Bulletin of the National Research Centre

RESEARCH

Open Access

# Genetic composition, virulence factors, and antimicrobial resistance profiles of *Bacillus cereus* and *Bacillus subtilis* isolates from food vendors in Ondo State, Nigeria: implications for food safety

Aderonke Mary Fayanju[1*], Bamidele Juliet Akinyele[1], Babayemi Olawale Oladejo[1] and Ayodeji Charles Osunla[2]

## Abstract

**Background** This study investigated *Bacillus cereus* and *Bacillus subtilis* from food vendors in Ondo State, Nigeria.

**Methods** A comprehensive whole-genome sequencing (WGS) analysis of *Bacillus* genomes, including genome assembly, plasmid prediction, species identification, antimicrobial resistance (AMR) gene identification, virulence gene identification, and multilocus sequencing typing, was conducted.

**Results** The genome assembly revealed a *B. cereus* genome with 87 contigs, a length of 5,798,917 base pairs, and a GC content of 34.79%, whereas *B. subtilis* had a genome length of 4,238,143 bp and was composed of 253 contigs with a contig L50 of 24, a contig N50 of 55,053, and a GC content of 43.14904%. Plasmid prediction revealed the absence of prominent plasmids in the assembled *B. cereus* genome, whereas the repUS12 plasmid was recognized with an identity of less than 95.63% for the *B. subtilis* genome. Species identification via the average nucleotide identity (ANI) calculation confirmed that *Bacillus cereus* had a 98.97% ANI value, whereas a 98.39% ANI value was confirmed for *B. subtilis* WAUSV36. AMR genes were identified, with virulence genes such as the alo, cytK, and hbl genes also detected in *B. cereus*, whereas clpX, codY, purA, and purB genes were detected in *B. subtilis.* Multiple-locus sequence typing (MLST) revealed that *B. cereus* belongs to sequence type 73 with 100% identity, identifying housekeeping gene alleles, including glp_13, gmk_8, and ilv_9, whereas *B. subtilis* belongs to sequence type 130, with the ilvD gene showing a perfect match and the highest allele length of 471 for the housekeeping genes identified.

**Conclusions** This detailed WGS analysis provides valuable insights into the genetic composition, potential virulence factors, and resistance profiles of *B. cereus* and *B. subtilis,* enhancing the understanding of their pathogenicity and epidemiology. The genomic analysis of *B. cereus* and *B. subtilis* revealed potential genomic applications in the context of food safety.

**Keywords** Whole-genome sequencing (WGS), Antimicrobial resistance (AMR), Virulence genes, Multilocus sequencing typing (MLST), Food safety

*Correspondence:
Aderonke Mary Fayanju
Fayanjuaderonke@gmail.com
Full list of author information is available at the end of the article

## Background

*Bacillus cereus*, a common spore-forming facultatively anaerobic gram-positive bacterium, has been isolated from patients suffering from various diseases and foodborne illnesses (Dietrich et al. 2021). It is widely distributed in nature, behaves as an opportunistic pathogen and is often linked to two distinct forms of human foodborne illness: symptoms such as diarrhea and abdominal discomfort and nausea and vomiting. *Bacillus subtilis* is a gram-positive bacterium with a rod-shaped structure. While it can produce spores that are resistant to heat, it is not known to cause infections in humans. Among the Bacillus species, *B. subtilis* and *B. velezensis* have garnered significant interest in the food industry because of their recognized safety and ability to compete with other microorganisms in natural settings, potentially influencing microbiota selection.

While *B. cereus* may not cause severe issues in healthy individuals, it can pose a significant risk to people with certain underlying conditions, including those who are immunocompromised or in the process of postsurgery recovery (Nguyen and Tallent 2019). Notably, some proteins previously believed to be unique to *B. cereus* have been discovered in *B. thuringiensis* isolates. In addition, the cross-talk among these species and the genetics of their association with *Bacillus thuringiensis* have also interested researchers working on genes that govern their differing roles in nature and pathogenicity (Ehling-Schulz et al. 2019).

*Bacillus cereus* and *Bacillus subtilis* are common contaminants in food, posing risks to human health because of their potential to produce toxins and exhibit antimicrobial resistance. Understanding the genetic makeup, virulence factors, and antimicrobial resistance profiles of these isolates is crucial for ensuring food safety and preventing foodborne illnesses, as demonstrated by Adamski et al. (2023). A recent study by Hurtado-Bautista et al. (2021) emphasized the importance of genetic analysis in elucidating bacterial evolution and adaptation to diverse environments. The formation of distinct clades by the *B. cereus* and *B. subtilis* lineages indicates the genetic diversity within these bacterial species. The genetic diversity, virulence factors, and antimicrobial resistance profiles vary among different strains of *Bacillus* species.

Furthermore, the presence of virulence factors in Bacillus species highlights the importance of genetic characterization. A study by Bianco et al. (2021) pinpointed specific virulence genes in *B. cereus* and *B. subtilis* that contribute to their pathogenicity. Moreover, the *cytK* and *hbl* genes were detected in *B. cereus,* whereas the *clpX, codY, purA,* and *purB* genes were identified in *B. subtilis,* highlighting the diverse virulence mechanisms employed by these bacteria. In addition to virulence factors, the antimicrobial resistance profiles of Bacillus strains play a critical role in determining their pathogenicity and potential impact on food safety. Research by Tagne et al. (2023) evaluated the antibiotic susceptibility of Bacillus strains isolated from environmental sources, shedding light on the potential influence of seasonal variations on antimicrobial resistance patterns. This underscores the dynamic nature of antimicrobial resistance in Bacillus species and the necessity for ongoing surveillance.

Moreover, the genetic composition of Bacillus strains, including plasmid content and species identification, is vital for understanding their epidemiology and transmission dynamics. The utilization of whole-genome sequencing (WGS) for characterizing *B. cereus* and *B. subtilis* isolates offers a comprehensive view of their genetic diversity and evolutionary relationships. Species identification through the average nucleotide identity (ANI) calculation and multilocus sequence typing (MLST) techniques further enhances the classification of these bacterial isolates, as conducted by Bianco et al. (2021).

By elucidating the genetic underpinnings of these bacterial strains, researchers can better evaluate the risks associated with their presence in food environments and develop targeted interventions to mitigate these risks. This study aims to offer insights into the genetic makeup, virulence factors, and antimicrobial resistance profiles of *Bacillus cereus* and *Bacillus subtilis* from food vendors to enhance the understanding of their pathogenicity and implications for food safety.

## Methods

### Isolation and identification of *Bacillus* species collected from food vendors

The process typically begins with aseptic transfer of 1 g of watermelon, pineapple, cooked cheese, pies, beef, turkey, chicken, and Naira swabs to tenfold serial dilutions of the samples in sterile saline solution as described by the Health Protection Agency (2009). The initial step in the standardized laboratory technique involves the preparation of selective media suitable for the isolation of *Bacillus* species in HiCrome *Bacillus* agar, as demonstrated by (Alippi and Abrahamovich 2019), and has been validated for the presumptive identification of *Bacillus* species. Following the preparation of the selective media, selected diluents of the serially diluted food samples were inoculated onto agar plates following the even distribution of the sample material on the agar surface. The use of Tryptic Soy agar (TSA) and nutrient agar (NA) media, as indicated by (Kim et al. 2022), has been effective in detecting Bacillus species through culture-dependent methods. Hence, we will incorporate these media into

our technique to increase the recovery of *Bacillus* colonies from diverse sample sources.

### Genome assembly and annotation of the *Bacillus* cereus sequence

Unassembled raw reads (R1 and R2) of the whole genome were uploaded in FASTQ format to the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) server to perform assembly of the genome (Olsen et al. 2023). The assembled genome was annotated via the Rapid Annotations using Subsystems Technology (RAST) toolkit on the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) server, which uses a FASTA-format contig file.

### Prediction of plasmids in *Bacillus* cereus and *Bacillus* subtilis

The plasmid prediction was performed by uploading the assembled genome contig file derived from BV-BRC to the Plasmid Finder 2.1 server. The server enables the detection of plasmids in complete and partially sequenced bacterial isolates by recognizing and characterizing plasmid replicons in whole-genome sequencing (WGS) data (Caratolli and Hasman 2020).

### Identification of *Bacillus* species (ANI calculator)

The average nucleotide identity (ANI) calculator was employed to ensure precise species identification. The ANI calculates ANI values, and the ANI calculates the mean nucleotide identity by considering both the top matches (one-way ANI) and mutually best matches (two-way ANI) between two genomic datasets of genomes of the same species that exhibit a similarity threshold of over 95%. (Yoon et al. 2017).

### Determination of antimicrobial resistance genes in *Bacillus* cereus and *Bacillus* subtilis

The antimicrobial resistance genes were identified and extracted from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) server and ResFinder 4.1, which is a database for detecting antimicrobial resistance genes within an isolated whole-genome dataset (Florensa et al. 2022).

### Determination of virulence genes in *Bacillus* cereus and *Bacillus* subtilis

The virulence genes were identified and extracted from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) server.

### Determination of multilocus sequence typing (MLST) in *Bacillus* cereus and *Bacillus* subtilis

Multilocus sequence typing of the assembled genome was performed via the MLST 2.0 tool. This tool distinguishes the species and strains of the bacteria and the sequence type.

## Results

### Presumptive and whole-genome sequencing profile of bacterial strains from food samples and naira notes

Presumptively identified *Bacillus* species were divulged after their genome sequencing analysis as *Bacillus cereus* AH676, and *B. subtilis* WAUSV36, respectively, as illustrated in Table 1.

**Table 1** The presumptive and sequencing profile of bacterial isolates from food samples and naira notes

| S/N | Presumptive identification | 16S rRNA sequence identification |
|---|---|---|
| 1 | *Bacillus* species | *Bacillus cereus* AH676 |
| 2 | *Bacillus* species | *Bacillus subtilis* ATCC 49188 |

**Table 2** Genome statistics for *Bacillus cereus*

| | ***Bacillus cereus*** | ***Bacillus cereus*** |
|---|---|---|
| *Genome statistics* | | |
| Contigs | 87 | 253 |
| Genome Length | 5,798,917 | 4,238,143 |
| GC Content | 34.79077 | 43.14904 |
| Contig L50 | 6 | 24 |
| Contig N50 | 437,167 | 55,053 |

**Table 3** Annotation statistics of *Bacillus cereus* and *Bacillus subtilis*

| | ***Bacillus cereus*** | ***Bacillus subtilis*** |
|---|---|---|
| *Annotation statistics* | | |
| tRNA | 71 | 36 |
| rRNA | 5 | 5 |
| CDS | 5950 | 4771 |
| CDS Ratio | 1.0260537 | 1.1257288 |
| Hypothetical CDS | 1501 | 894 |

### Genome assembly and annotation in *Bacillus cereus* and *Bacillus subtilis*

The genome identified on the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) server was *Bacillus cereus,* with a genome ID of 1396.4273. The complete genome has the following genome and annotation statistics, as shown in Tables 2 and 3.

### Identification of the average nucleotide identity values of *Bacillus* cereus and *Bacillus* subtilis

Compared with the reference genome, the *Bacillus cereus* whole genome has a 98.97% ANI value; the *Bacillus cereus* AH676 chromosome has an accession number of CM000738, as shown in Fig. 1. Compared with that of *Bacillus subtilis* WAUSV36, the genome of *Bacillus subtilis* has a 98.39% ANI value, indicating a high degree of genetic similarity, as illustrated in Fig. 2.

### Prediction of plasmids in *Bacillus* cereus and *Bacillus* subtilis strains

As the PlasmidFinder 2.0 server predicted, the assembled genome containing Zero (0) prominent plasmids in *Bacillus cereus* and the *repUS12* plasmid was recognized by the plasmid finder in *Bacillus subtilis*. The identity is < 100%, specifically 95.63%, as displayed in Fig. 3.

### Determination of antimicrobial resistance genes in *Bacillus* cereus and *Bacillus* subtilis strains

The antimicrobial resistance genes identified in *Bacillus cereus* on the BV-BRC server and the classes in which the resistant drugs exist are recorded in Table 4. Beta-lactam (*blaZ*), fosfomycin (*fosB1* and *MurA*), aminoglycoside (*gidB, S12p*), and fluoroquinolones (*gyrB* and *gyrA*) are some of the antibiotic resistance genes detected in the *Bacillus cereus* strain. *Bacillus subtilis* was resistant to fosfomycin (*MurA, dxr*), fluoroquinolones (*gyrA*), and macrolides *(RlmA(II),* as illustrated in Table 5. Different sets of virulence genes were also found when the result was removed from the BV-BRC.

### Detection of virulence genes in *Bacillus* cereus and *Bacillus* subtilis strains

The number of virulence genes identified in the whole genome of *Bacillus cereus* is presented in Table 6. They include *Alo, asbA* and *clpX*. In contrast, *B. subtilis* WAUSV36 shows a more limited virulence gene profile with only six genes (*bslA/yuaB, clpX, codY, purA, purB, recA*) were also found to be encoded as virulence factors in *Bacillus subtilis* when the result was out on BV-BRC, as displayed in Table 7.

### Detection of multilocus sequence typing (MLST) in B. *cereus* and B. subtilis

The MLST results revealed that the *Bacillus cereus* strain belongs to sequence type 73 with an identity of 100%. The housekeeping gene alleles were identified as glp_13, gmk_8, ilv_9, pta_14, pur_9, pyc_12 and tpi_31, as shown in Table 8. After following the default procedure on the website, the results revealed that the *Bacillus subtilis strain* belongs to sequence type 130. Table 9 shows that the *ilvD* gene had a perfect match, with a percentage identity of 100%. Additionally, alleles for housekeeping genes were successfully identified.

## Discussion

Genetic makeup, virulence factors, and antimicrobial resistance profiles enhance the understanding of *Bacillus cereus* and *Bacillus subtilis* pathogenicity and implications for food safety. The genome assembly of *Bacillus cereus* revealed a genome with 87 contigs spanning 5,798,917 base pairs and a GC content of 34.79%, which is consistent with the findings of Bianco et al. (2021), who explored the characterization of *Bacillus cereus* group isolates from human bacteremia through whole-genome sequencing (WGS). This research emphasizes the effectiveness of WGS in rapidly characterizing *B. cereus* group strains, providing comprehensive insights into their genetic epidemiology. By utilizing WGS, this study revealed crucial information about the presence of virulence factors and antimicrobial genes within these isolates. The findings shed light on the potential risks associated with these strains, highlighting the importance of understanding and addressing this often underestimated threat in the context of food safety. In contrast, *Bacillus subtilis* WAUSV36 has a genome length of 4,238,143 base pairs, consisting of 253 contigs with a contig L50 of 24 and a contig N50 of 55,053. It exhibits a higher GC content of 43.15%. This aligns with findings from Ehling-Schulz et al. (2019), who highlighted the need for refining the taxonomic classification and risk assessment of *B. cereus* AH676 through advancements in computational and microbiological methods. Species identification via the average nucleotide identity (ANI) calculation confirmed that *Bacillus cereus* AH676 had a 98.97% ANI value and 98.39% ANI value for *Bacillus subtilis* WAUSV36, similar to the findings of Bianco et al. (2021) on the effectiveness of WGS in rapidly characterizing *B. cereus* group strains, providing comprehensive insights into their genetic epidemiology.

Plasmid prediction in the assembled *B. cereus* genome revealed the absence of prominent plasmids, whereas the *repUS12* plasmid was identified with less than 95.63% identity in the *B. subtilis* genome, as similarly reported

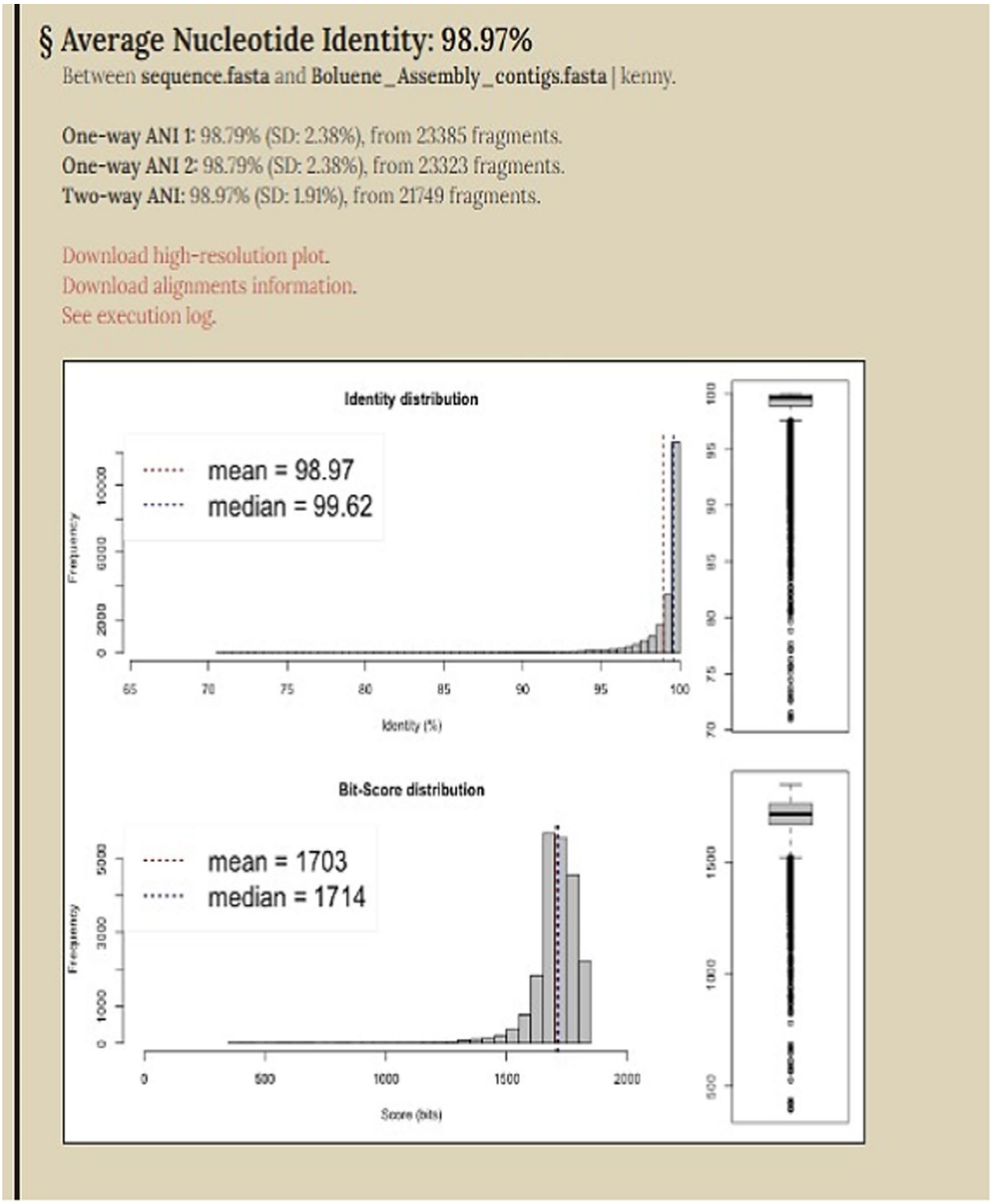



**Fig. 1** Average nucleotide identity of *Bacillus cereus* AH676

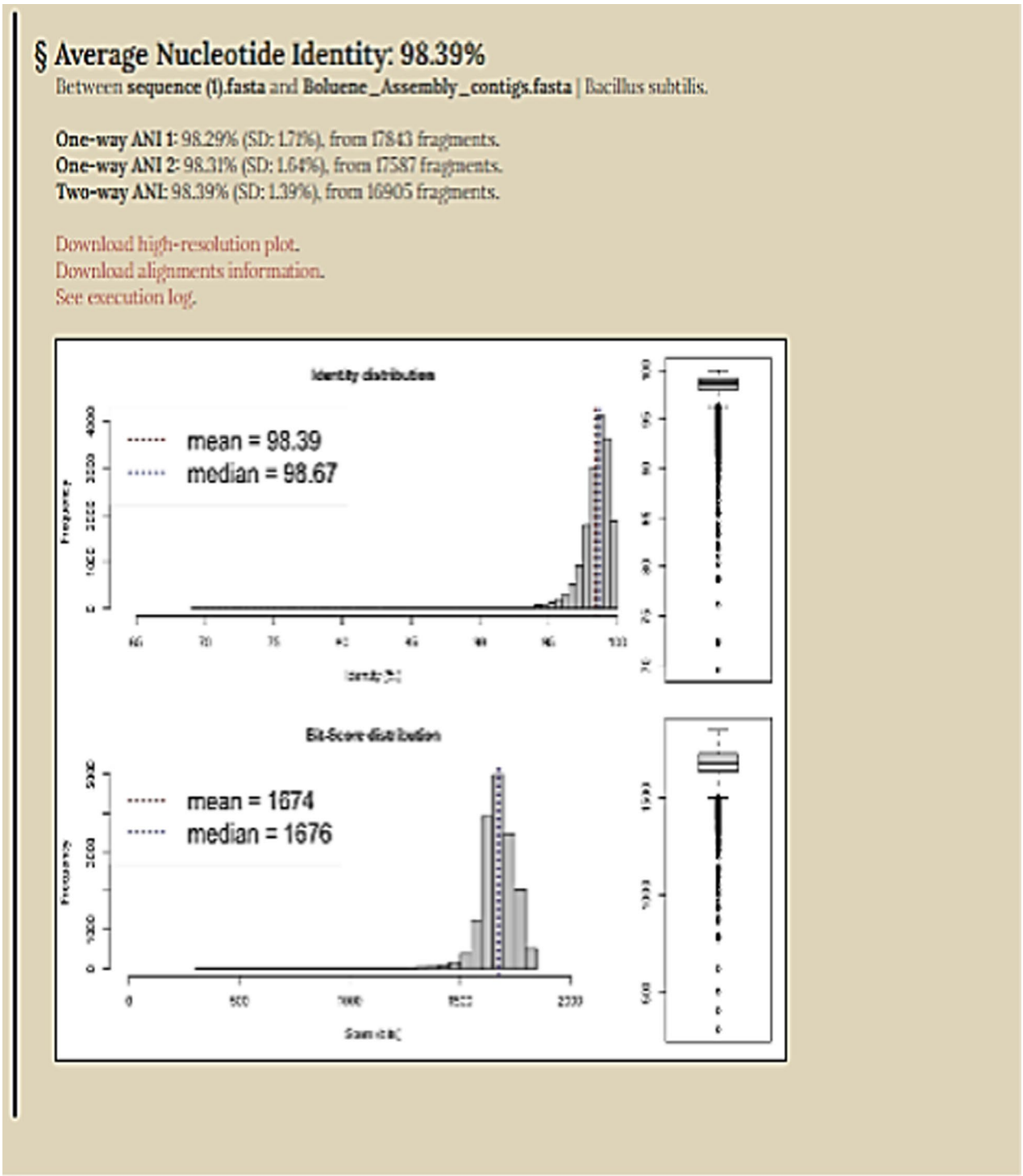
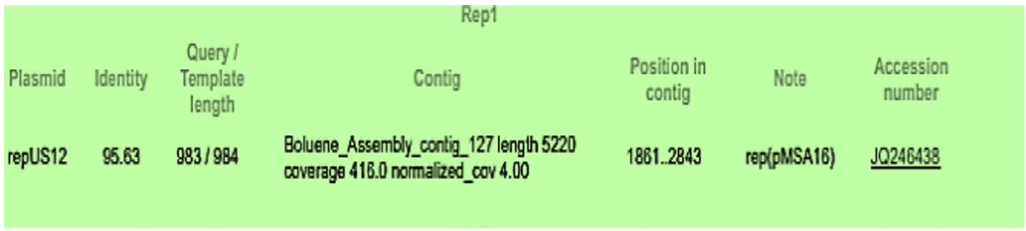



**Fig. 2** Average *nucleotide identity of B. subtilis* WAUSV36

by Bianco et al. (2021). The presence of virulence factors in *Bacillus* species underscores the importance of genetic characterization in understanding their pathogenicity. Hurtado-Bautista et al. (2021) conducted a study focusing on the intriguing realm of phenotypic plasticity and the evolution of thermal tolerance in Bacillus species originating from diverse environments as opposed to food sources in this study, specifically temperate and



Rep1

| Plasmid | Identity | Query / Template length | Contig | Position in contig | Note | Accession number |
|---|---|---|---|---|---|---|
| repUS12 | 95.63 | 983 / 984 | Boluene_Assembly_contig_127 length 5220 coverage 416.0 normalized_cov 4.00 | 1861..2843 | rep(pMSA16) | JQ246438 |

**Fig. 3** Identification of the Plasmid Type in *Bacillus subtilis* WAUSV36

**Table 4** Antimicrobial drug classes and resistance genes detected in *Bacillus cereus* AH676

| Drug class | Resistant genes |
|---|---|
| Beta-lactam | *blaZ* |
| Fosfomycin | *fosB1, MurA* |
| Aminoglycosides | *gidB, S12p* |
| Diaminopyrimidines | *folA, Dfr* |
| Glycopeptide | *VanF/M-type* |
| Myxopyronins Corallopyronins | *rpoC* |
| Cycloserine | *Ddl, Alr* |
| Mupirocin | *Iso-tRNA* |
| Fluoroquinolones | *gyrB, gyrA* |
| Peptide antibiotics | *PgsA, BcrA, GdpD, LiaR, LiaF, BcrC, LiaS,* |

**Table 5** Predicted antimicrobial resistance genes detected in *Bacillus subtilis* WAUSV36

| Antibiotics phenotype | Resistant genes |
|---|---|
| Peptide antibiotics | *LiaF, LiaR, PgsA, GdpD, BcrC* |
| Aminoglycosides | *ANT(6)-I* |
| Cycloserine | *Alr* |
| Tetracyclines, Aminoglycosides, Phenicol | *YkkCD* |
| Elfamycins | *EF-Tu* |
| Fosfomycin | *MurA, dxr* |
| Fluoroquinolones Quinolones Quinolines | *gyrA* |
| Macrolides, Lincosamides | *RlmA(II)* |
| Mupirocin | *Iso-tRNA* |
| Myxopyronins Corallopyronins, Peptide antibiotics | *rpoC* |
| Cycloserine | *Ddl* |

hot springs. This study focused on two bacterial lineages, *Bacillus cereus* sensu lato and *Bacillus subtilis* sensu lato, which have evolved in distinct habitats. By examining the growth and reaction norms to temperature of

**Table 6** Virulence genes and their number of occurrences detected in *Bacillus cereus* AH676

| Virulence genes | Number of occurrence |
|---|---|
| *Alo* | 1 |
| *asbA* | 1 |
| *BAS3109* | 1 |
| *clpX* | 1 |
| *codY* | 1 |
| *cytK* | 1 |
| *GBAA4766* | 1 |
| *hblA* | 1 |
| *inhA* | 1 |
| *nheA* | 1 |
| *nheB* | 1 |
| *nheC* | 1 |
| *Nos* | 1 |
| *phnX* | 1 |
| *sigB* | 1 |
| *sodA1* | 1 |
| *sodA2* | 1 |
| *sodC* | 1 |
| *inhA* | 1 |
| *hblC* | 1 |
| *hblD* | 1 |

**Table 7** Sets of virulence genes detected in *Bacillus subtilis* WAUSV36

| Row/label | Number of genes |
|---|---|
| *bslA/yuaB* | 1 |
| *clpX* | 1 |
| *codY* | 1 |
| *purA* | 1 |
| *purB* | 1 |
| *recA* | 1 |

**Table 8** Multilocus sequence typing of *Bacillus cereus*

| Locus | Identity | Coverage | Alignment Length | Allele length | Gaps | Allele |
|---|---|---|---|---|---|---|
| glp | 100 | 100 | 372 | 372 | 0 | glp_13 |
| gmk | 100 | 100 | 504 | 504 | 0 | gmk_8 |
| ilv | 100 | 100 | 393 | 393 | 0 | ilv_9 |
| pta | 100 | 100 | 414 | 414 | 0 | pta_14 |
| pur | 100 | 100 | 348 | 348 | 0 | pur_9 |
| pyc | 100 | 100 | 363 | 363 | 0 | pyc_12 |
| tpi | 100 | 100 | 435 | 435 | 0 | tpi_31 |

**Table 9** Multilocus sequence typing of *Bacillus subtilis* AH676

| Locus | Identity | Coverage | Alignment length | Allele length | Gaps | Allele |
|---|---|---|---|---|---|---|
| glpF | 100 | 100 | 384 | 384 | 0 | glpF_1 |
| ilvD | 100 | 100 | 471 | 471 | 0 | ilvD_1 |
| pta | 100 | 100 | 414 | 414 | 0 | pta_35 |
| purH | 100 | 100 | 399 | 399 | 0 | purH_85 |
| pycA | 100 | 100 | 399 | 399 | 0 | pycA_56 |
| rpoD | 100 | 100 | 384 | 384 | 0 | rpoD_3 |
| tpiA | 100 | 100 | 420 | 420 | 0 | tpiA_4 |

these bacterial strains, research has shed light on how these bacteria adapt to varying thermal conditions. The significant implications of thermal conditions on the risk posed by *Bacillus cereus* and *Bacillus subtilis* for food safety highlight the need for comprehensive strategies to mitigate the risks associated with these bacteria. Understanding the adaptive mechanisms, survival strategies, and biofilm-forming abilities of *B. cereus* and *B. subtilis* in response to temperature variations in food processing is crucial for implementing effective control measures and ensuring the safety of food products for consumers (Hurtado-Bautista et al. 2021). A study by Bianco et al. (2021) identified the alo, cytK, and hbl genes in *B. cereus*, whereas the *clpX, codY, purA,* and *purB* genes were detected in *B. subtilis*, highlighting the diverse virulence mechanisms employed by these pathogens. Antimicrobial resistance (AMR) genes were detected in both *Bacillus* species subjected to whole-genome sequencing (WGS) analysis in this study, covering various drug classes, such as beta-lactams, aminoglycosides, and fluoroquinolones, as similarly reported by Bianco et al. (2021). Notably, virulence genes such as the *alo, cytK,* and *hbl* genes were detected in *B. cereus,* whereas *clpX, codY, purA,* and *purB* were detected in *B. subtilis,* as corroborated by Bianco et al. (2021). This finding highlights the importance of genetic analysis in revealing the virulence factors of Bacillus species and their implications for pathogenicity. Additionally, a study by Qu et al. (2021) revealed the distribution of virulence genes in *Bacillus cereus* strains isolated from lettuce farms in China, with genes such as *nheA, nheB, nheC, hblA, hblC, hblD, entFM,* and *cytK* being prevalent among the strains. This highlights the pathogenic potential of *Bacillus cereus* strains in foodborne illnesses.

Moreover, in a study by Tagne et al. (2023), the antimicrobial resistance profiles of *Bacillus* strains isolated from environmental sources were evaluated, shedding light on the potential impact of seasonal variations on antimicrobial resistance patterns. This research emphasizes the dynamic nature of antimicrobial resistance in Bacillus species and underscores the need for continuous surveillance to monitor and address resistance mechanisms. By linking genetic characterization with antimicrobial resistance profiles, a study by Sornchuer et al. (2022) also provided valuable insights into the pathogenic potential of *Bacillus* strains and their implications for food safety and public health.

Multilocus sequence typing (MLST) further characterized *B. cereus* AH676 as belonging to sequence type 73 with 100% identity, identifying housekeeping gene alleles, including glp_13, gmk_8, and ilv_9, whereas *Bacillus subtilis* WAUSV36 was classified as sequence type 130, with the ilvD gene showing a perfect match and the highest allele length of 471 for housekeeping genes identified as similarly reported by Bianco et al. (2021). A study by Bianco et al. (2021) emphasized the importance of

whole-genome sequencing and MLST in characterizing *Bacillus cereus* isolates, highlighting the genetic diversity within the species.

In a study by Yu et al. (2020), the prevalence and characterization of *Bacillus cereus* in ready-to-eat foods in China revealed antimicrobial resistance patterns, with isolates showing resistance to β-lactam antibiotics and rifamycin. These findings underscore the potential public health risks associated with the presence of antimicrobial-resistant *Bacillus cereus* strains in food products. Furthermore, Wang et al. (2022) provided insights into *Bacillus cereus* associated with infant foods in Beijing, identifying various virulence gene carriage patterns in *B. cereus* strains, with genes such as *nhe* and *entFM* being highly prevalent. This study highlights the potential risks posed by *Bacillus cereus* in food products targeted at vulnerable populations such as infants. Additionally, Hashhash (2023) highlighted the risk assessment of *Bacillus cereus* in cooked meat products, emphasizing the importance of accurate detection methods such as VITEK®2 and PCR for food safety.

Moreover, Carroll (2020) delved into the evolutionary history of Group III *Bacillus cereus* sensu lato, elucidating the transition between emetic and diarrheal foodborne pathogens, particularly focusing on cereulide synthetase acquisition and loss events within the species. This research provides valuable insights into the pathogenic mechanisms of *Bacillus cereus* strains causing foodborne illnesses (Carroll 2020).

## Conclusions

By aligning the findings of the genetic makeup of *Bacillus cereus* and *B. subtilis* from food vendors in our study, the comprehensive genomic analysis of Bacillus species inferred a more holistic understanding of the genetic underpinnings of pathogenicity, antimicrobial resistance, and virulence in Bacillus strains. This information can contribute to the broader knowledge of Bacillus species, their genetic diversity, and the factors influencing their pathogenic potential, thereby informing strategies for the control and management of these bacteria in various settings. The genomic characteristics, antimicrobial resistance profiles, virulence gene distributions, and clinical implications of *Bacillus cereus* highlight the diverse nature of this bacterium and its importance in various settings, including food safety, healthcare-associated infections, and environmental contamination. Understanding the genetic makeup and pathogenic potential of *Bacillus cereus* is crucial for developing effective control and prevention strategies to mitigate the risks associated with this versatile bacterium in food contamination for food safety.

**Acknowledgements**
The authors appreciate the practical input of the technical staff of the Microbiology Department, Federal University for Technology, Akure, Nigeria, during the course of the research.

**Author contributions**
BJA designed the study concept. AMF conducted the study, collated and analyzed the study results and wrote the original draft. BOO provided administrative support, supervised the study, and previewed and corrected the first draft. ACO previewed and perused the final draft of the manuscript. All authors approved the final draft of the manuscript.

**Funding**
None was received.

**Availability of data and materials**
Not applicable.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
None to declare.

**Author details**
[1]Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria. [2]University of Saskatchewan Toxicology Centre, Saskatchewan, Canada.

Received: 17 July 2024 Accepted: 7 September 2024
Published online: 16 September 2024

**References**

Adamski P, Byczkowska-Rostkowska Z, Gajewska J, Zakrzewski A, Kłębukowska L (2023) Prevalence and antibiotic resistance of *Bacillus* sp. isolated from raw milk. Microorganisms. 11(4):1065. https://doi.org/10.3390/microorganisms11041065

Alippi A, Abrahamovich E (2019) Hi-crome Bacillus agar for presumptive identification of bacillus and related species isolated from honey samples. Intl J Food Microbiol 305:108245. https://doi.org/10.1016/j.ijfoodmicro.2019.108245

Aoyagi T, Oshima K, Endo S, Baba H, Kanamori H, Yoshida M et al (2020) Ba813, which harbors *Bacillus cereus and is* genetically closely related to *Bacillus anthracis*, causes nosocomial bloodstream infections: bacterial virulence factors and clinical outcomes. PLoS ONE 15(7):e0235771. https://doi.org/10.1371/journal.pone.0235771

Bianco A, Capozzi L, Monno M, Sambro L, Manzulli V et al (2021) Characterization of *Bacillus cereus* group isolates from human bacteremia by whole-genome sequencing. Front Microbiol. https://doi.org/10.3389/fmicb.2020.599524

Carattoli A, Hasman H (2020) PlasmidFinder and In Silico pMLST: identification and typing of plasmid replicons in whole-genome sequencing (WGS). In: de Cruz F (ed) Horizontal gene transfer: Methods in molecular biology, vol 2075. Humana, New York. https://doi.org/10.1007/978-1-4939-9877-7_20

Carroll L (2020) Cereulide synthetase acquisition and loss events within the evolutionary history of group III *Bacillus cereus* sensu lato facilitate the transition between emetic and diarrheal foodborne pathogens. Mbio. https://doi.org/10.1128/mbio.01263-20

Dietrich R, Jessberger N, Ehling-Schulz M, Märtlbauer E, Granum PE (2021) The food poisoning toxins of *Bacillus cereus*. Toxins 13(2):98. https://doi.org/10.3390/toxins13020098

Ehling-Schulz M, Lereclus D, Koehler T (2019) The *Bacillus cereus* group: Bacillus species with pathogenic potential. Microbiol Spect. https://doi.org/10.1128/microbiolspec.gpp3-0032-2018

Florensa AF, Kaas RS, Clausen PTLC, Aytan-Aktug D, Aarestrup FM (2022) ResFinder: an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. Microbial Genom 8(1):000748. https://doi.org/10.1099/mgen.0.000748

Hashhash A (2023) Risk assessment of *Bacillus cereus* in cooked meat products by using VITEK®2 and PCR. Benha Veter Med J. 44(1):9–12. https://doi.org/10.21608/bvmj.2023.198628.1647

Health Protection Agency (HPA) (2009) https://assets.publishing.service.gov.uk/media/5a7efde0e5274a2e8ab497a4/Guidelines_for_assessing_the_microbiological_safety_of_ready-to-eat_foods_on_the_market.pdf

Hurtado-Bautista E, Sánchez L, Islas-Robles A, Santoyo G, Olmedo-Álvarez G (2021) Phenotypic plasticity and evolution of thermal tolerance in bacteria from temperate and hot spring environments. J Life Env Sci 9:e11734. https://doi.org/10.7717/peerj.11734

Kim T, Heo S, Na H, Lee G, Kim J, Kwak M et al (2022) Bacterial community of Galchi-baechu kimchi based on culture-dependent and culture-independent investigations and selection of starter candidates. J Microbiol Biotechnol 32(3):341–347. https://doi.org/10.4014/jmb.2112.12003

Nguyen AT, Tallent SM (2019) Screening food for *Bacillus cereus* toxins via whole-genome sequencing. Food Microbiol 78:164–170

Olson RD, Assaf R, Brettin T, Conrad N, Cucinell C et al (2023) Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): a resource combining PATRIC. IRD and ViPR Nucleic Acids Res 51(D1):D678–D689. https://doi.org/10.1093/nar/gkac1003

Qu Y, Wei C, Dai X, Bai Y, Zhao X et al (2021) The possible transmission and potential enterotoxicity of *Bacillus cereus* on lettuce farms in five Chinese provinces. Front Microbiol. https://doi.org/10.3389/fmicb.2021.746632

Sornchuer P, Saninjuk K, Prathaphan P, Tiengtip R, Wattanaphansak S (2022) Antimicrobial susceptibility profile and whole-genome analysis of a strong biofilm-forming *Bacillus* sp. b87 strain isolated from food. Microorg. 10(2):252. https://doi.org/10.3390/microorganisms10020252

Tagne M, Nougang M, Tamnou E, Njoya A, Bahebeck P et al (2023) Antibiotic susceptibility of some Bacillus strains isolated from ground and rainwater in urbanized areas in Cameroon (Central Africa) and the potential impact of seasonal changes. J Adv Microbiol Res. 4(1):86–95. https://doi.org/10.22271/micro.2023.v4.i1b.72

Wang P, Zhao X, Qu T, Liang L, Ji Q et al (2022) Insight into *Bacillus cereus* associated with infant foods in Beijing. Foods 11(5):719. https://doi.org/10.3390/foods11050719

Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110(10):1281–1286. https://doi.org/10.1007/s10482-017-0844-4

Yu S, Yu P, Wang J, Li C, Guo H et al (2020) A study on prevalence and characterization of *Bacillus cereus* in ready-to-eat foods in China. Front Microbiol. https://doi.org/10.3389/fmicb.2019.03043

Zhang Y, Miao J, Zhang N, Wang X, Li Z et al (2023) Analysis of the function, diversity, and evolution of the bacillus phage genome. BMC Microbiol. https://doi.org/10.1186/s12866-023-02907-9

## Publisher's Note