, and the predicted protein–protein networks involving arsenic-associated genes for *B. thetaiotaomicron* VPI-5482. The two arsD genes (BT_0117 and BT_0801) in *B. thetaiotaomicron* VPI-5482 are located on two transcription units, which

Table 3. Comparison of gene function order in arsenic resistance operon in selected Human Microbial Project reference genomes.

GENOME	GENE CLUSTER IDENTIFIER*	GENE ORDER				
		1	2	3	4	5
Citrobacter sp. 30_2	CSAG_00049-CSAG_00053	arsR	arsD	arsA	arsB	arsC
	CSAG_00054-CSAG_00055	arsA	arsD			
	CSAG_04238-CSAG_04242	arsR	arsD	arsA	arsB	arsC
	CSAG_04243-CSAG_04243	arsA				
Escherichia coli MS 115-1	HMPREF9540_00432-HMPREF9540_00434	arsC	arsB	arsR		
	HMPREF9540_04807-HMPREF9540_04810	arsD	arsA	arsC	arsB	
	HMPREF9540_04811-HMPREF9540_04813	arsA	arsD	arsR		
Escherichia coli MS 198-1	HMPREF9552_02903-HMPREF9552_02907	arsR	arsC	arsB	arsA	arsD
	HMPREF9552_02908-HMPREF9552_02908	arsR				
Lactobacillus fermentum ATCC 14931	HMPREF0511_1131-HMPREF0511_1134	arsR	arsA	arsB	arsD	
Providencia stuartii ATCC 25827	PstuA_020100016300-PstuA_020100016320	arsC	arsB	arsA	arsD	arsR

Note: *Start and end genes are used to identify gene clusters.

are, respectively, labeled as TUJXV-83 and TUJXV-442 in BioCyc, respectively. These transcription units have nine and six genes, respectively (Fig. 4A and B). As shown in Figure 4C, both transcription units have the genes for homologs of *arsA* (BT_0116; BT_0802), *acr3* [homologous to *arsB*] (BT_0114; BT_0803), and *arsD* (BT_0117; BT_0801). TUJXV-83 is unique for a permease (BT_0113), mercuric transport protein (BT_0114), and arsenic reductase (BT_0115). Three additional proteins encoded in both transcription units are cytochrome *c* biogenesis protein (BT_0118; BT_0800), protein with thioredoxin-like fold (BT_0119; BT_0799), and redox-active disulfide protein (BT_0120; BT_0798).

In both the transcription units, the *arsD* gene was adjacent to the gene for a cytochrome *c* biogenesis protein DsbD_2 (Pfam13386) and an anion-transporting ATPase arsA (Pfam06953). Using the IMG system, a search of chromosomal cassettes with Pfam06953 and Pfam13386 in 2,841 finished bacterial genomes identified cassettes in the following nine genomes: *B. thetaiotaomicron* VPI-5482; *Bacteroides vulgatus* ATCC 8482; *Bacteroides xylanisolvens* XB1A; *Porphyromonas asaccharolytica* VPI 4198, DSM 20707; *Prevotella melaninogenica* ATCC 25845; *Shewanella putrefaciens* 200; *S. putrefaciens* CN-32; *Shewanella* sp. ANA-3; and *Shewanella* sp. W3-18-1. It was only in the Bacteroidetes genomes (*Bacteroides*, *Poryphyromonas*, and *Prevotella*) that genes for ArsD and DsbD_2 were adjacent.

The gene for ArsD proteins (BT_0117 and BT_0801) of *B. thetaiotaomicron* VPI-5482 was selected as input proteins for generation of protein–protein interaction network. As expected, the generated networks (Fig. 4D) include the genes in the transcription units that had the neighborhood evidence (green line). The interaction between BT_0117 (*arsD*)

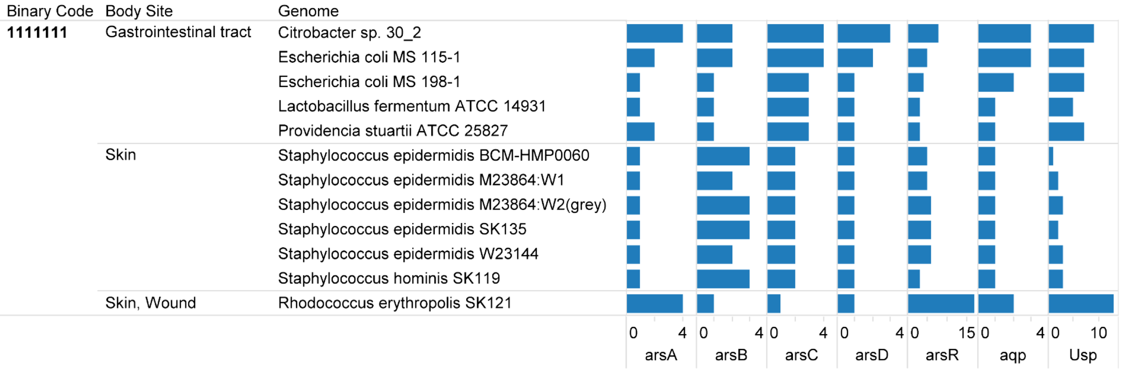


Figure 2. Genomes in the Human Microbiome Project (HMP) genomes collection with genes for arsenic operon, aquaporin, and universal stress protein.
Notes: Binary code is based on the presence of seven protein families: Pfam02374 [anion-transporting ATPase (ArsA)]; Pfam02040 [arsenical pump membrane protein (ArsB)]; Pfam03960 [arsenate reductase and related proteins, glutaredoxin family (ArsC)]; Pfam06953 [arsenical resistance operon trans-acting repressor (ArsD)]; Pfam01022 [As(III)-responsive transcriptional repressor (ArsR)]; Pfam00230 [major intrinsic protein family (MIP/AQP)]; and Pfam00582 [universal stress protein domain (Usp)].

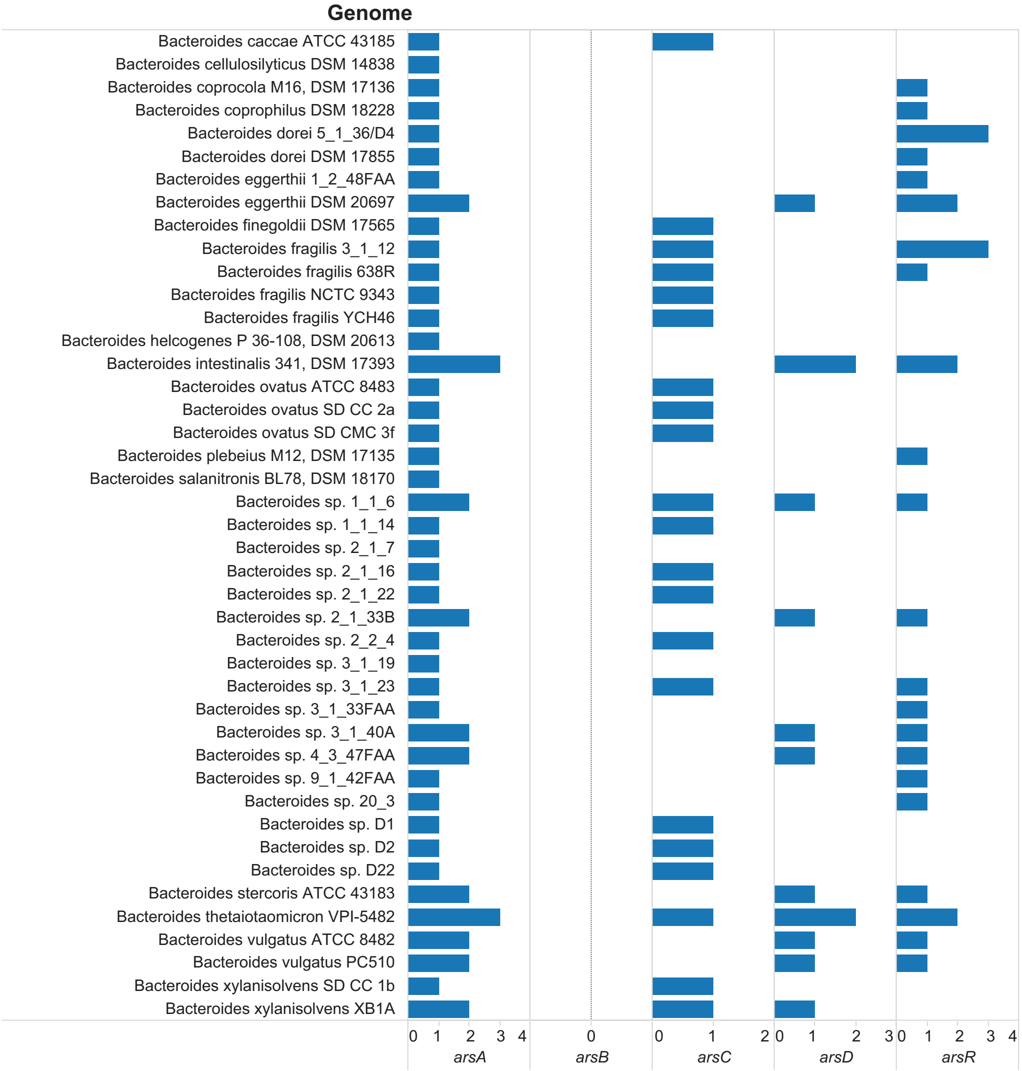


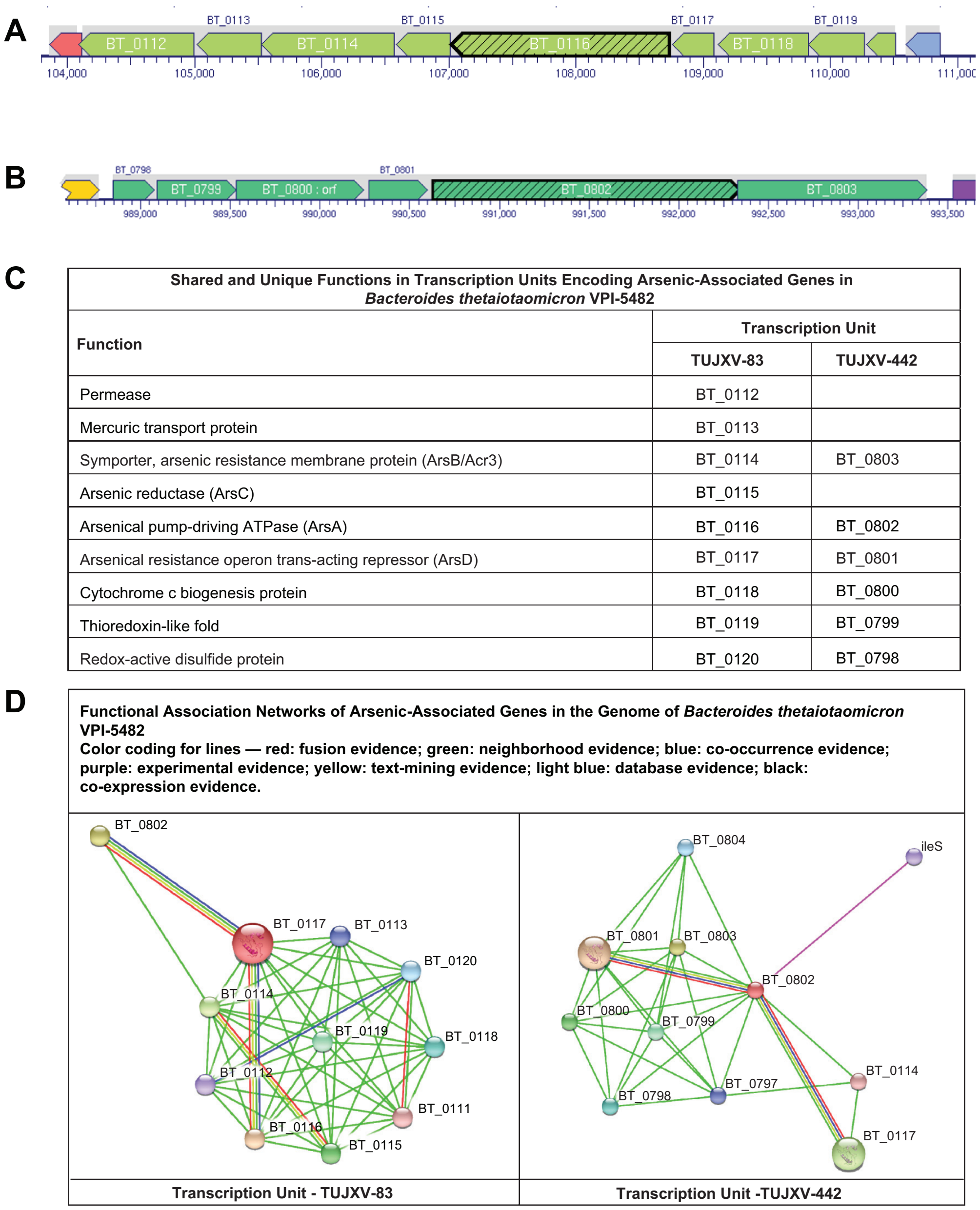
Figure 3. Abundance of genes for arsenic-associated genes in genomes of *Bacteroides* species.
Notes: The horizontal axis has the count for arsenic-associated genes in the genome. The scale for each Pfam family varies according to the maximum abundance observed in the genomes evaluated. The genes and their Pfam encoding are *arsA*, Pfam02374 (anion-transporting ATPase); *arsB*, Pfam02040 (arsenical pump membrane protein); *arsC*, Pfam03960 (arsenate reductase and related proteins, glutaredoxin family); *arsD*, Pfam06953 (arsenical resistance operon trans-acting repressor); and *arsR*, Pfam01022 (As(III)-responsive transcriptional repressor).

and BT_0116 (*arsA*) has multiple types of evidence and is expected. A predicted interaction of BT_0120 (redox-active disulfide protein) had co-occurrence and fusion evidence types with BT_0112 (a permease) and BT_0110 (hypothetical protein), respectively. There was experimental evidence for the interaction between homologs of BT_0802 (arsA) and ileS (BT_0806; isoleucyl-tRNA synthetase).

Discussion

We have provided an integrated view of relevance of 62 genomes from 18 genera with genes for arsenic operon, aquaporin, and USPs (Fig. 2). A set of 12 bacteria genomes in the HMP collection[5] was identified to have genes encoding seven protein families defined in this research as relevant to arsenic sensing and regulation (Table 1). The following bacteria in the HMP reference genomes are associated with the gastrointestinal tract: *Citrobacter* sp. 30_2, *E. coli* MS 115-1, *E. coli* MS 198-1, *L. fermentum* ATCC 14931, and *P. stuartii* ATCC 25827. *Citrobacter* sp. 30_2 is a Gram-negative isolate from an intestinal biopsy specimen of patient with Crohn's disease.[36]

The *Citrobacter* genus are rod shaped, motile, non-spore forming members of the family Enterobacteriaceae that use citrate as their sole source of carbon.[37,38] In terms of arsenic metabolism, *Citrobacter* sp. NC-1 isolated from soil contaminated with arsenic at levels as high as 5,000 mg. As kg^{-1} was able to reduce 20 mM arsenate within 24 h.[39] A comparison of *Citrobacter* sp. 30_2 and *Citrobacter* UC1CIT strains from a premature infant revealed the presence of an arsenic operon unique to strain 30_2.[36] The two *E. coli* strains identified are part of the project on human gut microbiota and Crohn's



Shared and Unique Functions in Transcription Units Encoding Arsenic-Associated Genes in *Bacteroides thetaiotaomicron* VPI-5482

Function	Transcription Unit	
	TUJXV-83	TUJXV-442
Permease	BT_0112	
Mercuric transport protein	BT_0113	
Symporter, arsenic resistance membrane protein (ArsB/Acr3)	BT_0114	BT_0803
Arsenic reductase (ArsC)	BT_0115	
Arsenical pump-driving ATPase (ArsA)	BT_0116	BT_0802
Arsenical resistance operon trans-acting repressor (ArsD)	BT_0117	BT_0801
Cytochrome c biogenesis protein	BT_0118	BT_0800
Thioredoxin-like fold	BT_0119	BT_0799
Redox-active disulfide protein	BT_0120	BT_0798

Functional Association Networks of Arsenic-Associated Genes in the Genome of *Bacteroides thetaiotaomicron* VPI-5482
Color coding for lines — red: fusion evidence; green: neighborhood evidence; blue: co-occurrence evidence; purple: experimental evidence; yellow: text-mining evidence; light blue: database evidence; black: co-expression evidence.

Transcription Unit - TUJXV-83 | **Transcription Unit -TUJXV-442**

Figure 4. Transcription units and functional associations of arsenic resistance operon in *Bacteroides thetaiotaomicron* VPI-5482. The web pages for the transcription units are http://biocyc.org/BTHE226186/NEW-IMAGE?type=OPERON&object=TUJXV-83 and http://biocyc.org/BTHE226186/NEW-IMAGE?type=OPERON&object=TUJXV-442.

disease (http://genome.wustl.edu/projects/). An indication that the genomic composition of *E. coli* MS 115-1 is equipped for environmental fitness is the presence of the *clpK* gene for thermal resistance in *Klebsiella pnuemoniae*.[36] *E. coli* MS 115-1 is one of the two *E. coli* strains with the *clpK* gene. In Table 3, we observed that *arsA* and *arsD* were adjacent for all the four Gram-negative bacteria. However, in *L. fermentum* ATCC 14931, the *arsB* and *arsD* were adjacent. A systematic evaluation of more than 19,000 bacterial genomes could provide additional examples of this gene adjacency. Functional analysis with molecular techniques could also elucidate impact of the adjacency on arsenic extrusion.

We have included the USPs as markers for arsenic exposure because of (i) prior research that support induction of USPs by arsenic and (ii) proximity of arsenic-associated genes and genes for USPs. Arsenite early exposure (15 minutes) induced the transcription of two *usp* genes in *Herminiimonas arsenicoxydans*, a bacterium isolated from arsenic-contaminated sludge.[40] A *usp* gene and an arsenic resistance operon are located on an antibiotic-resistant island in the genome of *Acinetobacter baumannii*, an opportunistic pathogen that causes nosocomial infections.[41] We have observed that the genome of *Bacillus cereus* Q1 contains a *usp* gene that is adjacent and in the same transcription direction with a gene with predicted function for HTH ArsR-type DNA-binding domain (InterPro Database Identifier: IPR001845).[9] Further research is needed to better define the relationship between expression of USP genes and the level of arsenic exposure. Additionally, investigations are desired in the context of arsenic sensing to compare the speed of expression of USP genes and arsenic resistance operon (ars) genes.

In the Gram-negative colon inhabiting *B. thetaiotaomicron* VPI-5482, two transcription units include arsenic resistance genes (ars) (Fig. 4). Investigations to confirm these genes would help define mechanisms for arsenic sensing and regulation by *B. thetaiotaomicron* VPI-5482, which is able to acquire and utilize indigestible dietary polysaccharides.[42] Multiple copies of *ars* genes in *B. thetaiotaomicron* VPI-5482 are consistent with expansion of paralogous genes and the species environmental sensing abilities needed to adapt to changing ecosystems.[33] Only genomes categorized under the phylum Bacteroidetes contain genes encoding for the cytochrome c biogenesis protein adjacent to the arsD gene protein is adjacent to the *arsD* gene only genomes of the Bacteroidetes phylum (Fig. 3). Cytochrome *c*, produced by a posttranslational process, consists of heme-containing proteins important for cellular energy production and signaling.[28,29] The *arsD* controls the maximal expression of the arsenic-resistant operon (*arsRDABC*).[43] The ArsD metallochaperone protein delivers arsenite to ArsA efflux pump.[44,45] The significance of the adjacency of cytochrome *c* biogenesis protein and the metallochaperone needs further investigation. In *Shewanella putrefaciens* strain CN-2, a subunit of *c*-type cytochrome (CymA) that is present in anaerobic conditions functions in conjunction with a known respiratory arsenate reductase.[46]

Through additional functional annotation data curation, we noted the presence of a gene for mercuric transport protein (Locus Tag: BT_0113; UniProt Accession: Q8ABJ7) in one of the BioCyc transcription units (TUJXV-83) of the *B. thetaiotaomicron* VPI-5482 (Fig. 4). Predictions available at OrthoDB indicate that the gene encodes a mercuric transport protein (http://cegg.unige.ch/orthodb/results?searchtext=Q8ABJ7).[47] Genes BT_0112 and BT_0114 encode transport functions. The proteins encoded by the genes BT_0112, BT_0113, and BT_0114 could be investigated for mechanisms of heavy metal transport in *B. thetaiotaomicron*.

The focus of the evaluative profiling scheme was limited to seven Pfam annotations. Thus, certain functions that are arsenic associated would not be evaluated. For example, in the *Bacteroides* genomes, annotation for ArsB (Pfam02040; arsB) was not observed (Fig. 3). The annotation available in the genomes was the ACR3 form. Furthermore, in *L. fermentum* ATCC 14931, *arsC* (HMPREF0511_1135) in the arsenic resistance operon (HMPREF0511_1131 to HMPREF0511_1135) was not annotated with the Pfam family Pfam0396, but the annotation observed was Pfam01451. Our evaluative scheme assessed the arsenic reductase genes annotated with Pfam03960. Further development of the evaluative profiling for arsenic sensing and regulation would be more comprehensive using the arsenic-related gene families: cytoplasmic AsV reduction (ars), periplasmic AsV reduction (arr), arsenite oxidation (aio), and arsenite methylation (arsM).[48] Finally, the evaluative profiles will account for instances where multiple Pfam families map to a gene as with *arsC* and *arsB*.

Conclusion

In conclusion, we have developed a suite of bioinformatics and visual analytics methods to evaluate the availability (presence or absence) and abundance of functional annotations in a microbial genome for seven Pfam protein families: As(III)-responsive transcriptional repressor (ArsR); anion-transporting ATPase (ArsA); arsenical pump membrane protein (ArsB); arsenate reductase (ArsC); arsenical resistance operon trans-acting repressor (ArsD); water/glycerol transport protein (aquaporins); and USP. We identified 62 genomes from 18 genera that have genes for all the seven protein families. Our list included 12 genomes in the HMP reference genomes from the following genera *Citrobacter*, *Escherichia*, *Lactobacillus*, *Providencia*, *Rhodococcus*, and *Staphylococcus*. The use of visual analytics methods makes it possible to include additional arsenic-associated protein families in the profiling scheme. Finally, investigations are desired on the arsenic sensing and regulatory systems in members of the Bacteroidetes phylum.

Author Contributions

Conceived and designed the experiments: RDI, UKU, SSS, ALH, AEC, and ON. Analyzed the data: RDI, UKU, SSS, ALH, AEC, SAO, OAH, ZAK, OAE, DEF, and ON. Wrote the first draft of the manuscript: UKU, RDI, and SSS. Contributed to the writing of the manuscript: RDI, UKU, SSS, ALH, AEC, SAO, OAH, ZAK, OAE, DEF, and ON. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Robinson CJ, Bohannan BJ, Young VB. From structure to function: the ecology of host-associated microbial communities. *Microbiol Mol Biol Rev*. 2010;74(3): 453–476.
2. Lu K, Mahbub R, Cable PH, et al. Gut microbiome phenotypes driven by host genetics affect arsenic metabolism. *Chem Res Toxicol*. 2014;27(2):172–174.
3. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett C, Knight R, Gordon JI. The human microbiome project: exploring the microbial part of ourselves in a changing world. *Nature*. 2007;449(7164):804.
4. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207–214.
5. Human Microbiome Jumpstart Reference Strains Consortium. A catalog of reference genomes from the human microbiome. *Science*. 2010;328(5981):994–999.
6. Wallace BD, Redinbo MR. The human microbiome is a source of therapeutic drug targets. *Curr Opin Chem Biol*. 2013;17(3):379–384.
7. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature*. 2013;500(7464):541–546.
8. Kong LC, Tap J, Aron-Wisnewsky J, et al. Gut microbiota after gastric bypass in human obesity: increased richness and associations of bacterial genera with adipose tissue genes. *Am J Clin Nutr*. 2013;98(1):16–24.
9. Williams BS, Isokpehi RD, Mbah AN, et al. Functional annotation analytics of *Bacillus* genomes reveals stress responsive acetate utilization and sulfate uptake in the biotechnologically relevant *Bacillus megaterium*. *Bioinform Biol Insights*. 2012;6:275–286.

10. Biswas A, Deb D, Ghose A, et al. Dietary arsenic consumption and urine arsenic in an endemic population: response to improvement of drinking water quality in a 2-year consecutive study. *Environ Sci Pollut Res*. 2014;21(1):609–619.
11. Johnson MO, Cohly HH, Isokpehi RD, Awofolu OR. The case for visual analytics of arsenic concentrations in foods. *Int J Environ Res Public Health*. 2010; 7(5):1970–1983.
12. Beauchamp EM, Serrano R, Platanias LC. Regulatory effects of arsenic on cellular signaling pathways: biological effects and therapeutic implications. In: Kumar R, ed. *Nuclear Signaling Pathways and Targeting Transcription in Cancer*. New York: Springer; 2014:107–119.
13. Sharples JM, Meharg AA, Chambers SM, Cairney JW. Mechanism of arsenate resistance in the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. *Plant Physiol*. 2000;124(3):1327–1334.
14. Diorio C, Cai J, Marmor J, Shinder R, DuBow MS. An *Escherichia coli* chromosomal ars operon homolog is functional in arsenic detoxification and is conserved in gram-negative bacteria. *J Bacteriol*. 1995;177(8):2050–2056.
15. Fu SF, Chen PY, Nguyen QT, et al. Transcriptome profiling of genes and pathways associated with arsenic toxicity and tolerance in *Arabidopsis*. *BMC Plant Biol*. 2014;14(1):94.
16. Oberoi S, Barchowsky A, Wu F. The global burden of disease for skin, lung and bladder cancer caused by arsenic in food. *Cancer Epidemiol Biomarkers Prev*. 2014; 23(7):1–8.
17. Alava P, Tack F, Laing GD, Van de Wiele T. Arsenic undergoes significant speciation changes upon incubation of contaminated rice with human colon micro biota. *J Hazard Mater*. 2013;262:1237–1244.
18. Van de Wiele T, Gallawa CM, Kubachka KM, et al. Arsenic metabolism by human gut microbiota upon in vitro digestion of contaminated soils. *Environ Health Perspect*. 2010;118:1004–1009.
19. Wang L, Jeon B, Sahin O, Zhang Q. Identification of an arsenic resistance and arsenic-sensing system in *Campylobacter jejuni*. *Appl Environ Microbiol*. 2009; 75(15):5064–5073.
20. Castillo R, Saier MH. Functional promiscuity of homologues of the bacterial ArsA ATPases. *Int J Microbiol*. 2010;2010:187373.
21. Yang H-C, Cheng J, Finan TM, Rosen BP, Bhattacharjee H. Novel pathway for arsenic detoxification in the legume symbiont *Sinorhizobium meliloti*. *J Bacteriol*. 2005;187(20):6991–6997.
22. Mbah AN, Mahmud O, Awofolu OR, Isokpehi RD. Inferences on the biochemical and environmental regulation of universal stress proteins from Schistosomiasis parasites. *Adv Appl Bioinform Chem*. 2013;6:15.
23. Isokpehi RD, Simmons SS, Cohly HH, Ekunwe SI, Begonia GB, Ayensu WK. Identification of drought-responsive universal stress proteins in viridiplantae. *Bioinform Biol Insights*. 2011;5:41–58.
24. Nachin L, Nannmark U, Nyström T. Differential roles of the universal stress proteins of *Escherichia coli* in oxidative stress resistance, adhesion, and motility. *J Bacteriol*. 2005;187(18):6265–6272.
25. Kvint K, Nachin L, Diez A, Nyström T. The bacterial universal stress protein: function and regulation. *Curr Opin Microbiol*. 2003;6(2):140–145.
26. Sacheti P, Bhonsle H, Patil R, Kulkarni MJ, Srikanth R, Gade W. Arsenomics of *Exiguobacterium* sp. PS (NCIM 5463). *RSC Adv*. 2013;3(25):9705–9713.
27. Van Dyk TK, Smulski DR, Reed TR, Belkin S, Vollmer AC, LaRossa RA. Responses to toxicants of an *Escherichia coli* strain carrying a uspA': lux genetic fusion and an *E. coli* strain carrying a grpE': lux fusion are similar. *Appl Environ Microbiol*. 1995;61(11):4124–4127.
28. Travaglini-Allocatelli C. Protein machineries involved in the attachment of heme to cytochrome *c*: protein structures and molecular mechanisms. *Scientifica*. 2013;2013:505714.
29. Mavridou DA, Ferguson SJ, Stevens JM. Cytochrome *c* assembly. *IUBMB Life*. 2013;65(3):209–216.
30. Riley T, Mee B. Susceptibility of *Bacteroides* spp. to heavy metals. *Antimicrob Agents Chemother*. 1982;22(5):889–892.
31. Pavlidis P, Noble WS. Matrix2png: a utility for visualizing matrix data. *Bioinformatics*. 2003;19(2):295–296.
32. Markowitz VM, Chen IM, Palaniappan K, et al. IMG: the integrated microbial genomes database and comparative analysis system. *Nucleic Acids Res*. 2012; 40(D1):D115–D122.
33. Comstock LE, Coyne MJ. *Bacteroides thetaiotaomicron*: a dynamic, niche-adapted human symbiont. *Bioessays*. 2003;25(10):926–929.
34. Caspi R, Altman T, Billington R, et al. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Res*. 2014;42(D1):D459–D471.
35. Franceschini A, Szklarczyk D, Frankild S, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res*. 2013;41(D1):D808–D815.
36. Morowitz MJ, Denef VJ, Costello EK, et al. Strain-resolved community genomic analysis of gut microbial colonization in a premature infant. *Proc Natl Acad Sci U S A*. 2011;108(3):1128–1133.
37. Borenshtein D, Schauer DB. The genus *Citrobacter*. In: Dworkin M, et al, eds. *The Prokaryotes*. New York: Springer; 2006:90–98.
38. O'Hara CM, Roman SB, Miller JM. Ability of commercial identification systems to identify newly recognized species of *Citrobacter*. *J Clin Microbiol*. 1995; 33(1):242–245.
39. Chang YC, Nawata A, Jung K, Kikuchi S. Isolation and characterization of an arsenate-reducing bacterium and its application for arsenic extraction from contaminated soil. *J Ind Microbiol Biotechnol*. 2012;39(1):37–44.
40. Cleiss-Arnold J, Koechler S, Proux C, et al. Temporal transcriptomic response during arsenic stress in *Herminiimonas arsenicoxydans*. *BMC Genomics*. 2010; 11(1):709.
41. Post V, White PA, Hall RM. Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother*. 2010;65:1162–1170.
42. Xu J, Bjursell MK, Himrod J, et al. A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. *Science*. 2003;299(5615):2074–2076.
43. Chen Y, Rosen BP. Metalloregulatory properties of the ArsD repressor. *J Biol Chem*. 1997;272(22):14257–14262.
44. Lin Y-F, Yang J, Rosen BP. ArsD residues Cys12, Cys13, and Cys18 form an As (III)-binding site required for arsenic metallochaperone activity. *J Biol Chem*. 2007;282(23):16783–16791.
45. Yang J, Salam A, Ajees A, Rosen BP. Genetic mapping of the interface between the ArsD metallochaperone and the ArsA ATPase. *Mol Microbiol*. 2011;79(4): 872–881.
46. Murphy JN, Saltikov CW. The cymA gene, encoding a tetraheme c-type cytochrome, is required for arsenate respiration in *Shewanella* species. *J Bacteriol*. 2007;189(6):2283–2290.
47. Waterhouse RM, Tegenfeldt F, Li J, Zdobnov EM, Kriventseva EV. OrthoDB: a hierarchical catalog of animal, fungal and bacterial orthologs. *Nucleic Acids Res*. 2013;41(D1):D358–D365.
48. Li X, Zhang L, Wang G. Genomic evidence reveals the extreme diversity and wide distribution of the arsenic-related genes in Burkholderiales. *PLoS One*. 2014;9(3):e92236.