Whole-Genome Sequencing and Comparative Genome Analysis Provided Insight into the Predatory Features and Genetic Diversity of Two *Bdellovibrio* Species Isolated from Soil

Omotayo Opemipo Oyedara, Aldo Segura-Cabrera, Xianwu Guo, Temidayo Oluyomi Elufisan, Rafael Alejandro Cantú González, and Mario A. Rodríguez Pérez

1 Instituto Politécnico Nacional, Centro de Biotecnología Genómica, 88710 Reynosa, TAMPS, Mexico
2 Department of Biological Sciences, College of Science, Engineering and Technology, Faculty of Basic and Applied Science, Osun State University, PMB 4494, Osogbo, Osun State, Nigeria
3 Red de Estudios Moleculares Avanzados, Instituto de Ecología, A.C., Xalapa Enriquez, VER, Mexico
4 National Center for Technology Management, Agency of the Federal Ministry of Science and Technology (FMST), Obafemi Awolowo University, Ile-Ife, Nigeria

Correspondence should be addressed to Omotayo Opemipo Oyedara; tayooyedara@yahoo.com

Received 12 September 2017; Revised 24 January 2018; Accepted 19 February 2018; Published 10 April 2018

Academic Editor: Marco Gerdol

Copyright © 2018 Omotayo Opemipo Oyedara et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Bdellovibrio* spp. are predatory bacteria with great potential as antimicrobial agents. Studies have shown that members of the genus *Bdellovibrio* exhibit peculiar characteristics that influence their ecological adaptations. In this study, whole genomes of two different *Bdellovibrio* spp. designated SKB1291214 and SSB218315 isolated from soil were sequenced. The core genes shared by all the *Bdellovibrio* spp. considered for the pangenome analysis including the epibiotic *B. exovorus* were 795. The number of unique genes identified in *Bdellovibrio* spp. SKB1291214, SSB218315, W, and *B. exovorus* JJS was 1343, 113, 857, and 1572, respectively. These unique genes encode hydrolytic, chemotaxis, and transporter proteins which might be useful for predation in the *Bdellovibrio* strains. Furthermore, the two *Bdellovibrio* strains exhibited differences based on the % GC content, amino acid identity, and 16S rRNA gene sequence. The 16S rRNA gene sequence of *Bdellovibrio* sp. SKB1291214 shared 99% identity with that of an uncultured *Bdellovibrio* sp. clone 12L 106 (a pairwise distance of 0.008) and 95–97% identity (a pairwise distance of 0.043) with that of other culturable terrestrial *Bdellovibrio* spp., including strain SSB218315. In *Bdellovibrio* sp. SKB1291214, 174 bp sequence was inserted at the host interaction (hit) locus region usually attributed to prey attachment, invasion, and development of host independent *Bdellovibrio* phenotypes. Also, a gene equivalent to *Bd0108* in *B. bacteriovorus* HD100 was not conserved in *Bdellovibrio* sp. SKB1291214. The results of this study provided information on the genetic characteristics and diversity of the genus *Bdellovibrio* that can contribute to their successful applications as a biocontrol agent.

1. Background

Studies on predatory bacteria have received much attention recently because of the possibility to harness their potentials for the biocontrol of pathogenic bacteria. *Bdellovibrio* spp. are versatile predatory bacteria that specialize in preying upon a wide range of Gram-negative bacteria, utilizing the resulting molecules from their attack for growth and reproduction [1]. Based on the mechanism of predation, there are two species of the genus *Bdellovibrio*, namely, *B. bacteriovorus* and *B. exovorus*. The former invade the periplasmic space of its prey while the latter attaches to the external surface (epibiotic) to derive its nutrients [2, 3]. Members of the genus *Bdellovibrio* are diverse with some of them exhibiting...
unique features that can influence their ecological adaptations. For instance, *B. bacteriovorus* strain W has the unique ability to develop a dormant structure called bdellocyst which can help them survive unfavorable conditions [4]. *B. bacteriovorus* is an obligate predatory bacterium. However, a strain isolated from Tiber River (*B. bacteriovorus* strain Tiberius) has shown the unique ability to grow simultaneously in the presence and absence of prey [5]. *B. bacteriovorus* strains that replicate and grow on nutrient-rich media without bacterial prey, usually called host-independent (HI) phenotypes often have mutations at a region of their genomes known as host interaction (hit) locus, tagged gene *Bd0108* in *B. bacteriovorus HD100*. The hit locus has been proposed to regulate the formation of type IV pilus needed for prey attachment and invasion [6].

Ancient and recent lateral gene transfers have been reported to occur in *Bdellovibrio* spp., and this may play a crucial role in their evolution probably leading to the development of unique features that can impact on their predatory lifestyle [5, 7, 8]. Thus, whole-genome sequence analysis can provide an in-depth understanding of variations in predation traits and evolution of *Bdellovibrio* spp. in turns helping in their successful application as biocontrol agents against bacterial pathogens. For instance, acquisition of pathogenic islands and alteration in their genomic structure via horizontal gene transfer may have an impact that can influence their application as biocontrol agents.

*Bdellovibrio* spp. found in soil are heterogeneous with different populations coexisting in the soil [9]. In our previous study, we isolated two different strains of *Bdellovibrio* spp. designated SKB1291214 and SSB218315 from soil samples in the same environment. The strains exhibited different phenotypes based on the time required to form plaque on Gram-negative bacteria prey lawns and prey range which was limited to some members of the family Enterobacteriaceae in *Bdellovibrio* sp. SKB1291214 [10]. Furthermore, the amplification of host interaction (hit) locus in *Bdellovibrio* sp. SKB1291214 using the PCR technique was unsuccessful. Therefore, we use whole-genome sequencing and comparative genomics as a tool to understand the genetic variations between these two strains and determine their relatedness with other reported genomes retrieved from the NCBI database.

### 2. Materials and Methods

#### 2.1. Bacterial Strains and Genome Sequencing

*Bdellovibrio* spp. strains SKB1291214 and SSB218315 were isolated from soil samples obtained from different locations on a plot of land (26.069678N, −98.313108W and 26.069446N, −98.312902W) within the Center for Genomic Biotechnology, National Polytechnic Institute (IPN as in Spanish) located in the city of Reynosa, Mexico. The *Bdellovibrio* spp. were cultured as described in our earlier report [10]. The genomic DNA (gDNA) was extracted using the Wizard® Genomic DNA Purification Kit (Madison, Wisconsin, USA) according to the manufacturer’s instructions. The gDNA was subjected to optical density measurements in NanoDrop and Qubit (Thermo Fisher Scientific, Waltham, MA, USA). DNA migration in agarose gel electrophoresis was done to confirm the purity and concentration prior to fragmentation in Bioruptor (Diagenode Inc., Denville, NJ, USA). Fragmented gDNA was tested for size distribution and concentration using a 2200 Tapestation (Agilent Technologies Inc., Santa Clara, CA, USA) and subjected to Illumina library preparation using the Beckman SPRI-TE automated liquid handler and library prep reagents (Beckman Coulter, CA, USA). The resulting library was tested for size distribution and concentration by 2200, NanoDrop, and Qubit. The libraries were then loaded for Illumina NextSeq sequencing according to the standard operation. Paired-end 75 nucleotide (nt) reads were generated and checked for data quality using FASTQC (Babraham Institute, Cambridge, UK).

2.2. Genome Assembly and Annotation. The pair-end reads generated from the Illumina sequencing were trimmed using the Sickle tool 1.33 [11], assembled de novo using the SPAdes assembler version 3.10.0 [12], and then arranged into scaffolds using the MeDuSa scaffold 1.3 [13]. The resulting contigs were then improved using Iterative Mapping and Assembly for Gap Elimination (IMAGE) [14]. Quast software was used to assess the quality of the generated scaffold based on the number of contigs and the N50 [15]. The genome sequences were automatically annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/annotation/prok/) and Rapid Annotation using Subsystem Technology (RAST) server [16]. Prophage sequences and genomic islands were predicted from the genomes using PHASTER [17] and IslandViewer4 [18] online application, respectively.

2.3. Phylogenetic Tree Construction and Estimation of Pairwise Evolutionary Divergence between 16S rRNA Gene Sequences. The 16S rRNA gene sequences were aligned using the MUSCLE alignment tool with default parameters, and a phylogenetic tree was constructed using the maximum likelihood method based on the Kimura 2-parameter model. Bootstrap values were calculated to test the robustness of interior node support and were obtained by conducting 1000 pseudoreplicates using MEAGA® 6.0 software [19]. Pairwise evolutionary divergence (distance) was conducted in MEAGA® 6.0 software using the Kimura 2-parameter model with 1000 bootstrap replications.

2.4. Comparative Genome Analysis. For the whole-genome comparative study, genomes of eight *Bdellovibrio* spp. were retrieved from the NCBI database and compared with the genomes of the study *Bdellovibrio* strains (*Bdellovibrio* sp. SKB1291214 and *B. bacteriovorus* SSB218315). The retrieved genomes include that of the epibiotic *B. exovorus* JSS (NC_020813), *B. bacteriovorus* strains HD100 (NC_005363), W (NZ_CP002190), Tiberius (NC_019567), 109J (NZ_CP007656), R0 (LUKE00000000), EC13 (LUDK00000000), and BER2 (LUKF00000000).

The similarity among the genomes based on average amino acid identity (AAI) was inferred using the ANI/AAI-Matrix Genome-based distance matrix calculator [20]. A pangene analysis was carried out with the bacterial
3.1. Genomic Features of Bdellovibrio spp. Strains SKB1291214 and SSB218315 and B. bacteriovorus HD100, SKB1291214, and SSB218315 were compared by constructing a genome map using the KBase online software (https://kbase.us/), followed by BLASTP analysis of the hit regions in ExPASy Bioinformatics Resource Portal (https://www.expasy.org/). Alignment of the regions corresponding to the hit locus in the different Bdellovibrio strains was done using the multiple sequence alignment tool, Clustal Omega [23].

Table 1: Genomic features of B. bacteriovorus strains SSB218315 and Bdellovibrio sp. SKB1291214.

| Genome information          | B. bacteriovorus SSB218315 | Bdellovibrio sp. SKB1291214 |
|-----------------------------|----------------------------|-----------------------------|
| Chromosome size             | 3,769,537 bp                | 3,730,590 bp                |
| Number of contigs           | 1                          | 20                          |
| N50                        | 199,513                     |                             |
| GC content                 | 50.5%                       | 44.80%                      |
| Total RNA                  | 4                           | 4                           |
| Complete rRNAs             | 3                           | 3                           |
| tRNAs                      | 34                          | 33                          |
| Noncoding RNAs             | 4                           | 4                           |
| Total genes                | 3620                        | 3677                        |
| Total CDS                  | 3579                        | 3637                        |
| Coding CDS                 | 3536                        | 3588                        |
| Phage                      | 1 (incomplete)              | 1 (incomplete)              |
| Genomic island             | 69                          | 146                         |

2.5. Nucleotide Sequence Accession Numbers. The whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank databases under the accession NELQ0000000 for Bdellovibrio sp. SKB1291214 (the version described in this paper is version NELQ01000000). The complete genome sequence of B. bacteriovorus SSB218315 was deposited in the same databases under accession number CP020946.

3. Result and Discussion

3.1. Genomic Features of Bdellovibrio spp. Strains SKB1291214 and SSB218315. The genomic features of B. bacteriovorus strains SSB218315 and Bdellovibrio sp. strain SKB1291214 are summarized in Table 1. The genome size of B. bacteriovorus SSB218315 and Bdellovibrio sp. SKB1291214 is 3,769,537 bp and 3,730,590 bp, respectively. Bdellovibrio spp. are small but have large genomes (approximately 3.7 Mb) that encode predation factors presumed important to seek and lyse prey cells [24]. The percentage GC content in Bdellovibrio sp. SKB1291214 (44.8%) is low compared to B. bacteriovorus SSB218315 (50.5%). Lambert et al. [25] reported some genes expressed during predation in B. bacteriovorus HD100. These include genes that are up- and down-regulated at the early stage of B. bacteriovorus HD100 (30 minutes) infection as it switches from the motile preys-seeking attack stage to the intraperiplasmic phase, when it establishes itself in the prey cell. And because of the phenotypic differences observed between Bdellovibrio spp. SKB1291214 and SSB218315 [10], genome analysis was done to identify and compare the gene equivalent described by Lambert et al. [25] in the study strains. From BLASTP analysis results, B. bacteriovorus SSB218315 have all the described gene equivalent (Additional file 1a). However, among the 75 described upregulated genes, Bd1230 (lamb), Bd0487, and Bd2298 equivalents in B. bacteriovorus HD100 were absent in the genome of Bdellovibrio sp. SKB1291214. The Bd1230 (lamb) gene encodes maltoporin, an outer membrane protein that is important for sugar transport in Gram-negative bacteria, and it is usually expressed when Bdellovibrio degrades its prey. The genes Bd0487 and Bd2298 are found only in the genome of Bdellovibrio, and they are significantly upregulated when Bdellovibrio enters the periplasmic phase of growth [25]. Furthermore, eight out of the forty-one reported downregulated gene equivalents implicated in the attack phase of Bdellovibrio were absent in the genome of SKB1291214. These genes include the equivalent of Bd3260, Bd2608, Bd2400, Bd0737, and Bd0992 (cwlJ) encoding putative membrane proteins and enzymes (putative lipase and cell wall hydrolase) that play a role in prey attachment and penetration. Two gene equivalents Bd0880 and Bd0931 encoding stress response proteins, a homologue of periplasmic adaptor protein CpxP and transcriptional regulator, and MerR family were also absent in the genome of Bdellovibrio sp. SKB1291214. B. bacteriovorus uses type IV pilus to attach and subsequently invade prey cells. The gene equivalent of Bd0108 which encode proteins that function in regulating type IV pilus secretion in Bdellovibrio was also not present in the genome of SKB1291214. The missing gene equivalents described above might be playing important roles during Bdellovibrio predation. And thus, the absence of these genes in the genomes of Bdellovibrio sp. SKB1291214 can affect its rate of predation.

The RAST annotation server also predicted some genes presumed to enhance predation in Bdellovibrio spp. (Additional files 1b–f). These include genes encoding motility and chemotaxis factors, transport system including type IV pilus, stress response proteins, degradative proteins, and siderophores, and other defense factors.

The rapid motility of Bdellovibrio helps in prey location [26]. From RAST annotation and manual curation, about 75 genes encoding motility and chemotaxis factors were identified in the study Bdellovibrio strains. Among these factors are five adventurous gliding motility factors R, S, T, U, V, and MglA used by Bdellovibrio spp. to glide on solid surfaces and find prey in environments with a low water content such as biofilms [27, 28]. The RAST annotation server also predicted a sequence called diguanylate cyclase/phosphodiesterase (GGDEF and EAL domains) with PAS/PAC sensor(s) in Bdellovibrio spp. SKB1291214 (B9G69_13450, B9G69_
4 International Journal of Genomics

14735, B9G69_01735, and B9G69_08345) and SSB218315 (B9G79_16530, B9G79_14755, B9G79_11600, B9G79_00860, and B9G79_03750) as stress response proteins. Proteins that possess this GGDEF sequence secrete cyclic di-GMP, a signalling protein that controls *Bdellovibrio* to grow either as a predator that require prey for survival or a host-independent phenotype that can replicate on nutrient-rich medium. Four enzymatically competent GGDEF protein domains designated DgcA, DgcB, DgcC, and DgcD have been reported [29]. In the study of Hobley et al. [29], ΔdgcA mutants became a nonmotile host-independent strain that can grow axenically on nutrient-rich medium only. The ΔdgcA mutants can invade, replicate, and septate inside prey cells but cannot glide out of the prey cells to look for new prey. The ΔdgcB mutants became flagellated host-independent strains. The ΔdgcC mutants developed into predatory strains that are not capable of growing as host-independent (HI) strains. However, for ΔdgcC to grow axenically, they require additional or secondary mutation. Mutation of the dgcD gene did not result in any phenotypic alteration with mutants ΔdgcD growing both as host-dependent (HD) and HI. BLASTP analysis showed that GGDEF protein domains DgcA, DgcB, and DgcC are conserved in the two *Bdellovibrio* strains. However, the DgcD is not conserved in *Bdellovibrio* sp. SKB1291214, a similar result observed in *B. bacteriovorus* Tiberius [29].

*Bdellovibrio* spp. have been described to be nonpathogenic to human [30]. However, genomes of *Bdellovibrio* spp. SKB129124 and SSB218315 and other *Bdellovibrio* strains encode genes annotated as collagenase and hemolysin, virulence factors associated with some pathogens of human such as *Staphylococcus aureus* [31] and *Vibrio vulni-

ficus* [32]. The genome of *B. bacteriovorus* SSB218315 also encodes genes annotated as RTX toxins, a factor that has different biological functions such as pore-forming leukotoxin, metalloprotease, and lipase activities [33]. BLAST2GO software was used to carry out a BLASTP search and assign gene ontology (GO) to the gene products of the sequences annotated as hemolysin, collagenase, and RTX toxins (Additional file 1g). The GO of the sequences annotated as hemolysin III is cytolysis, and the BLASTP analysis revealed a conserved hemolysin III-related protein domain. The BLASTP analysis of the annotated collagenase revealed a conserved U32 family peptidase protein domain. However, collagenase belongs to the U32 family peptidase [34]. Identification of the biological roles of hemolysin III and collagenase in *Bdellovibrio* spp. will aid in their successful application as biocontrol agents against human pathogens. Analysis using the Pfam database [35] revealed that the RTX-toxin sequences did not have toxin domain but rather a protein domain identified as a regulator of chromosome condensation (RCC1) repeat. Thus, the annotated RTX toxin might be performing a different role than being involved in toxin production.

**Bacteria** can acquire genomic islands (GEIs) via horizontal gene transfer (HGT). These GEIs can confer adaptive features such as antibiotic resistance, survival features, and metabolic activities, metabolism of complex compounds on the bacteria [36]. Some distinguishing features of GEIs include association with genes encoding tRNA, integrase, or transposase, and possession of the percentage G + C content that is different from another part of the genome [37]. Predicted GEIs of *Bdellovibrio* strains SKB1291214 and SSB218315 include hypothetical proteins, peptidase, seption protein spoVG (in *B. bacteriovorus* SSB218315), and survival protein *surA* which can aid the survival of bacteria at the stationary growth phase (Additional file 2).

3.2. Phylogeny and Amino Acid Identity of *Bdellovibrio* Species. The phylogenetic analysis was done to compare the 16S rRNA gene sequences of *Bdellovibrio* sp. SKB1291214 and *B. bacteriovorus* SSB218315 with sequences of other members of the genus *Bdellovibrio* and their relatives that belong to the genus *Bacteriovorax, Peredibacter, and Halo-

bacteriovorax* (Figure 1). The 16S rRNA sequence of strains SKB1291214 and SSB218315 showed 96% similarity with a pairwise evolutionary distance of 0.043 (Additional file 3). The strain SKB1291214 shared 99% identity with an uncontested *Bdellovibrio* sp. clone12 L 106 (pairwise distance of 0.008) while strain SSB218315 shared 100% identity with other culturable terrestrial *B. bacteriovorus* which include *B. bacteriovorus* strain HD100 (pairwise distance 0.001) and Tiberius (pairwise distance 0.004). The phylogenetic tree showed that the two *Bdellovibrio* strains SKB1291214 and SSB218315 are phylogenetically different despite being isolated from soil samples in the same environment. Further species delineation was done to examine the AAI among the *Bdellovibrio* strains. For strains to belong to the same species, they must have ANI and AAI ≥ 95%, <10 Karlin genomic signature, and >70% in silico GGDH [38]. The AAI between strain SKB1291214 and other strains was very low (63.70–67.68%) while strain SSB218315 shared a high AAI value of 95% with *B. bacteriovorus* strains HD100, Tiberius, and 109J (Figure 2). The result showed that strain SSB218315 is closely related to HD100, Tiberius, and 109J and thus, they can conveniently be grouped as the same species. Meanwhile, considering the percentage GC content, phylogenetic tree clustering pattern, and AAI value, strain SKB1291214 could be grouped as a novel species; however, further analysis is needed.

3.3. Pangenome Analysis. A bacterial pangenome analysis (BPGA) tool was used to carry out pangenome analysis of eight *Bdellovibrio* spp. The pangenome is made up of 8134 genes, and the *Bdellovibrio* spp. shared 795 genes as core genomes (Figure 3, Additional file 4a). The BPGA predicted the pangenome of *Bdellovibrio* sp., as open based on the power law regression of the program (Additional file 4b). The total number of unique genes found in *Bdellovibrio* spp. SKB1291214 and SSB218315 is 1343 and 113, respectively (Table 2). The epibiotic *B. exovorus* JJS and bdellocyst-forming *B. bacterio-

vorus* W have a total of 1572 and 857 unique genes, respectively. The GO of the unique genes in *Bdellovibrio* sp. SKB1291214 revealed that they are rich in proteins involved in molecule transport, oxidation-reduction process, signal transduction, hydrolase activity phosphorylation, and nucleotide and ion binding (Additional files 4c–f). *B. exovorus* JJS has the highest number of unique genes (1572), and among these are three genes encoding type II
CRISPR-associated endonuclease Cas1, CRISPR-associated Cas2, and type II CRISPR RNA-guided endonuclease Cas9 which usually act to defend prokaryotes against any invading foreign genetic material. These CRISPR genes are however absent in the genome of the periplasmic *Bdellovibrio* spp.

A comparative genomic study by Pasternak et al. [39] identified protein families that are specific to predatory bacteria and differentiate them from the nonpredatory bacteria. All the fifteen protein families reported to be specific to predatory bacteria were present in *Bdellovibrio* sp. SKB1291214 and SSB218315. Homologue of genes encoding two protein families, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (B9G69_00295; mean similarity value = 57; *E* value = 1.33 *E* − 134) and indole-3-glycerol phosphate synthase (B9G69_04640; mean similarity value = 74; *E* value = 0.0), reported to be specific to nonpredatory bacteria was found among the unique genes of *Bdellovibrio* sp. SKB1291214 (Additional file 4c). Predatory bacteria are different from the nonpredators based on the pathway utilized for the biosynthesis of isoprenoids. While the nonpredators use the deoxy-d-xylulose 5-phosphate (DOXP) or nonmevalonate pathway, the predators use the mevalonate pathway for the biosynthesis of isoprenoids [39]. The 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase is an enzyme required for the nonmevalonate pathway synthesis of isoprenoid, and its presence in *Bdellovibrio* sp. SKB1291214 presumably is a result of horizontal gene transfer (HGT).

One of the differences between *B. exovorus* and *B. bacteriovorus* is the mechanism they use for prey attack [2, 3]. The latter is characterized by the invasion of the prey periplasm while the former are not capable of penetrating into their prey. During prey invasion in *B. bacteriovorus* HD100, three genes tagged *Bd0816*, *Bd3459*, and *Bd3460* play an important role [26, 40, 41]. The *Bd0816* and *Bd3459* encode D-alanyl-D-alanine carboxypeptidase usually expressed at the point of prey entry while *Bd3460* encodes a protein called ankyrin which protects *Bdellovibrio* hydrolytic enzymes which it secretes during prey invasion.

Genes encoding D-alanyl-D-alanine carboxypeptidase were present among the unique genes of *Bdellovibrio* sp. SKB1291214 (B9G69_09970 and B9G69_09965) and *B. exovorus* JJS (A11Q_2041) (Additional files 4c and d). However, gene encoding ankyrin was absent in the genome of *B. exovorus* JJS but present in the genome *Bdellovibrio* sp. SKB1291214 and SSB218315 which are closer to the epibiotic *B. exovorus*. While the presence of genes encoding D-alanyl-D-alanine carboxypeptidase is a general feature of the genus *Bdellovibrio* spp., the ankyrin-encoded genes are limited to the periplasmic members of the genus *Bdellovibrio*. The predation mechanism
of *B. exororus* does not require prey invasion, hence, the possible reason why it does not have the gene equivalent of Bd3460 in its genome. Furthermore, the unique genes of SKB1291214 also contain the Autographivirinae Erwinia phage-associated region coding for protein *AmsF* (B9G69_00395) which is involved in amylovoran biosynthesis. Amylovoran is an exopolysaccharide that plays a role in the pathogenesis of *Erwinia carotovora* [42].

The presence of genes encoding integrases (among predicted GEIs), transposases, phage-associated protein *AmsF*, and nonpredatory bacteria-associated 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (among the unique genes) suggests the occurrence of HGT in *Bdellovibrio* sp. SKB1291214. From the BLASTP analysis done with the BLAST2GO software using an E value threshold of 1E−6, there is an indication that some of the unique genes are acquired horizontally from bacteria that belong to groups other than class Deltaproteobacteria. These groups include the Alphaproteobacteria (B9G69_00040, DUF4334 domain-containing and B9G69_13200, AraC family transcriptional regulator), Betaproteobacteria (B9G69_13210, Com family DNA-binding transcriptional regulator and B9G69_11675, FAD: FMN transferase), Gammaproteobacteria (B9G69_13180, glyoxalase bleomycin resistance dioxygenase; B9G69_13235, terminase small subunit; B9G69_13225, AlpA family transcriptional regulator; and B9G69_13230, bacteriophage), Epsilonproteobacteria (B9G69_13210, Com family DNA-binding transcriptional regulator and B9G69_11675, FAD: FMN transferase), Gammaproteobacteria (B9G69_13180, glyoxalase bleomycin resistance dioxygenase; B9G69_13235, terminase small subunit; B9G69_13225, AlpA family transcriptional regulator; and B9G69_13230, bacteriophage), Epsilonproteobacteria (B9G69_13210, Com family DNA-binding transcriptional regulator and B9G69_11675, FAD: FMN transferase), Gammaproteobacteria (B9G69_13180, glyoxalase bleomycin resistance dioxygenase; B9G69_13235, terminase small subunit; B9G69_13225, AlpA family transcriptional regulator; and B9G69_13230, bacteriophage). This result corroborates the earlier findings of Gophna et al. [7]. A study on the extent and frequency of HGT in *Bdellovibrio* spp will provide useful information that can aid their successful application as biocontrol agents.

3.4. Analysis of the Host Interaction (hit) Locus. *B. bacteriovorus* has been described to have the ability to switch from being predatory usually referred to as host dependent (HD) to growing on nutrient-rich medium axenically, sometimes
referred to as host independent (HI). Mutation at a region identified as host interaction (hit) locus has been reported to be responsible for the conversion from the HD to HI phenotypes. The hit locus has been described to be made up of an open reading frame (ORF) tagged Bdo108 and part of ORF tagged Bdo109 encoding a putative cell wall-associated protein in B. bacteriovorus HD100 [6]. There are pil genes located upstream of the hit locus (Figure 4(a)). These pil genes encode structural proteins for the formation of the type IV pilus system needed for prey adherence and colonization. The genes Bdo113 and Bdo114 are responsible for the pilus assembly while the TadA (Bdo110) and TadB (Bdo111) encode ATPase that provides energy for the type IV pilus secretion [43]. And downstream of the hit locus are genes tagged BD_RS00505 (new locus tag for B. bacteriovorus HD100 genes) and Bdo103 in B. bacteriovorus HD100; both genes encode hypothetical proteins of unknown function. The above-described genes (the Bdo108, Bdo109, pil genes, BD_RS00505, and Bdo103) are inserted into two genes Bdo102 and Bdo121 encoding chemotaxis factors. In our previous study, hit locus was successfully amplified in B. bacteriovorus SSB218315 using the PCR technique. The negative result obtained from the PCR amplification of the hit locus in Bdellovibrio sp. SKB1291214 made us construct genomic maps to compare the hit locus region between the hit locus region of two Bdellovibrio spp. HD100, SSB218315, and SKB1291214 (Figures 4(a)–4(c)). From the result of the BLASTP and multiple sequence alignment analysis, the region corresponding to the Bdo108 (hit locus) is not conserved in Bdellovibrio sp. SKB1291214. (Additional file 5). Furthermore, a fragment of 174 bp absent in HD100 and SSB218315 was found inserted between gene equivalent Bdo102 and BD_RS00505 in SKB1291214 (Figure 4(b)). This fragment produces an insignificant E value with BLASTP analysis. Comparative analysis revealed that the gene equivalent Bdo109 is conserved among the Bdellovibrio spp. including Bdellovibrio sp. SKB1291214 and B. exovorus JSS. Thus, this suggests that Bdo109 gene may have an important role in the predatory activities of Bdellovibrio spp. Also, variations in the sequence of Bdo108 may not be sufficient to hinder prey predation Bdellovibrio spp. Because Bdellovibrio spp. that have a mutation at the hit locus can be cultured axenically [6], we attempted to culture Bdellovibrio spp. SKB1291214 and SSB218315 on nutrient-rich medium in the absence of prey using three different techniques described by Ferguson et al. [44], Lambert and Sockeyt [45], and Seidler and Starr [46]. However, we could not successfully isolate the HI phenotypes using the three approaches, though all the yellow bacterial colonies obtained from the method exhibited the phenotypic characteristics described in the previous research.

4. Conclusion

Members of the genus Bdellovibrio have been reported to have potential applications as biocontrol agents against pathogens. This study focused on the whole-genome sequencing and comparative analysis of two Bdellovibrio spp. that showed phenotypic differences. The comparative analysis showed that B. bacteriovorus SSB218315 is genetically related to the soil-derived B. bacteriovorus HD100. We also observed that the Bdellovibrio sp. SKB1291214 is distinctively different from the epibiotic B. exovorus; although SKB1291214 showed traits associated with the intraperiplasmic predatory lifestyle, it is still different from SSB218315 and HD100 based on the 16S rRNA gene sequencing analysis, GC content, and AAI. The diversity was observed among the members of the genus Bdellovibrio thus suggesting the need to review the taxonomy of the genus Bdellovibrio in the nearest future. The pangenome analysis revealed that genomes of Bdellovibrio spp. have genes encoding different predation factors including signal transduction, hydrolytic, proteolytic, transport, and transport proteins that can help them survive as a bacterial predator. However, some factors such as hemolysin III and collagenase observed in the genomes need to be studied and characterized so that they will not have counterproductive effects when Bdellovibrio spp. are considered for applications as a biocontrol agent of pathogens in humans. Finally, Bdellovibrio sp. SKB1291214 have GEIs with atypical percent GC, AmsF protein, and a homologue of 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase among its unique genes and an insertion of a 174 bp fragment in its hit locus region. These occurrences are presumptive indications of HGT in Bdellovibrio sp. SKB1291214.

| Genome number | Strain | Number of core genes | Number of accessory genes | Number of unique genes | Number of exclusively absent genes |
|---------------|--------|-----------------------|----------------------------|------------------------|-----------------------------------|
| 1             | B. bacteriovorus HD100 | 795                  | 2641                       | 65                     | 1                                 |
| 2             | B. bacteriovorus strain Tiberius | 795                  | 2579                       | 301                    | 1                                 |
| 3             | B. bacteriovorus W | 795                  | 1063                       | 857                    | 54                                |
| 4             | B. bacteriovorus 109J | 795                  | 2635                       | 132                    | 1                                 |
| 5             | B. bacteriovorus SSB218315 | 795                  | 2575                       | 113                    | 6                                 |
| 6             | B. exovorus JSS | 795                  | 200                        | 1572                   | 725                               |
| 7             | Bdellovibrio sp. SKB1291214 | 795                  | 1366                       | 1343                   | 27                                |
Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Omotayo Opemipo Oyedara and Mario A. Rodríguez Pérez contributed equally to this manuscript.

Acknowledgments
This work was funded by Secretaría de Investigación y Posgrado of Instituto Politécnico Nacional (Grant nos. 20161059 and 20170752). Consejo Nacional de Ciencia Y Tecnología (CONACYT), Mexico is acknowledged for providing Omotayo Opemipo Oyedara a doctoral scholarship (no. 595082/326342). Xianwu Guo and Mario A. Rodríguez Pérez hold scholarships from Comisión de Operación y Fomento de Actividades Académicas, Instituto Politécnico Nacional (COFAA-IPN). The publication fee for the manuscript was funded by COFAA-IPN.

Supplementary Materials
Supplementary 1. Additional file 1a: genome analysis of Bdellovibrio spp. SKB1291214 and SSB218315 for the genes expressed by *B. bacteriovorus* HD100 during predation as reported by Lambert et al. [25]. Additional files 1b–f: presumed predation-enhancing factors in *Bdellovibrio* spp. SKB1291214 and SSB218315 predicted by RAST annotation.
server. (1b) Flagellar and chemotaxis factor. (1c) Transport and type IV pilus proteins. (1d) Stress response proteins. (1e) Factors associated with the production of degradative enzymes for the metabolism of molecules. (1f) Siderophores and defense factors. Additional file 1g: BLASTp analysis of genes annotated as hemolysin, collagenase, and RTX toxin in the genomes of *Bdellovibrio* spp.

**Supplementary 2.** Additional files 2a and b: predicted genomic islands in *Bdellovibrio* spp. SKB1291214 and SSB218315, respectively.

**Supplementary 3.** Additional file 3: pairwise evolutionary distance among *Bdellovibrio* spp.

**Supplementary 4.** Additional files 4a–f: pangenome analysis of *Bdellovibrio* spp. using the BPGA pipeline. (4a) The core genes identified in the *Bdellovibrio* spp. (4b) The power and exponential fit law to predict the nature of *Bdellovibrio* spp. pangenome as open or close. (4c–f) The unique genes identified in *Bdellovibrio* spp. SKB1291214, JSS, W, and SSB218315, respectively.

**Supplementary 5.** Additional 5a and b: multiple sequence alignment of the Bd0108 and Bd0109 genes of hit locus, respectively.

**References**

[1] R. E. Sackett, “Predatory lifestyle of *Bdellovibrio bacteriovorus*,” *Annual Review of Microbiology*, vol. 63, no. 1, pp. 523–539, 2009.

[2] H. Stolp and M. P. Starr, “*Bdellovibrio bacteriovorus* gen. et sp. nov., a predatory, ectoparasitic, and bacteriolytic microorganism,” *Antonie Van Leeuwenhoek*, vol. 29, no. 1, pp. 217–248, 1963.

[3] S. F. Koval, S. H. Hynes, R. S. Flannagan, Z. Pasternak, Y. Davidov, and E. Jurkevitch, “*Bdellovibrio exovorus* sp. nov., a novel predator of *Caulobacter crescentus*,” *International Journal of Systematic and Evolutionary Microbiology*, vol. 63, Part 1, pp. 146–151, 2013.

[4] J. J. Tudor and S. F. Conti, “Characterization of germination and activation of *Bdellovibrio* bdellocyts,” *Journal of Bacteriology*, vol. 133, no. 1, pp. 130–138, 1978.

[5] L. Hobley, T. R. Lerner, L. E. Williams et al., “Genome analysis of a simultaneously predatory and prey-independent, novel *Bdellovibrio bacteriovorus* from the River Tiber, supports in silico predictions of both ancient and recent lateral gene transfer from diverse bacteria,” *BMC Genomics*, vol. 13, no. 1, p. 670, 2012.

[6] M. J. Capeness, C. Lambert, A. L. Lovering et al., “Activity of *Bdellovibrio* hit locus proteins, Bd0108 and Bd0109, links Type IVa pilus extrusion/retraction status to prey-independent growth signalling,” *PLoS One*, vol. 8, no. 11, article e79759, 2013.

[7] U. Gopnha, R. L. Charlebois, and W. F. Doolittle, “Ancient lateral gene transfer in the evolution of *Bdellovibrio bacteriovorus*,” *Trends in Microbiology*, vol. 14, no. 2, pp. 64–69, 2006.

[8] A. Pan, I. Chanda, and J. Chakrabarti, “Analysis of the genome and proteome composition of *Bdellovibrio bacteriovorus*: indication for recent prey-derived horizontal gene transfer,” *Genomics*, vol. 98, no. 3, pp. 213–222, 2011.

[9] E. Jurkevitch, D. Minz, B. Ramati, and G. Barel, “Prey range characterization, ribotyping, and diversity of soil and rhizosphere *Bdellovibrio* spp. isolated on phytopathogenic bacteria,” *Applied and Environmental Microbiology*, vol. 66, no. 6, pp. 2365–2371, 2000.

[10] O. O. Oyedara, E. J. de Luna-Santillana, O. Olguin-Rodriguez et al., “Isolation of *Bdellovibrio* sp. from soil samples in Mexico and their potential applications in control of pathogens,” *Microbiology Open*, vol. 5, no. 6, pp. 992–1002, 2016.

[11] N. A. Joshi and J. N. Fass, “Sickle: A Sliding-Window, Adaptive, Quality-BasedTrimming Tool for FastQ Files,” 2011, (Version 1.33) [Software]. https://github.com/najoshi/sickle.

[12] A. Bankevich, S. Nurk, D. Antipov et al., “SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing,” *Journal of Computational Biology*, vol. 19, no. 5, pp. 455–477, 2012.

[13] E. Bosi, B. Donati, M. Galardini et al., “MeDuSa: a multi-draft based scaffold,” *Bioinformatics*, vol. 31, no. 15, pp. 2443–2451, 2015.

[14] I. J. Tsai, T. D. Otto, and M. Berriman, “Improving draft assemblies by iterative mapping and assembly of short reads to eliminate gaps,” *Genome Biology*, vol. 11, no. 4, article R41, 2010.

[15] A. Gurevich, V. Saveliev, N. Vyahhi, and G. Tesler, “QUAST: quality assessment tool for genome assemblies,” *Bioinformatics*, vol. 29, no. 8, pp. 1072–1075, 2013.

[16] R. K. Aziz, D. Bartels, A. A. Best et al., “The RAST server: rapid annotations using subsystems technology,” *BMC Genomics*, vol. 9, no. 1, p. 75, 2008.

[17] D. Arndt, J. R. Grant, A. Marcu et al., “PHASTER: a better, faster version of the PHAST phage search tool,” *Nucleic Acids Research*, vol. 44, no. W1, pp. W16–W21, 2016.

[18] C. Bertelli, M. R. Laird, K. P. Williams et al., “IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets,” *Nucleic Acids Research*, vol. 45, no. W1, pp. W30–W35, 2017.

[19] K. Tamura, G. Stecher, D. Peterson, A. Filipski, and S. Kumar, “MEGA6: molecular evolutionary genetics analysis version 6.0,” *Molecular Biology and Evolution*, vol. 30, no. 12, pp. 2725–2729, 2013.

[20] K. T. Konstantinidis and J. M. Tiedje, “Genomic insights that advance the species definition for prokaryotes,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 7, pp. 2567–2572, 2005.

[21] N. M. Chaudhari, V. K. Gupta, and C. Dutta, “BPGA - an ultra-fast pan-genome analysis pipeline,” *Scientific Reports*, vol. 6, no. 1, article 24373, 2016.

[22] S. Götz, J. M. García-Gómez, J. Terol et al., “High-throughput functional annotation and data mining with the Blast2GO suite,” *Nucleic Acids Research*, vol. 36, no. 10, pp. 3420–3435, 2008.

[23] F. Sievers, A. Wilm, D. Dineen et al., “Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega,” *Molecular Systems Biology*, vol. 7, no. 1, p. 539, 2011.

[24] S. Rendulic, P. Jagtap, A. Rosinus et al., “A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective,” *Science*, vol. 303, no. 5658, pp. 689–692, 2004.

[25] C. Lambert, C. Y. Chang, M. J. Capeness, and R. E. Sackett, “The first bite—profiling the predatome in the bacterial pathogen *Bdellovibrio*,” *PLoS One*, vol. 5, no. 1, article e8599, 2010.
