Genomic insights from Paraclostridium bifermentans HD0315_2: General features and pathogenic potential

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Background: Paraclostridium bifermentans is the most diverse distributed species of Paraclostridium and can cause fatal human infections under rare conditions. However, its pathogenic mechanisms and adaptation ability behind infections remain unclear. Herein, we reported the complete genome sequence of P. bifermentans HD0315_2 isolated from the feces of a patient with Crohn’s disease. Then, we performed genomic analyses to understand its pathogenic mechanisms and adaptation ability.

Results: The de novo assembly revealed that the HD0315_2 strain carried a circular chromosome of 3.27 Mb and six circular plasmids (19.41 to 139.50 kb). The phylogenomic analysis assigned the HD0315_2 strain as P. bifermentans and reclassified some previously non-P. bifermentans strains into this clade. The general genomic features showed that this species harbored a flexible genomic pool characterized by variable genome length and multiple plasmids. Then, the HD0315_2 strain was predicted as a human pathogen with high probability, and Listeria LIPI-1 virulence proteins were identified on its genome. Besides, abundant antibiotics/metal/stress resistant genes, such as asrABCH, cat, mccF, macB, entS, albA, bcrA, and tetB, were carried by either the genome or the plasmids. Furthermore, we proposed that transposase-directed horizontal gene transfer was responsible for the distribution of multiple copies of the hin gene in the plasmids.

Conclusion: The flexible genomic pool of P. bifermentans encodes abundant functions for antimicrobial or oxidative stress resistance, helping it successfully inhabit and adapt to diverse environments. Moreover, P. bifermentans HD0315_2 might infect hosts via a Listeria LIPI-1-like cycle, with the help of a plasmid expressing the Hin DNA invertase to evade host immune responses.

KEYWORDS
Paraclostridium, P. bifermentans, antimicrobial resistance, pathogenicity, Listeria LIPI-1, multiple plasmids, phylogenomic tree
Introduction

*Paraclostridium* spp. belongs to the class *Clostridia* of Gram-positive, rod-shaped, and obligate anaerobe bacteria that reside in various mesophilic conditions, such as soil, marine habitats, polluted waters, and clinical specimens (Jyothsna et al., 2016). To date, only two species have been formally published under this genus (i.e., *P. bifermentans* and *P. benzoelyticum*) (Jyothsna et al., 2016). Among them, the most frequently isolated and best-characterized species is *P. bifermentans* (Hale et al., 2016). Additionally, in 2016, *P. bifermentans* was reclassified from *Clostridium bifermentans* (Jyothsna et al., 2016). This species has been previously recognized as nonpathogenic unless co-inhabited with *Clostridium perfringens*, producing putrid lesions in guinea pigs (Weinberg and Séguin, 1918). However, 15 cases of *P. bifermentans* infections in humans have been recently summarized and included brain abscess, lymphadenitis, necrotizing endometritis, a prosthetic knee joint infection, empyema, and endocarditis (Kolander et al., 1989; Edagiz et al., 2015; Hale et al., 2016; Biswas et al., 2018; Barrett et al., 2020). Kutsuna et al. have shown that *P. bifermentans* PAGU1678 exacerbates mouse’s pathological conditions in a dextran sulfate sodium-induced colitis model (Kutsuna et al., 2018). Although *P. bifermentans* has emerged as a rare pathogen in humans, it thrives as a human pathogen under specific conditions and rises to major health hazards. Nevertheless, no report has determined any suggestive genes causing its pathogenicity, although various draft genomes have been published. On the other hand, the high similarity of 16S rRNA sequences within the genus (e.g., *P. bifermentans* and *P. benzoelyticum* share a 16S rRNA sequence similarity > 99%) is difficult for their taxonomic identification and characterization of biological traits. Herein, we isolated *P. bifermentans* strains from the feces of patients with Crohn’s disease (CD) for the first time. Additionally, we showed that *P. bifermentans* strains were only isolated from patients with CD but not from patients with ulcerative colitis (UC), underlying the potential involvement of this species in CD. Then, the complete genome of *P. bifermentans* HD0315_2, isolated from the feces of a young male patient with CD, was assembled de novo using reads from Illumina and Nanopore sequencing. Furthermore, whole-genome sequence-based taxonomy identification and comparative genome analyses were performed to clarify the general genome function and its specificities regarding virulence, adaptation, and pathogenic effects.

Materials and methods

Isolation and characterization of strains

Anaerobic conditions (90% N₂, 5% CO₂, and 5% H₂) were used during the isolation and culture of *P. bifermentans* strains. First, fresh fecal samples were collected from a 24-year-old Chinese man with active CD from Guangdong (China) who suffered from recurrent abdominal pain, changes in stool traits, and perianal exudate. Briefly, 10 ml of sterile PBS was added per gram of fresh feces to a 50-ml conical tube, then vortexed and left to settle. Next, the feces suspension was transferred to anaerobic blood culture bottles (BD, BACTECTM Lytic/10 Anaerobic/F Culture Vials, America) supplemented with sterile sheep blood and rumen fluid. Bottles were incubated under anaerobic conditions at 37°C for 30 d as described by Lagier’s culturaomics strategy (Lagier et al., 2018). Then, 1 ml of suspension was steriley aspirated from the incubated culture and serially diluted (10 to 10⁻¹²). Finally, 100 µl of each dilution was evenly plated on Brain-Heart Infusion (BHI) or reinforced clostridia agar plates to harvest colonies. Purification was further conducted by streaking. The harvested colonies were enriched in BHI medium at 37°C for 3 d and identified by MALDI Biotyper RTC (Bruker Daltonics, Germany). Single and sufficiently grown colonies were directly transferred to the MALDI Biotyper RTC (Bruker, Germany), with 350 bp insert size and sequenced using the PE150 flow cell (Oxford Nanopore Technologies, UK) and low-quality reads (i.e., scores < 7) were removed. The short-read sequencing library was constructed using the SQK-LSK109 kit (Oxford Nanopore Technologies, UK) and the Illumina NovaSeq platform (Novogene, Nanjing, China), respectively. The long-read sequencing library was constructed using the SQRK-LSK109 kit (Oxford Nanopore Technologies, UK) according to the manufacturer’s instructions. Sequencing and base calling were performed using MinKNOW v1.15.4 with the FLO-MINSPI5 flow cell (Oxford Nanopore Technologies, UK) and low-quality reads (i.e., scores < 7) were removed. The short-read sequencing library was constructed with a 350 bp insert size and sequenced using the PE150 strategy. Adapter trimming and low-quality reads (Phred score ≤ 20) were removed for quality control. The de novo genome assembly was conducted using the Unicycler.
v0.4.9b assembler (Wick et al., 2017), with the default hybrid assembly pipeline.

Phylogenomic characterization and plasmid detection

Taxonomy assignment was further curated using the gtdbtk_wf workflow implemented in GTDB-Tk (Parks et al., 2018). The average nucleotide identity (ANI) between the HD0315_2 strain and the phylogenomic close genomes were calculated using fastANI (Jain et al., 2018). A phylogenomic tree based on the whole genome protein sequences was constructed using CVTree3 with default parameters (K-tuple length 3, 4, 5, 6, 7) (Zuo and Hao, 2015). Plasmids were predicted using PlasForest v1.2 (https://github.com/leaemiliepradier/PlasForest) and mlplasmids v2.1.0 (https://gitlab.com/sirarredondo/analysis_mlplasmids) based on machine learning from sequence homology and pentamer frequencies.

Comprehensive genome annotation

Comprehensive genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al., 2016). Functional genome annotation with multiple databases, including Carbohydrate-Active enZymes (CAZy), Cluster of Orthologous Groups (COG), and Kyoto Encyclopedia of Genes and Genomes (KEGG), was performed using eggNOGMapper v2.1.5 (Huerta-Cepas et al., 2017). Comprehensive genome analysis, including the annotation of subsystems, identification of specialty genes (transporters, virulence factors, drug targets, antibiotic resistance genes, and antimicrobial resistance genes), and phylogenetic analysis, was conducted using PATRIC v3.6.10 (https://www.patricbrc.org/). Predominance of phylogeny analysis was performed using PathogenFinder v1.1 (https://cge.cbs.dtu.dk/services/PathogenFinder/). Genomic islands (GI) were identified using IslandViewer 4 (Bertelli et al., 2017).

Quality assurance

The HD0315_2 strain was repeatedly sub-cultured on broth agar plates to confirm the obtainment of a single colony before sequencing. Taxa assignments were cross-validated by both MALDI Biotyper RTC and full-length 16S rRNA sequencing, which determined that the HD0315_2 strain belongs to the Paraclostridium genus (Supplementary Tables S1, S2). Quality control was conducted by using CheckM v1.0.12 to evaluate the assembly quality and using samtools v1.9 and bcftools v1.10.2 to check the variants through mapping the Illumina reads to the contigs. Then, the assembled genome sequence was loaded onto GTDB-Tk for taxonomy assignments.

Results and discussion

Genomic features and phylogenomic tree

The hybrid assembly using Nanopore and Illumina reads generated seven complete circular contigs without Ns. The results showed that the (1) high-quality contigs were assembled using the Unicycler assembler (contamination = 0.70%, completeness = 99.30%, Strain heterogeneity = 0.00%), (2) the pair-end Illumina reads showed an overall 99.61% alignment rate to the assembled contigs, and (3) only three-point sites were determined as SNP sites, all located in the chromosome (i.e., contig HD0315_2) (Supplementary Table S3). The plasmid identification analysis showed that the biggest contig was the chromosome sequence, while the remaining six contigs were plasmids. The taxon identification workflow implemented in GTDB-Tk identified the HD0315_2 strain as P. bifermentans based on the sequence ANI (> 96) from the biggest contig (chromosome). The six smaller contigs did not return the bacteria marker genes when loaded into the workflow, which validated them as plasmids. The whole-genome sequencing of P. bifermentans HD0315_2 comprehends a circular chromosome with 3,265,124 bp, 3, 366 CDSs, 51 rRNA, 103 tRNAs, and a G + C content of 28.8%; and six plasmids with lengths, G + C contents, and CDSs of the six plasmids are between 19,414–139,499 bp, 24.6–27.6%, and 23–162, respectively (Figure 1A).

Table 1 contains information about the genomic features between these strains are listed in Table 1. Currently, only two complete genomes of the Paraclostridium genus have been released in the NCBI genome database, and both belong to P. bifermentans species. Similar to the HD0315_2 strain, these two genomes carry multiple plasmids and close genome G + C content (<0.5% variations). Moreover, the chromosome lengths in this species varied from 3.27 to 3.64 Mb, indicating a flexible genomic pool not only from plasmid sequences but also from genome accessory sequences. Although the chromosome length of the HD0315_2 strain is the shortest and has a lower G/G+C proportion, it carries more rRNAs/tRNAs and is associated with more plasmids and might represent a more efficient protein synthesis capability of HD0315_2, in contrast to the Cbm strain that is characterized by an opposite pattern. Detailed comparisons of the genomic features between these strains are listed in Table 1.

The genome sequence alignment among HD0315_2, DSM14991, and Cbm strains indicated that large sequence rearrangement/transversion events happened in Cbm, close to the homologous replicon origin region of HD0315_2, which might result in the variations of rRNAs and tRNAs gain or loss (Figure 1A). Five GIs were predicted in the genome sequence,
TABLE 1  Genomic features of the strains in the genus *Paraclostridium* with completed genome sequences.

| Strain            | Accession | Type                  | Length (bp) | GC%  | CDS  | rRNA  | tRNA  | Other RNA | Total Length (bp) | G/G+P (%) |
|-------------------|-----------|-----------------------|-------------|------|------|-------|-------|-----------|-------------------|-----------|
| *P. bifermentans* HD0315_2 | CP094935  | Chromosome            | 3,265,124   | 28.8 | 3,366| 51    | 103   | 4         | 3,639,934         | 89.7      |
| pH0315_2-1        | CP094934  | Plasmid               | 139,499     | 26.3 | 162  |       |       |           | 2,102,952         |           |
| pH0315_2-2        | CP094935  | Plasmid               | 109,009     | 26.8 | 140  |       |       |           | 2,122,603         |           |
| pH0315_2-3        | CP094936  | Plasmid               | 49,869      | 24.6 | 58   |       |       |           | 1,612,391         |           |
| pH0315_2-4        | CP094937  | Plasmid               | 32,029      | 25.8 | 39   |       |       |           | 1,642,900         |           |
| pH0315_2-5        | CP094938  | Plasmid               | 24,990      | 27.6 | 34   |       |       |           | 1,622,601         |           |
| pH0315_2-6        | CP094939  | Plasmid               | 19,414      | 27.2 | 23   |       |       |           | 1,592,377         |           |
| *P. bifermentans* DSM14991 | CP079737  | Chromosome            | 3,304,778   | 28.8 | 3,346| 48    | 102   | 4         | 3,566,216         | 92.7      |
| Unnamed 1         | CP079738  | Plasmid               | 142,041     | 26.6 | 162  |       |       |           | 2,122,603         |           |
| Unnamed 2         | CP079739  | Plasmid               | 52,569      | 26.5 | 76   |       |       |           | 1,622,601         |           |
| Unnamed 3         | CP079740  | Plasmid               | 47,028      | 24.2 | 48   |       |       |           | 1,602,088         |           |
| Unnamed 4         | CP079741  | Plasmid               | 19,800      | 26.8 | 21   |       |       |           | 1,592,377         |           |
| *P. bifermentans* Cbm | CP032452  | Chromosome            | 3,638,572   | 28.5 | 3,486| 28    | 52    | 3         | 3,770,185         | 96.5      |
| pPbmMP            | CP032455  | Plasmid               | 26.1        | 101  |      |       |       |           |                   |           |
| pPbm2_2           | CP032454  | Plasmid               | 26.4        | 5    |      |       |       |           |                   |           |
| pPbm14_8          | CP032453  | Plasmid               | 34.4        | 12   |      |       |       |           |                   |           |

*G/G+P represents the chromosome length proportion to the whole genomic length containing the chromosome and plasmids.

The phylogenomic relationship between these strains was further validated by the ANI analysis (Figure 1C).

**General gene functions**

A total of 1,188 subsystem features were revealed by RAST in the genome of *P. bifermentans* HD0315_2 (Supplementary Figure S2). Most features were assigned to “Protein Metabolism (237)” and “Amino Acids and Derivatives (179).” The wide distribution or habitats of *Paraclostridium* spp. have suggested that this genus has an excellent ability to survive in various environments (Rai et al., 2015), which was endowed by the genomic function. We found that 75 genes were tightly correlated to the adaptation of HD0315_2, including 42 assigned to “Virulence, Disease and Defense,” 7 assigned to “Phages, Prophages, Transposable elements, Plasmids,” and 26 assigned to “Stress Response” (Supplementary Figure S2). The main resistance coding genes were those sub-assigned to tetracycline (4), fluoroquinolones (2), cobalt-zinc-cadmium (11), copper (2), and multidrug-compounds (12). The virulence factor coding genes were mainly sub-assigned to *Mycobacterium*-involved virulence operon (10) and *Listeria* LIPI-1-extended proteins (3). Stress-response coding genes were mainly sub-assigned to osmotic stress (4) and oxidative stress (15) (Supplementary Table S5). However, the plasmid annotation using RAST presented poor results, and only 15 genes in the plasmids were assigned to the subsystem (data not shown).
The circular display of the complete genome sequence feature and phylogenomic tree originated from *P. bifermentans* HD0315_2. (A) From outer to inner rings: genome sequence of *P. bifermentans* Cbm, the genome sequence of *P. bifermentans* DSM14991, CDSs on the forward/reverse strand, GC skew, GC content, and genome islands. RNAs and repeat sequence regions are displayed in the CDSs circles. GI represents genome islands. (B) Phylogenomic tree constructed from WGS of the phylogenetic close strains. Numbers marked on the tree branch represent four Clades divided by phylogenomic analyses. Leaf labels with red color are those reclassified as *P. bifermentans* members in this study. Previously documented taxonomic information of them are listed in Supplementary Table S1. (C) Heatmap displaying the ANI between the phylogenetically close *Paraclostrium* spp. The color bar on the right indicates the ANI value calculated using fastANI. Numbers marked alongside the dendrogram branch represent four Clades divided by clustering. Strains names marked with blue color are type strains of *P. bifermentans* those with different genome versions or from different institution collections (e.g., ATCC or DSM). Strain HD0315_2 is marked with green color.
Among them, two genes were in pHD0315_2-1 code bile hydrolysis (1) and cobalt-zinc-cadmium resistance genes (1). *Paraclostridium* strains have been observed expressing multiple antibiotic-resistance genes (e.g., chloramphenicol, tetracycline, and gentamicin) (Liang et al., 2020), metal/metalloid (e.g., Se, Hg, As, and Zn) (Wang et al., 2021; Zhang et al., 2022).
or toxic compounds (e.g., ciprofloxacin) (Fang et al., 2021) and were also deduced from the genomic coding functions in this study. Importantly, coding genes identified as *Listeria* LIPI-1-extended proteins in the HD0315_2 genome have been well-characterized for the infection cycle processes of “Internalization,” “Escape from the vacuole,” and “Reinfection” (see Supplementary Figure S3 for details). Overall, the functions encoded by the HD0315_2 genome contribute to survival in diverse environments. Meanwhile, for the multiple plasmids, the identification of functions was difficult due to the poor annotations.

**Pathogenicity and survival features**

Since various pathogenicity or virulence factor coding genes were annotated in the genome sequence, we further checked the possibility of the HD0315_2 strain being a human pathogen using PathogenFinder. The results have suggested that the HD0315_2 strain is a human pathogen with a high probability (0.77 and 0.98), besides 11 and one pathogenic protein family detected in the genome of HD0315_2 respectively (Supplementary Table S1). Interestingly, all pathogenic proteins detected in the chromosome sequence were homologs [e.g., putative malate-2H(+)/Na(+)-lactate antiporter, PTS-system-phospho carrier protein, and ribonuclease Ph] of the *Clostridium difficile*, and the pathogenic protein detected in the HD0315_2 strain was an amino acid/peptide transporter homolog of *Clostridium botulinum* (see Supplementary Table S4 for details). However, well-characterized toxins (e.g., enterotoxins and cytoxins) that can cause diarrhea and inflammation by *C. difficile* (Butler et al., 2016) were not detected in the HD0315_2 strain. The detected pathogenic proteins were related to pathogen virulence and surviving regulation. For example, ribonuclease Ph is present in many pathogen ns and has been proven to help them withstand oxidative stress or oxidative bursts of neutrophils and/or macrophages from hosts (Lawal et al., 2011). Moreover, the Hin DNA invertase coding gene (i.e., hin) was carried by five out of the six plasmids (Figure 2). The Hin DNA invertase has been found to regulate flagellar phase variations in pathogens (Merrickel and Johnson, 2001), which would help the HD0315_2 strain to evade host immune responses. Other features, not in the RAST subsystem but annotated by PGAP, included antimicrobial/metal resistance genes (e.g., *asr*ABC, *cat*, *mccF*, *macB*, *ents*, *alba*, *bcrA*, and *tetB*), transposase coding genes [e.g., *tnXo19* (Tn3 family), and *ISDku1* (IS256 family)]. (Figures 2A–D). Moreover, four *hin* genes were linked with *tnXo19*, indicating a high transfer of the *hin* gene by transposase-directed horizontal gene transfer (HGT) events, which would also help the HD0315_2 strain to evade host immune responses. Meanwhile, *mccF*, *macB*, *ents*, *alba*, and *bcrA* (coding microcin C7 self-immunity protein, macrolide export ATP-binding/permease protein, enterobactin exporter, antilisterial bacteriocin subtilosin biosynthesis protein, and bacitracin transport ATP-binding protein, respectively) might help the HD0315_2 strain to resist microcin produced from *Paraclostridium* members or microbial toxins from other bacteria. Consequently, these plasmid features would make this strain not only resistant to host responses but also to harsh environments. Hence, *P. bifermentans* HD0315_2 might live under antimicrobial or oxidative stress and might infect the host cell via a *Listeria* LIPI-1-like cycle. Thus, the chromosome or plasmid-encoded functions would help *P. bifermentans* HD0315_2 survive during the whole infection cycle by evading host immune responses or resisting medical compounds.

**Conclusion**

In summary, we reported the complete genome sequence of *P. bifermentans* HD0315_2 and analyzed its general genomic features, phylogenomic relationship, and pathogenic potential. The results revealed that multiple plasmids are present in *P. bifermentans* strains. Additionally, some species previously identified as non-*P. bifermentans* were reclassified as *P. bifermentans* by the phylogenomic analysis. The pathogenicity proteins encoded by the HD0315_2 strain genome suggested that it can infect host cells via a *Listeria* LIPI-1-like cycle. Moreover, both the chromosome and plasmids were found coding abundant antimicrobial or oxidative stress resistance functions. Additionally, transposase-directed HGT generated the distribution of multiple copies of the *hin* gene in the plasmids, which might help the strain evade host immune responses. Therefore, in the present study, we expanded the knowledge regarding the general genomic features of *P. bifermentans*, revealed some of its environmental/host adaptation mechanisms, and proposed, for the first time, its pathogenicity model at the genomic level.

**Data availability statement**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

**Ethics statement**

This study was approved by our Institutional Review Board (July 24, 2019; reference No. K-2019-146-01) and was compliant with all relevant ethical regulations. The patients/participants provided their written informed consent to participate in
this study. Written consent form was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

HZ performed the bacteria isolation and taxon identification. YP and XC performed the WGS-based analysis. HZ, JW, and XC drafted the manuscript. YP, YL, and WH reviewed and revised the manuscript. HH and YN designed the whole study. All authors made substantial and direct contributions to the work, and read and approved the final version of the manuscript.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.928153/full#supplementary-material

References

Barrett, I. F., Saragadam, S. D., DiMaria, C. N., and Delgado-Daza, A. (2020). Infection of a prothetic knee joint with Clostridium bifermentans. Orth Med Care Reports. 2020, omaa057. doi: 10.1093/omcr/omaa057

Bertelli, C., Laird, M. R., Williams, K. P., Group, S. F. U. R. C., Lau, B. Y., Hoad, G., et al. (2017). IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. Nucleic Acids Res. 45, W30–W33. doi: 10.1093/nar/gkx343

Biowan, R., Raja, S., Siofka, S., Gopalakrishnan, M., and Szeema, S. K. (2018). Brain abscess and cervical lymphadenitis due to Paraclostridium: a report of two cases. Anaerobe. 51, 8–11. doi: 10.1016/j.anero.2018.03.006

Butler, M., Olson, A., Drekonja, D., Shaukat, A., Schwehr, N., Shipee, N., et al. (2016). Early diagnosis, prevention, and treatment of Clostridium difficile. Update [Internet]. Rockville, MD: Agency for Healthcare Research and Quality.

Choksket, S., Jain, A., Sharma, D., Grover, V., and Korpole, S. (2020). Paraclostridium dentum, a novel species with pathogenic features isolated from human dental plaque sample. Anaerobe. 65, 102239. doi: 10.1016/j.anero.2020.102239

Edagie, S., Lagace-Wien, P., Embil, J., Karlovsky, J., and Walkty, A. (2015). Emphysema caused by Paraclostridium bifermentans: a case report. Can. J. Infect. Dis. Med. Microbiol. 26, 105–109. doi: 10.1155/2015/481076

Fang, H., Oberoi, A. S., He, Z., Khanal, S. K., and Lu, H. (2021). Ciprofloxacin-degrading Paraclostridium sp. isolated from sulfate-reducing bacterial-enriched sludge: optimization and mechanism. Water Res. 191, 116808. doi: 10.1016/j.watres.2021.116808

Hale, A., Kirby, J. E., and Albrecht, M. (2016). Fatal spontaneous Clostridium bifermentans necrotizing endometritis: a case report and literature review of the pathogen. Open Forum Infect Dis. 3, ofw095. doi: 10.1093/ofid/ofw095

Huerta-Cepas, J., Kröll, K., Coelho, L., Gilbert, M., Taylor, J., Von Mering, C., et al. (2017). Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol. Biol. Evol. 34, 2115–2122. doi: 10.1093/molbev/msx148

Jain, C., Rodriguez-R, L. M., Phillippy, A. M., Konstantinidis, K. T., and Aluru, S. (2018). High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat. Commun. 9, 1–8. doi: 10.1038/s41467-017-07641-9

Jyothsna, T. S., Tushar, L., Sasikala, C., and Ramana, C. V. (2016). Paraclostridium benzoyleticum gen. nov., sp. nov., isolated from marine sediment and reclassification of Clostridiomycetaceae as Paraclostridium bifermentans comb. nov. Proposal of a new genus Paeniclostridium gen. nov. to accommodate Clostridium aerobactin and Clostridium ghonii. Int. J. Syst. Evol. Microbiol. 66, 1268–1274. doi: 10.1099/ijsem.0.008874

Kolander, S. A., Cosgrove, E. M., and Molavi, A. (1899). Clostridial endocarditis: report of a case caused by Clostridium bifermentans and review of the literature. Arch. Intern. Med. 149, 455–456. doi: 10.1001/archinternmed.1989.0390020143032

Kutsuna, R., Miyoshi-Akiyama, T., Mori, K., Hayashi, M., Tomida, J., Morita, Y., et al. (2019). Description of Paraclostridium bifermentans subsp. muricitolitidis subsp. nov., emended description of Paraclostridium bifermentans (Sasi Jyothsna et al. 2016), and creation of Paraclostridium bifermentans subsp. bifermentans subsp. nov. Microb. Immunol. 63, 1–10. doi: 10.1111/miu.12416

Kutsuna, R., Tomida, J., Morita, Y., and Kawamura, Y. (2018). Paraclostridium bifermentans exacerbates pathosis in a mouse model of ulcerative colitis. PloS ONE. 13, e0197668. doi: 10.1371/journal.pone.0197668

Lagier, J.-C., Dubourg, G., Million, M., Cadoret, F., Bilen, M., et al. (2018). Culturing the human microbiota and culturomics. Nat. Rev. Microbiol. 16, 540–550. doi: 10.1038/s41579-018-0041-0

Lewal, A., Jejelowo, O., Chopra, A. K., and Rosenzweig, J. A. (2011). Ribonucleases and bacterial virulence. Microb. Biotechnol. 4, 558–571. doi: 10.1111/j.1751-7915.2010.00411.x

Lawal, A., Jejelowo, O., Chopra, A. K., and Rosenzweig, J. A. (2011). Ribonucleases and bacterial virulence. Microb. Biotechnol. 4, 558–571. doi: 10.1111/j.1751-7915.2010.00411.x

Liang, Z., Zhang, Y., He, T., Yu, Y., Liao, W., Li, G., et al. (2020). The formation mechanism of antibiotic-resistance genes associated with bacterial communities during biological decomposition of household garbage. J. Hazard. Mater. 398, 122973. doi: 10.1016/j.jhazmat.2020.122973

Merrick, S. K., and Johnson, R. C. (2001). “In Situ-Gen-Mediated Site-Specific DNA Inversion,” in Encyclopedia of Genetics, eds S. Brenner, J. H. Miller (Academic Press: New York), 938–942. doi: 10.1101/rwgn.2001.0566

Parks, D. H., Chuvochina, M., Waite, D. W., Rinke, C., Skarszewski, A., Chaumeil, P., et al. (2018). A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat. Biotechnol. 36, 996–1004. doi: 10.1038/nbt.4229

Rai, A., Ramana, C. V., Uppada, J., and Sasikala, C. (2015). Paraclostridium. Bergey’s Manual of Systematics of Archaea and Bacteria. 2015, 1–12. doi: 10.1002/9781118960608.gbn01635

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10.3389/fmicb.2022.928153
Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E. P., Zaslavsky, L., et al. (2016). NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 44, 6614–6624. doi: 10.1093/nar/gkw569

Wang, Y., Wei, D., Li, P., Jiang, Z., Liu, H., Qing, C., et al. (2021). Diversity and arsenic-metabolizing gene clusters of indigenous arsenate-reducing bacteria in high arsenic groundwater of the Hetao Plain, Inner Mongolia. Ecotoxicology 30, 1680–1688. doi: 10.1007/s10646-020-02305-1

Weinberg, M., and Séguin, P. (1918). La gangrene Gazeuse, Monographies de l’Institut Pasteur. Paris, Masson and Cie.

Wick, R. R., Judd, L. M., Gorrie, C. L., and Holt, K. E. (2017). Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13, e1005595. doi: 10.1371/journal.pcbi.1005595

Zhang, X., Wang, L., Zeng, T., Liu, Y., Wang, G., Liu, J., et al. (2022). The removal of selenite and cadmium by immobilized biospheres: Efficiency, mechanisms and bacterial community. Environ. Res. 211, 113025. doi: 10.1016/j.envres.2022.113025

Zuo, G., and Hao, B. (2015). CVTree3 web server for whole-genome-based and alignment-free prokaryotic phylogeny and taxonomy. Genom. Proteom. Bioinform. 13, 321–331. doi: 10.1016/j.gpb.2015.08.004