Comparative Genomics of Host–Symbiont and Free-Living *Oceanobacillus* Species

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Abstract

Survival in a given environment requires specific functions, so genomic variation is anticipated within in individual taxonomic groups that exhibit a large diversity in lifestyles. In this study, we sequence and assemble the genome of *Oceanobacillus faecalis* strain HM6, a resident of the human gut. Using the genus *Oceanobacillus* and the HM6 draft genome sequence, we explore the functional requirements for survival in a symbiotic arrangement within the human gut, in contrast to free living in the environment. Comparative genomics of seven available *Oceanobacillus* complete genomes highlight a genomically heterogeneous group. Our analysis did not find strict phylogenetic separation between free-living and host–symbiont *Oceanobacillus* members. By comparing functional gene content between host-associated and free-living species, we identified candidate genes that are potentially involved in symbiotic lifestyles, including phosphotransferase genes, transporters and two component response regulators. This study summarizes genomic and phylogenetic differences in the *Oceanobacillus* genus. Additionally, we highlight functions that may be key for survival in the human gut community.

Key words: *Oceanobacillus*, comparative genomics, host–microbe symbiosis, gut microbiome.

Introduction

The human gastrointestinal tract (GIT) houses diverse bacteria in different sections of the complex organ, which are often involved in metabolite sharing and maintenance of homeostasis, among other functions (Gill et al. 2006; Wikoff et al. 2009; Round and Mazmanian 2009). Recent reports highlight that the human gut microbiota has codiversified with the host (Moeller et al. 2014, 2016), suggesting resident bacterial species to have acquired functions in their genomes which assist in colonizing. As we move into the translational era of genomic sciences, the microbial community and its members are expected to be used for diagnosis and treatment (Zeller et al. 2014; Shreiner et al. 2015). In this regard, it is important to understand the genomic suitability for a symbiont.

Species of the genus *Oceanobacillus* are known to inhabit the human gut (Roux et al. 2013; Lagier et al. 2015), however multiple other species of the same genus have been described as free-living organisms (Namwong et al. 2009; Romano et al. 2006; Raats and Halpern 2007; Nam et al. 2008; Tominaga et al. 2009; Amoozegar et al. 2014). In this study, we report the complete genome sequence of a human symbiont *Oceanobacillus faecalis* strain HM6. Additionally through comparative genomic analyses of the *Oceanobacillus* genus, we investigated the differentially abundant functions in the genomes of host–symbiont and free-living species, to

understand factors assisting survival in the two opposite environments.

Materials and Methods

Isolation and Characterization of *O. faecalis* Strain HM6

Oceanobacillus faecalis strain HM6 was cultured from fecal sample collected from a healthy individual (Age 29, Male; Blood Sugar 100–120 mg/dl; BP: 120/80), without history of prolonged illness or antibiotic treatment. It was cultured using Luria Bertini (LB) agar medium at 37 °C. Human ethical guidelines were followed strictly before engaging the volunteer for this study, with ethical clearance from the Human Ethical Committee of M. D. University, Rohtak, Haryana, India. Phylogenetic affiliation of purified microbes (HM6) was investigated with SSU rRNA gene analysis (Case et al. 2007). The physiological and metabolic activity of *O. faecalis* strain HM6 was assessed with biolog plate technique (Smalla et al. 1998). Fatty acids were extracted and methylated to form fatty acid methyl esters (FAME), for analysis using Gas Chromatography (6850) with the MIDI analytical framework (Osterhout et al. 1991).

De Novo Genome Sequencing, Assembly and Annotation

The genomic DNA of *O. faecalis* strain HM6 was sequenced with Roche 454 GS FLX+ following manufacturer's recommended protocol. Whole genome sequencing reads were analyzed using the GS assembler (GS Assembler version 2.9) with default parameters to construct genomic contigs (details of parameters is available in supplementary Methods, Supplementary Material online). Genomic fragments of <200 bases were removed from the final draft assembly. Assembled genome was submitted to the RAST server (Aziz et al. 2008) for annotation. tRNAscan-SE (version 1.4) (Lowe and Eddy 1997) was used to find tRNA genes, and rRNA gene clusters were discovered using the RNAmmer 1.2 Server (Lagesen et al. 2007).

Phylogenetic and Genomic Identity Analyses

For robust assessment of phylogenetic relationship within the *Oceanobacillus* genus members (supplementary table S1, Supplementary Material online), we analyzed their conserved genomic sequences. Details of phylogenetic analysis are available in supplementary Methods, Supplementary Material online.

Average Nucleotide Identity (ANI) was calculated using JSpecies (Richter and Rosselló-Móra 2009) with the BLAST alignment tool. In short, JSpecies splits the query and subject genomes into 1,020 bases long fragments and finds best hits between them using BLAST and averages their identity score to compute pairwise ANIb, referred to as ANI.

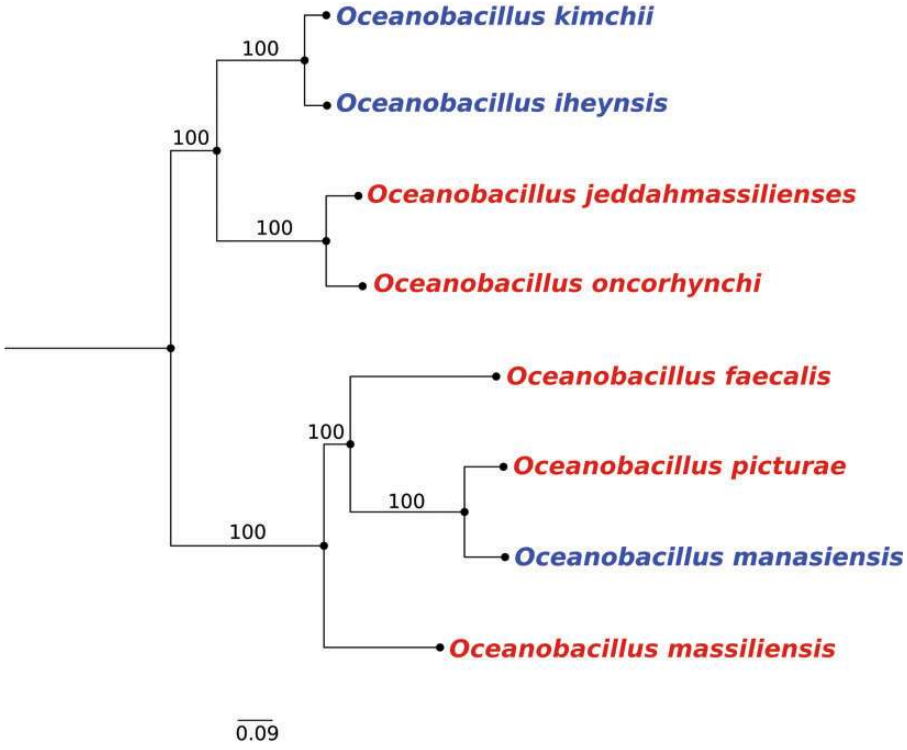

Fig. 1.—Maximum likelihood phylogenetic tree showing relation among human symbiont ("red") and free living ("blue") species of the genus *Oceanobacillus*.

Comparative Genomics: Pan-, Core-, and Accessory-Genome

Pan genome of the *Oceanobacillus* genus was constructed by clustering protein sequences from an aggregated pool of protein sequences from all strains. A Phylogenetic Profile Matrix (PPM) was created using sequence alignment tools as described in supplementary Methods, Supplementary Material online. The PPM was used to obtain pan, core, and accessory genomes of the entire *Oceanobacillus* genus, as well as of human symbiont and environmental species.

Results

Physiological and Taxonomic Summary of *O. faecalis* Strain HM6

Bacterial cells were gram-negative, rod shaped, aerobic, and nonspore forming. Colonies formed on LB agar plates after 48 h incubation at 37 °C. Salt tolerance was observed up to 2% with no visible growth beyond 2% NaCl in growth medium. Growth was inhibited in presence of different antibiotics except nalidixic acid. The fatty acid profile comprised mainly of glucose, trehalose, mannose, and raffinose as carbon sources but not maltose or lactose. It utilizes L-alanine and D-serine as nitrogen sources, but not Glycyl, L-Proline, L-Arginine and L-Serine (supplementary table S2, Supplementary Material online). FAME analysis identified presence of iso-C14: 0 (19.3%), iso-C15: 0 (10.56%), anteiso-C15: 0 (16.25%), C16: 0 (17.36%), C18: 0 (7.93%) as major cellular fatty acids, indicative of branched fatty acid dominance. The 16S rRNA gene from strain HM6 was sequenced and assembled to generate 1438bp sequence. Sequence comparison of the 16S rRNA gene showed 99% similarity with the corresponding genes of *Oceanobacillus* and *Virgibacillus*, from the Bacillus group. Phylogenetic analysis of the 16S rRNA of the isolated human gut microbe with its homologs verified its phylogeny of *Oceanobacillus*. Thus, we term it as *O. faecalis* strain HM6, and its general features have been described in supplementary table S3, Supplementary Material online.

Whole Genome Sequence of *O. faecalis* Strain HM6

The genome of *O. faecalis* was assembled into 225 contigs with a total of 3,581,422 bp (supplementary table S4, Supplementary Material online). We could predict 3,699

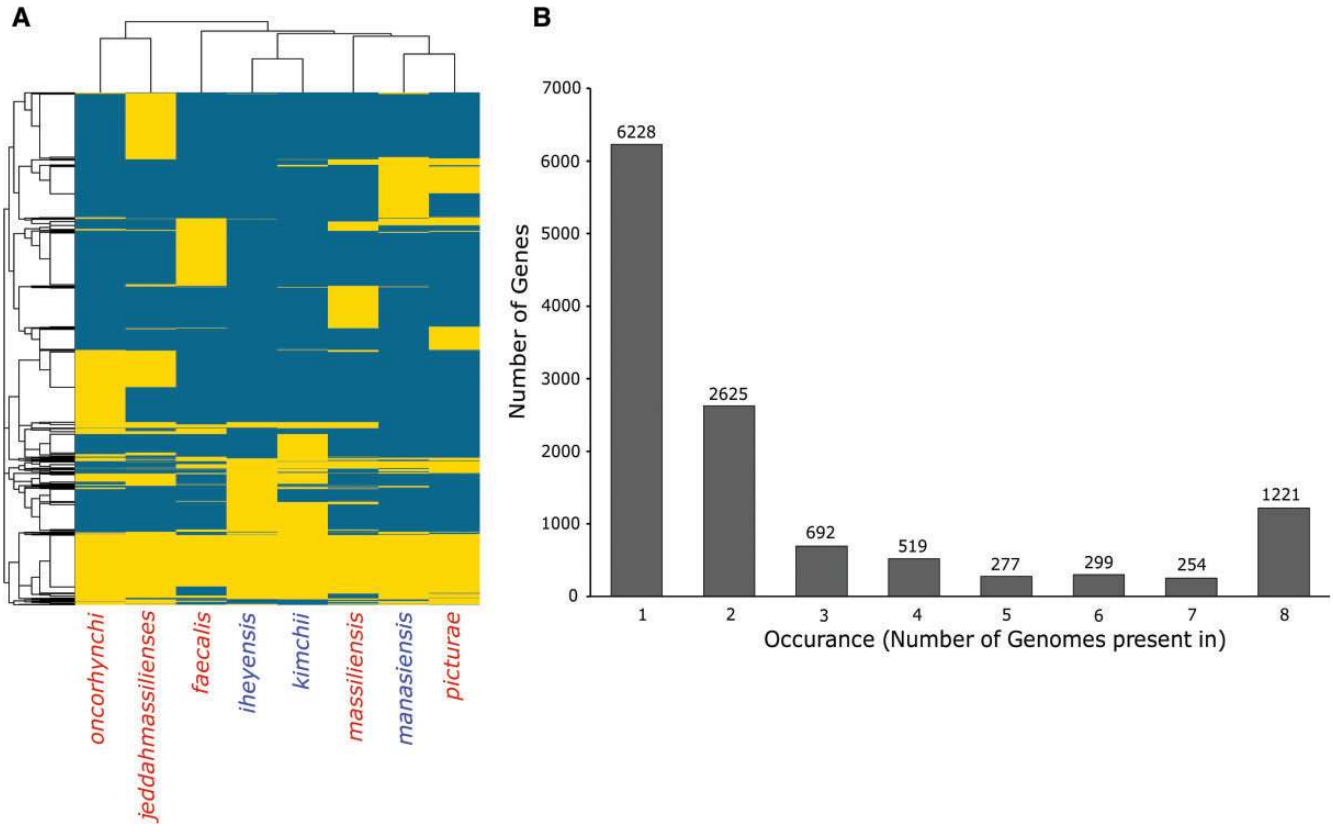

Fig. 2.—The pan genome of *Oceanobacillus*. (*A*) Heatmap showing the phylogenetic profile matrix for *Oceanobacillus* genus. Columns and rows of the heatmap represents individual *Oceanobacillus* species and pan genome OFUs, respectively. Cell color "yellow" signifies presence of a particular OFU, whereas "teal" denotes absence. Species names are colored with respect to human symbionts ("red") or environmental free living ("blue"). (*B*) OFU occurrence frequency, ranging from all genomes ($n = 8$, core genome) to a single genome ($n = 1$, genome specific OFU).

coding genes and 117 RNAs in the genome of *O. faecalis* strain HM6. Analysis using the RAST server yielded 427 subsystems, with majority of genes participating in carbohydrate metabolism (15.2%) and amino acids metabolism (13.9%). Complete details of subsystems annotation are available in supplementary table S5, Supplementary Material online.

Genomic Heterogeneity and Phylogenetic Association among Symbionts and Environmental *Oceanobacillus*

Comprehensive analysis of the *Oceanobacillus* genus was undertaken with strain HM6 and seven publically available genomes of *Oceanobacillus* (supplementary table S1, Supplementary Material online). *Oceanobacillus* have AT rich genomes with minimum %GC of 35.2 in *O. kimchi* and maximum %GC of 40.4 in *O. massiliensis*. Average genome size among *Oceanobacillus* is 4 Mb and an average 3,803 protein coding genes in each. Members had varying numbers of rRNA operon sets, with some having as many as seven (*O. iheyensis*) (supplementary table S1, Supplementary Material online). This presents an interesting scenario because number of rRNA operons in prokaryote genomes have been linked to their lifestyle (Klappenbach et al. 2000; Lim et al. 2012). Our observations indicate possible heterogeneity present in this genus. However, pairwise 16S rRNA gene identity was found to be between 98 and 100% for all organisms included in this study (supplementary table S6, Supplementary

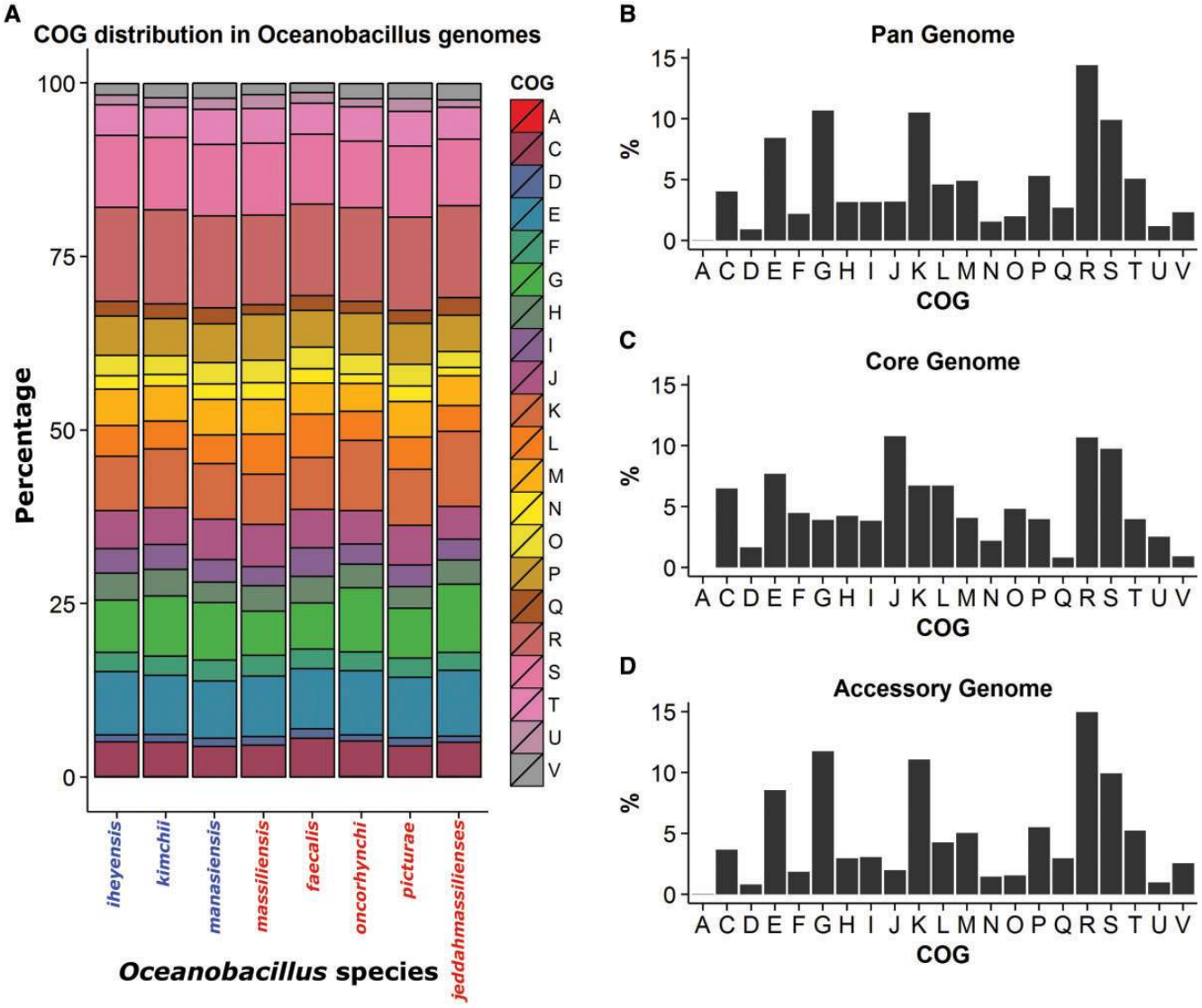

Fig. 3.—Functional diversity in *Oceanobacillus*. (*A*) Distribution of COG categories in genomes of individual species. Species names are colored with respect to human symbionts ("red") or environmental free living ("blue"). Abundance of COG categories in Pan (*B*), Core (*C*), and Accessory (*D*) genomes of the genus *Oceanobacillus*, respectively.

Material online), suggesting similar identity profiles within and across environmental as well as human symbiont groups.

In an attempt to infer the underlying phylogenetic relationship among species of this genus, we performed phylogenetic analysis of the conserved genomic regions. Our analysis revealed that *Oceanobacillus* species do not show a distinctly segregated phylogeny with respect to habitat. However, there were two major branches in the tree (fig. 1), one primarily containing organisms isolated from human gut (three out of four) and the other with two sub branches segregating into environmental and human gut isolates. It would be interesting to explore the phylogeny of this diverse group in future when more members of the genus would be sequenced and analyzed.

The average ANI value among all *Oceanobacillus* species was ~71%, which is towards the lower end of the 62–100% spectrum of interspecies variation within a genus (Kim et al. 2014), suggesting substantial genomic diversity. This observation was reaffirmed by functional feature conservation among member species, highlighted by a wide distribution of Phi coefficient (supplementary table S7, Supplementary Material online).

Oceanobacillus Genomes Characterized by a Small Core Genome and Diverse Gene Pool

The *Oceanobacillus* pan genome was found to contain 12,115 OFU, which is a strikingly large number considering only eight genomes were analyzed. Figure 3*A* contains distribution of the pan gene set across individual *Oceanobacillus* species. Of these, 1,221 OFU were common across all members, representing the core genome, that is, the basic set of functions that define an *Oceanobacillus.* We also found an interesting set of 6,228 OFUs that were unique to any organism, that is, not detected in any other species of the genus (fig. 2*B*). It suggests that around one tenth of all OFUs function as core with almost half being unique to an organism and the rest being shared by more than one member of the genus. This interesting distribution of functional groups in genomes of different *Oceanobacillus* may be related to their diverse lifestyles (figs. 2 and 3*A*).

We observed interesting trends in the distribution of various functional categories in the core and accessory genomes (fig. 3*C* and *D*). We found that both the pan and accessory genomes have similar COG compositions (fig. 3*B* and *D*). They were majorly constituted by COG G, COG K, and COG S classes, representing proteins involved in carbohydrate transport and metabolism (10.6%), transcription (10.4%), and amino acid transport and metabolism (8.4%). However, the core genome of *Oceanobacillus* had a very different composition, wherein the overrepresented class was COG J (translation, ribosomal structure, and biogenesis; 10.7%).We also noticed a major shift in the composition of COG V (defense mechanism; 0.8%), as compared with 3.1% and 2.3% in the pan genome, respectively.

Candidates for Habitat-Specific Functions in *Oceanobacillus Species*

To investigate whether surviving in different niches, free-living or human symbiont, require special features; we analyzed the gene pools of *Oceanobacillus species* with respect to lifestyle. We found that the core and accessory genomes for these sub groups within the same genus had similar distributions of

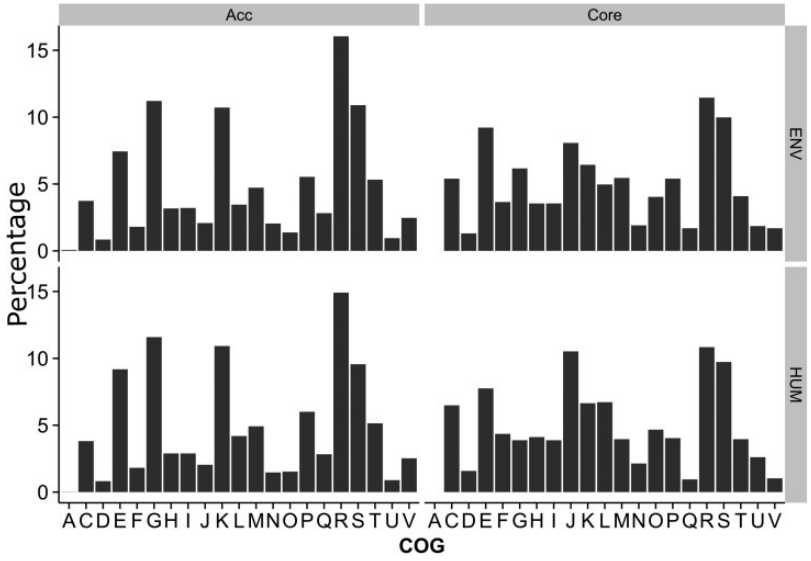

Fig. 4.—Distribution of COG classes in core and accessory genomes of environmental and symbiont *Oceanobacillus* species. Gene pools—accessory (Acc) and core (Core)—are represented in columns, whereas habitat specificity—environmental free-living (ENV) and human symbiont (HUM)—is mentioned in rows.

genes across COG functional categories, but there were also some potentially relevant differences. COG classes like COG V (defense mechanisms), COG P (inorganic ion transport and metabolism), COG G (carbohydrate transport and metabolism) and COG M (cell wall membrane envelop biogenesis) appeared to be under-represented in the core genome of human symbionts as compared with the free-living group (fig. 4). At the same time, COG U (intracellular trafficking, secretion, and vesicular transport), COG O (posttranslational modification, protein turnover, chaperones), COG L (replication, recombination, and repair) appeared to be over represented in the core genome of the human symbiont group (fig. 4).

Further we queried for functions selectively present in the human symbiont and free-living subgroups. Table 1 summarizes a list of functions uniquely present in the genomes of *Oceanobacillus* species isolated from human gut. Most of the

Table 1

Functions Selectively Associated with Isolates from Human Gut (Human Gut: $n = 4+/5$; Environmental: $n = 0/3$)

COG	COG Description	Class	Class Description
COG2855	Predicted membrane protein	S	Function unknown
COG1263	Phosphotransferase system IIC components, glucose/maltose/N-acetylglucosamine-specific	G	Carbohydrate transport and metabolism
COG1264	Phosphotransferase system IIB components	G	Carbohydrate transport and metabolism
COG3333	Uncharacterized protein conserved in bacteria	S	Function unknown
COG0642	Signal transduction histidine kinase	T	Signal transduction mechanisms
COG3730	Phosphotransferase system sorbitol-specific component IIC	G	Carbohydrate transport and metabolism
COG3181	Uncharacterized protein conserved in bacteria	S	Function unknown
COG4300	Predicted permease, cadmium resistance protein	P	Inorganic ion transport and metabolism
COG1136	ABC-type antimicrobial peptide transport system, ATPase component	V	Defense mechanisms
COG1522	Transcriptional regulators	K	Transcription
COG0476	Dinucleotide-utilizing enzymes involved in molybdopterin and thiamine biosynthesis family 2	H	Coenzyme transport and metabolism
COG2603	Predicted ATPase	R	General function prediction only
COG0745	Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain	TK	Multiple classes
COG5002	Signal transduction histidine kinase	T	Signal transduction mechanisms
COG0145	N-methylhydantoinase A/acetone carboxylase, beta subunit	EQ	Multiple classes
COG0387	Ca2+/H+ antiporter	P	Inorganic ion transport and metabolism
COG0785	Cytochrome c biogenesis protein	O	Posttranslational modification, protein turnover, chaperones
COG0789	Predicted transcriptional regulators	K	Transcription
COG1126	ABC-type polar amino acid transport system, ATPase component	E	Amino acid transport and metabolism
COG0745	Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain	TK	Multiple classes
COG0710	3-dehydroquinate dehydratase	E	Amino acid transport and metabolism
COG0745	Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain	TK	Multiple classes
COG0577	ABC-type antimicrobial peptide transport system, permease component	V	Defense mechanisms
COG1011	Predicted hydrolase (HAD superfamily)	R	General function prediction only
COG2217	Cation transport ATPase	P	Inorganic ion transport and metabolism
COG0709	Selenophosphate synthase	E	Amino acid transport and metabolism
COG1225	Peroxiredoxin	O	Posttranslational modification, protein turnover, chaperones
COG0436	Aspartate/tyrosine/aromatic aminotransferase	E	Amino acid transport and metabolism
COG0209	Ribonucleotide reductase, alpha subunit	F	Nucleotide transport and metabolism
COG1249	Pyruvate/2-oxoglutarate dehydrogenase complex, dihydrolipoamide dehydrogenase (E3) component, and related enzymes	C	Energy production and conversion
COG5002	Signal transduction histidine kinase	T	Signal transduction mechanisms
COG2076	Membrane transporters of cations and cationic drugs	P	Inorganic ion transport and metabolism

unique proteins are predicted to be involved in metabolism, transcription, and translation. Genes coding for phosphotransferase system proteins (COG1263, COG1264) were present in the human symbiont exclusive gene set, as were signal transduction histidine kinase (COG0642, COG5002), response regulator with CheY like receiver domain (COG0745). In addition to phosphotransferase system genes, several other transporter protein coding genes were found to be present in the human symbiont exclusive gene set. Genes within these functional modules in host–symbiont *Oceanobaclli* represent candidates that are potentially involved in interactions with the host and a symbiotic lifestyle. Investigations should be undertaken to discover the specific response pathways where these exclusive genes perform.

We also identified the subset of genes that were only present in the three free-living species (table 2). Exclusive functions in environmental *Oceanobacillus* species included UDP-glucose 6-dehydrogenase (COG1004), threonine efflux protein (COG1280), Arginine deiminase (COG2235), and L-rhamnose isomerase (COG4806) among others. We found genes coding for the three step pathway for conversion of L-arabinose to L-ribulose via the well-studied *ara* operon, to be present in the exclusive gene pool of free-living *Oceanobacillus*. These genes, L-arabinose isomerase (COG2160), sugar (pentulose and hexulose) kinases (COG1070), and Ribulose-5-phosphate 4-epimerase (COG0235) make up the *araABD* operons in bacteria that can utilize arabinose as a carbon source. In this regard, the differential presence of these functions, specially the arabinose degrading pathway genes should be explored further in *Oceanobacillus* and the potential consequences of its absence in species residing in the human gut.

Discussion

The genus *Oceanobacillus* is comprised of strains isolated from diverse habitats (Namwong et al. 2009; Roux et al. 2013; Lagier et al. 2015; Romano et al. 2006; Raats and Halpern 2007; Nam et al. 2008; Tominaga et al. 2009; Amoozegar et al. 2014). Standard methods of classification identify them in the same taxonomic group, but the observed diversity is significant, both phenotypically and genetically. Additionally, our analyses failed to observe a strict phylogenetic divide among environmental and human gut isolates. Extensive genomic analyses shows that different members of *Oceanobacillus* share little genomic and functional similarity. Average nucleotide identity across *Oceanobacillus* genomes is ~71%. Limited functional similarity coupled with a large pan genome suggests a diverse gene pool, customized to suit species-specific needs. Although a more conclusive view regarding the functional heterogeneity can be obtained only after analyzing more species of this genus.

Our analyses identified subsets of genes that were uniquely present in either free-living or host–symbiont isolates within our sample of eight *Oceanobacillus* species. Strains living in the human gut had fewer genes coding for proteins involved in defense mechanisms, inorganic ion metabolism and carbohydrate metabolism. Simultaneously accommodating unique genetic features, which could enable survival and adaptation within the host ecosystem. Many of these exclusive functions present in host gut inhabiting *Oceanobacillus* have been previously identified in the metagenomic functional pool of the human gut suggesting their prevalence and importance (Lagier et al. 2015). This study provides understanding on the genomic composition of *O. faecalis* strain HM6 and the general functional characteristics of the genus *Oceanobacillus*. We observe genomic heterogeneity and functional differences in the genomes of different species of this unique genera. We hypothesize these differences can be linked to the diverse lifestyle associated with respective species. Our results serve the basis for future studies of this truly unique bacterial genera.

Table 2

Functions Specifically Present in Free-Living *Oceanobacillus* (Human Gut: $n = 0/5$; Environmental: $n = 3/3$)

Hit	Description	Class	Class Description
COG4499	Predicted membrane protein	S	Function unknown
COG1028	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)	IQR	Multiple classes
COG1028	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)	IQR	Multiple classes
COG1070	Sugar (pentulose and hexulose) kinases	G	Carbohydrate transport and metabolism
COG3254	Uncharacterized conserved protein	S	Function unknown
COG1280	Putative threonine efflux protein	E	Amino acid transport and metabolism
COG4806	L-rhamnose isomerase	G	Carbohydrate transport and metabolism
COG3403	Uncharacterized conserved protein	S	Function unknown
COG2235	Arginine deiminase	E	Amino acid transport and metabolism
COG1004	Predicted UDP-glucose 6-dehydrogenase	M	Cell wall/membrane/envelope biogenesis
COG0235	Ribulose-5-phosphate 4-epimerase and related epimerases and aldolases	G	Carbohydrate transport and metabolism
COG4842	Uncharacterized protein conserved in bacteria	S	Function unknown
COG2160	L-arabinose isomerase	G	Carbohydrate transport and metabolism

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Author Contributions

N.S.C. and A.K.M. designed the project. J.K., R.P., and M.V. performed experiments and NGS sequencing. A.K.M., S.G., G.B., and N.S.C. performed data analyses. A.K.M., S.G., N.S.C., and R.P. wrote the manuscript. All authors have read and approve the manuscript.

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