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Complete genome of *Bacillus subtilis* subsp. *subtilis* KCTC 3135<sup>T</sup> and variation in cell wall genes of *B. subtilis* strains

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* Running title:

Complete genome sequence of *B. s. subtilis* KCTC 3135<sup>T</sup>
Abstract

The type strain *Bacillus subtilis* subsp. *subtilis* KCTC 3135$^T$ was deeply sequenced and annotated, replacing a previous draft genome in this study. The *tar* and *tag* genes were involved in synthesizing wall teichoic acids (WTAs), and these genes and their products were previously regarded as the distinguishing difference between *B. s. subtilis* and *B. s. spizizenii*. However, a comparative genomic analysis of *B. subtilis* spp. revealed that both *B. s. subtilis* and *B. s. spizizenii* had various types of cell walls. These *tar* and *tag* operons were mutually exclusive and the *tar* genes from *B. s. spizizenii* were very similar to the genes from non-*Bacillus* bacteria, unlike the *tag* genes from *B. s. subtilis*. The results and previous studies suggest that the *tar* genes and the *tag* genes are not inherited after subspecies speciation.

The phylogenetic tree based on whole genome sequences showed that each subspecies clearly formed a monophyletic group, while the tree based on *tar* genes showed that monophyletic groups were formed according to the cell wall type rather than the subspecies. These findings indicate that the *tar* genes and the presence of ribitol as a cell-wall constituent were not the distinguishing difference between the subspecies of *B. subtilis* and that the description of subspecies *B. s. spizizenii* should be updated.

Keywords:

*Bacillus subtilis* subsp. *subtilis* KCTC 3135$^T$

Complete genome sequence

Illumina sequencing

Cell-wall teichoic acids related genes
Introduction

Bacillus subtilis has been one of the best-known and most extensively studied Gram-positive bacteria for decades [1-3]. B. subtilis is highly ubiquitous and widely used in both industry and laboratory [4, 5]. Genetically engineered B. subtilis strains have been used to produce various biomolecules which can be used as enzymes [6], drugs [7] and raw chemicals [8]. Currently, B. subtilis has three subspecies [9, 10]. According to subspecies descriptions and previous studies, the presence of ribitol as a cell-wall constituent and the genetic structure of wall teichoic acids (WTAs) related genes are regarded as the major differences among B. s. subtilis and B. s. spizizenii [9, 11, 12]. Previous studies have shown that WTAs can be synthesized by tar (B. s. spizizenii strain W23) or tag genes (B. s. subtilis strain 168) and that these genes synthesize different repeat units [11-13]. Although most tar and tag genes are homologous, the genes have different genetic structures. The tar genes have a divergon, tarABJKL-tarDF, and produce a repeat unit with ribitol, while the tag genes have tagABC-tagDEF and produce a repeat unit with glycerol [13, 14]. In this study, a comparative genomic analysis of the complete genome sequence of B. s. subtilis KCTC 3135T and other B. subtilis genomes revealed that both B. s. subtilis and B. s. spizizenii have both genotypes of WTA-related genes, suggesting that B. s. subtilis and B. s. spizizenii cannot be distinguished by the presence of ribitol as a principal cell-wall constituent.

Materials and Methods

DNA extraction and complete genome sequencing

The strain of B. s. subtilis KCTC 3135T and B. s. spizizenii KCTC 3705T used here were
obtained from the Korean Collection for Type Cultures (KCTC) and cultured using Luria-Bertani (LB) medium (Difco) at 37˚C. The genomic DNA was extracted using a GenElute Bacterial Genomic DNA extraction kit (Sigma-Aldrich, USA). The library construction was performed by a TruSeq Nano DNA sample preparation kit and the genomic DNA was sequenced using HiSeq 2500 (Illumina, USA) and MiSeq (Illumina, USA) by a sequencing company (Chunlab, Korea).

Genome assembly and annotation

The raw sequences from HiSeq (150bp PE, 9.5Gbp) and MiSeq (300bp PE, 870 Mbp) were assembled using the SPAdes assembler 3.6.0 [15]. The ncRNA and rRNA genes were searched using Infernal 1.02 [16] based on the Rfam database 12.0 [17]. CRISPR repeats were identified by PilerCR 1.06 [18] and the CRISPR recognition tool 1.2 [19]. Gene prediction was carried out using Prodigal 2.6.2 [20] and the predicted genes were annotated using KEGG [21], Swissprot [22], EggNOG [23], SEED [24] databases and the NCBI prokaryotic genome annotation pipeline. Antibiotic resistant genes were identified using HMMER3.1b2 [25] with the core Resfams HMM database v1.2 [26]. HMMER3 was run using the hmmscan command with --tblout and --cut_ga parameters. A comparative genomic analysis was carried out using BIOCYC [27]. The presence of specific genes in the pathways was confirmed manually by blastp.

Reference genome sequences

The complete genome sequence of *B. s. subtilis* KCTC 3135T was deposited at GenBank
Other genomes of *B. subtilis* were retrieved from EzBioCloud [28]. The complete genomes of forty-one other *B. subtilis* subsp. *subtilis* strains were selected. Five complete and nine incomplete genomes of *B. subtilis* subsp. *spizizenii* strains were also selected. Three genomes of *B. subtilis* subsp. *inaquosorum* strains were chosen as reference genomes. The selection criteria of the reference genome of completeness and availability. All available complete genomes of *B. subtilis* subsp. *subtilis* strains were retrieved from EzBioCloud DB [28]. However only few complete genome sequences *B. s. spizizenii* and *B. s. inaquosorum* strains were available, incomplete genome of *B. s. spizizenii* of and *B. s. inaquosorum* strains were also retrieved. The genomes used in this study are listed in Table S1. The genome of *Staphylococcus aureus* subsp. *aureus* DSM 20231T (CP011526) was selected as an outgroup sequence.

Detection of putative horizontal gene transfer (HGT)

The putative HGT discovery processes of *B. s. subtilis* KCTC 3135T and *B. s. spizizenii* TU-B-10T were carried out using HGTector [29]. The protein sequences of each strain were used as a blastp query against GenBank nr database. The blastp results were used as input files for HGTector. The self group and close group were defined as *B. subtilis* and *Bacillus*, respectively. All other taxa were regarded as a distal group. The cutoff values of the close group and the distal group were 35.27 (KCTC 3135T)/39.78 (TU-B-10T) and 15.06 (KCTC 3135T)/15.05 (TU-B-10T), respectively.

Phylogenetic analysis
The genome sequences of 60 *B. subtilis* strains were obtained from EzBioCloud [28]. Most of the obtained genome sequences were complete genome sequences of *B. s. subtilis* and *B. s. spizizenii* while the rest were genomes of three *B. s. inaquosorum* strains. The genome of the *S. aureus* strain DSM 20231T was used as an outgroup (Table S1). The conserved genes (*glpF*, *ilvD*, *pta*, *purH*, *pycA*, *rpoD*, *tpiA*) of *B. subtilis* strains were designated as previous studies [30]. The tar/tagABDF gene sequences and conserved genes were retrieved from 60 genome sequences using the tblastn method of BLAST 2.2.30+ [31]. Among the retrieved genes, tarABD genes and/or the conserved genes were sequentially concatenated and used for a multi-locus sequence analysis (MLSA) [32]. The alignment was carried out using MUSCLE [33] and Neighbor-joining (NJ) tree was constructed using the Maximum Composite Likelihood method [34]. The Tar proteins from *B. s. spizizenii* W23 and the Tag proteins from *B. s. subtilis* 168 was used as reference queries. The accession numbers of the query proteins (Tar and Tag) and tblastn identities (similarities) of tar/tagABDF genes to the query proteins are shown in Table S2.

Among the 60 genomes, 25 unique genomes were selected to construct concise phylogenetic trees and a heatmap. The phylogenetic trees were constructed using the neighbor-joining (NJ) algorithm [35] with MEGA 7 [36]. The distance matrix was generated based on the whole genome similarity by OrthoANI [37] (Fig. 1) or on the distance based on MLSA using tarABD genes(Fig. 2A). Heatmaps (Fig. 2B and Fig. S3B) were generated based on the tblastn similarities of the tar/tagABDF target genes using R 3.4.2. The extensive trees and a heatmap are shown in Fig. S2 and S3. The distance matrix was constructed and sequence parsing was performed using an in-house script with Python 2.7.5 (on Linux) or Python 2.7.12 (on Windows).
Antibiotic susceptibility test

An antibiotic susceptibility test was carried out as previously described [38] with some modifications. The strains were inoculated into the LB medium at 37°C for one day and then spread onto LB agar plates. The plates were incubated at 37°C for three days.

Transmission electron microscopy (TEM)

Samples for TEM were prepared by a method described previously [39]. The ultrathin sections (80nm) were viewed under ZEISS LEO 912 and FEI Tecnai G2 Spirit Twin TEM at 120kV using Digital Micrograph software.

Results and discussion

General features of Bacillus subtilis subsp. subtilis KCTC 3135

The sequences were assembled into a single contig with approximately 2,485X coverage. The genome size of B. s. subtilis KCTC 3135 is 4,211,343bp with a G+C content of 43.51%. The numbers of rRNA operons, tRNAs and ncRNAs were 10, 86, and 5, respectively. The total number of genes predicted is 4,436, including 4,315 coding sequence (CDS). The genomic features of B. s. subtilis KCTC 3135 and B. s. spizizenii TU-B-10 are listed in Table 1 and Figure S1 describes the genome of B. s. subtilis KCTC 3135.

Based on EzBioCloud [28], 3775 genes (87.49% out of all 4,315 total CDS) were classified according to the COG category. Excluding 1,245 genes (32.98%) in category S (Function
unknown), the most abundant COG group is as follows; 301 genes (7.97% of COG-assigned 
genes) in Category E (Amino acid transport and metabolism); 288 genes (7.63% of COG-
assigned genes) in Category K (Transcription); 271 genes (7.18% of COG assigned-genes) in 
Category G (Carbohydrate transport and metabolism); 209 genes (5.54% of COG-assigned 
genes) in Category M (Cell wall/membrane/envelope biogenesis); and 207 genes (5.48% of 
COG-assigned genes) in Category P (Inorganic ion transport and metabolism). The overall 
COG distribution of strain KCTC 3135$^\text{T}$ was similar to the COG distributions of other related 
B. subtilis spp.. The COG distributions of these genomes are listed in Table S3. The result of 
the comparative genomic analysis showed that most of pathways were conserved in both B. s. 
subtilis KCTC 3135$^\text{T}$ and B. s. spizizenii TU-B-10$^\text{T}$. The pathways of the cell structure 
biosynthesis processes including peptidoglycan maturation ($\text{meso}$-diaminopimelate 
containing), lipoteichoic acid biosynthesis and UDP-N-acetylmuramoyl-pentapeptide 
biosynthesis ($\text{meso}$-diaminopimelate containing) were conserved in both strains. The pathway 
of the poly-(ribitol phosphate) wall teichoic acid biosynthesis was conserved only in B. s. 
spizizenii TU-B-10$^\text{T}$ while poly-(glycerol phosphate) wall teichoic acid biosynthesis 
processes was only in B. s. subtilis KCTC 3135$^\text{T}$. The biodegradation pathways including 
amines, amino acids, aromatic compounds, carbohydrate, carboxylate, fatty acids and protein 
degradation were also well conserved in both strains and did not show significant differences.

Genetic variation between B. s. subtilis KCTC 3135$^\text{T}$ and B. s. spizizenii 

The putative horizontal gene transfer events of B. s. subtilis KCTC 3135$^\text{T}$ and B. s. spizizenii 

TU-B-10$^\text{T}$ are shown in Table S4 and Table S5. There were 395 (9.2%) and 453 (10.5%) 
putative HGT events, respectively. The putative horizontal gene transfer percentages were
higher than those of other prokaryotic genomes [40]. The majority of putative HGT events were classified into the transcription (K) and carbohydrate transport and metabolism (G) categories (Table S6). Most genes classified into category G were phosphotransferase system (PTS) transporters of hexose and major facilitator superfamily (MFS) transporters. Previous studies showed that these genes in various bacteria can be acquired by HGT [41, 42].

According to the HGTector results, both tarABDF and tagABDF were not regarded as putative HGTs. However, both genes were mutually exclusive (Table S2). Previous study suggested ‘pseudoallelism’ of these genes in B. subtilis strains [43]. The interstrain hybrid of 168/W23 showed that these genes can be substituted between B. s. subtilis and B. s. spizizenii, indicating that the presence of the ribitol as a cell-wall constituent cannot be solid phenotype of B. s. spizizenii. The presence of poly-ribitol teichoic acid production genes (tarIJKL) in B. s. subtilis genomes also showed that the presence of ribitol as a cell-wall constituent were not a distinguishing characteristics between B. s. subtilis and B. s. spizizenii (Table S8). Both genomes of B. s. subtilis and B. s. spizizenii strains had one of the operons. Only exception was some B. s. subtilis strain. These strains did not show high similarity (approximately 60%) to either tarABDF or tagABDF genes (Table S8). Among these strains, strains VV2 had tarIJKL genes. These indicated that tag operon can be substituted with tar operon or vice versa in the genome of B. subtilis strains regardless of subspecies, showing the presence of ribitol is not a distinguishing characteristics of specific subspecies.

Phylogenetic analysis of the B. subtilis strains

Each B. subtilis subspecies was clearly distinguished and formed separate groups in OrthoANI trees and MLSA trees with conservative genes (Fig. 1, Fig. S2 and FigS4). It is
also clearly congruent with previous study [44]. However, most *B. subtilis* were clustered into
two clades according to the cell wall genotype in the tarABD MLSA NJ tree and the
combined MLSA tree (Fig. 2, Fig. S3 and Fig. S5). The genes in clade A were *tar*-like genes
while the genes in clade B were *tag*-like genes. However, some strains were not clustered into
clade A or clade B, and the genes of these strains did not show high levels of similarity to
either *tar* or *tag* genes (Table S2 and Table S8). Both clade A and clade B had both *B. s.*
subtilis and *B. s. spizizenii* strains as members, suggesting that the cell wall difference was
independent of speciation and could not be used to distinguish between *B. s. subtilis* and *B. s.*
spizizenii, as previously described [11, 12].

The OrthoANI tree showed that the overall degree of genome similarity between the strains
coincided with the subspecies classification. However, the *tarABD* MLSA NJ tree and
combined MLSA tree did not coincide with the OrthoANI tree. The MLSA trees showed that
each subspecies had multiple cell wall types, also indicating that the *B. subtilis* subspecies
cannot be distinguished by the presence of ribitol as a cell-wall constituent. It also indicated
that *tar* genes were not inherited after the speciation of *B. s. spizizenii*.

Antibiotic susceptibility and electron microscopy

*B. s. subtilis* KCTC 3135T and *B. s. spizizenii* KCTC 3705T showed different antibiotic
susceptibilities with rifampicin, streptomycin, ampicillin and tetracycline (Table 2). Compared
to *B. s. subtilis* KCTC 3135T, *B. s. spizizenii* KCTC 3705T was more susceptible to rifampicin,
streptomycin and tetracycline, and was resistant to ampicillin. However, the genetic structures
of specific antibiotics resistance genes or related genes did not contribute to the difference in
the antibiotic phenotypes. The distribution of antibiotic genes was not significantly different
among *B. s. subtilis* KCTC 3135\(^T\) and *B. s. spizizenii* TU-B-10\(^T\) (Table S7). Rifampicin is an antibiotic that inhibits RNA synthesis by binding *rpoB* [45]. The amino acid sequence difference of *rpoB* genes from *B. s. subtilis* KCTC 3135\(^T\), *B. s. spizizenii* TU-B-10\(^T\) were three residues, and these residues are not known as rifampicin binding sites [46]. These findings indicate that the difference in the antibiotic susceptibility levels did not arise from the variation of the *rpoB* genes. It was reported that the inhibition of cell wall synthesis increased the sensitivity to rifampicin though rifampicin did not target bacterial cell wall [47]. The mechanism of action of streptomycin is the inhibition of protein synthesis by binding the 30S subunit of ribosome, and streptomycin resistance is related to mutation of the S12 protein [48]. Although the levels of susceptibility of the two strains were different, the sequences of S12 proteins from both strains were identical. Ampicillin, in the family of β-lactam antibiotics, inhibits the formation of peptidoglycan by binding DD-transpeptidase [49, 50]. The modes of ampicillin resistance were the degradation of the β-lactam ring by β-lactamase and the alternation of the binding sites [51, 52]. Both *B. s. subtilis* KCTC 3135\(^T\) and *B. s. spizizenii* TU-B-10\(^T\) had class A and class D β-lactamase genes. The similarity rates of the class A and class D β-lactamase protein sequences of *B. s. subtilis* KCTC 3135\(^T\) and *B. s. spizizenii* TU-B-10\(^T\) were 91.2% and 94.3%, respectively. It was unclear whether the sequence differences in the lactamase genes were related to the different levels of ampicillin susceptibility of the strains. However, there were no non-sense mutations in the lactamase genes. It is known that the inhibition of WTA synthesis can sensitize MRSA to β-lactam [53]. The inhibition WTA synthesis led to the destabilization of penicillin binding protein, which had a crucial role in β-lactam resistance [53]. Tetracycline also binds to the 30S subunit of ribosome, and tetracycline resistance is were dependent on various tetracycline-resistant genes [54]. *B. s. subtilis* KCTC 3135\(^T\) was more susceptible to tetracycline though the presence of tetracycline-
resistant genes. It is unclear whether the differences in the ABC efflux pump were related to the differences in the antibiotic susceptibility levels of *B. s. subtilis* KCTC 3135<sup>T</sup> and *B. s. spizizenii* KCTC 3705<sup>T</sup>. Due to the extreme diversity of ABC efflux pump genes, it is difficult to discover all of the genes.

The bacterial cell walls of the strains, *B. s. subtilis* KCTC 3135<sup>T</sup> and *B. s. spizizenii* KCTC 3705<sup>T</sup> were examined by TEM. Although the ultrastructure of the bacterial cell walls was the visibly dissimilar (Fig. 3A and Fig. 3E), no significant morphological differences were observed. *B. s. subtilis* KCTC 3135<sup>T</sup> has a smooth outer membrane, whereas *B. s. spizizenii* KCTC 3705<sup>T</sup> has a rough outer membrane with electron-dense surface coats and spikes (Fig. 3F and Fig. 3H). The width of the outermost cell wall in *B. s. spizizenii* KCTC 3705<sup>T</sup> (Fig. 3H) is wider than that in *B. s. subtilis* KCTC 3135<sup>T</sup> (Fig. 3D), which provides evidence of the presence of electron-dense surface spikes in *B. s. spizizenii* KCTC 3705<sup>T</sup>. Electron microscopy of the cell wall revealed different cell wall structures according to the strains. Based on the previous reports, the morphology of *B. subtilis* cells was known to be affected by WTA [55, 56]. It is assumed that the different cell wall structures were related to the genetic structures of WTA biosynthesis genes. The initial step of WTA synthesis by both *tar* and *tag* genes were conserved, but the extension of the WTA polymer diverged [12]. The *tar* genes extended the polymer with ribitol while the *tag* genes extended the polymer with glycerol [12].

In conclusion, the overall genomic structures of *B. s. subtilis* KCTC 3135<sup>T</sup> and *B. s. spizizenii* KCTC 3705<sup>T</sup> were similar apart from the WTA synthesis genes, a difference which was previously regarded as a characteristic distinguishing *B. s. spizizenii* from *B. s. subtilis*. However, a comparative genomic analysis of multiple *B. subtilis* genomes showed that WTA with ribitol can be synthesized from both *B. s. spizizenii* and *B. s. subtilis*, indicating that the
subspecies description of *B. s. spizizenii* should be corrected. The cell wall morphologies of *B. s. subtilis* KCTC 3135$^\text{T}$ and *B. s. spizizenii* KCTC 3705$^\text{T}$ were examined using TEM, showing clear differences. Considering the overall genomic structures of *B. s. subtilis* KCTC 3135$^\text{T}$ and *B. s. spizizenii* KCTC 3705$^\text{T}$, the differences in the WTA synthesizing genes may contribute to the differences in the cell wall morphologies. The antibiotic susceptibility levels of *B. s. subtilis* KCTC 3135$^\text{T}$ and *B. s. spizizenii* KCTC 3705$^\text{T}$ were different, though the distribution of antibiotic genes were similar, suggesting that the difference in antibiotic resistance was not due to the presence of specific antibiotic genes but due to the other factor like difference in the cell-wall structures or the ABC efflux pumps, etc.

**Acknowledgments**

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33. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and


Figure legends

Figure 1. A neighbor-joining tree based on the OrthoANI distance matrix. *B. subtilis* with three subspecies contains 24 genomes, and *S. aureus* subsp. *aureus* DSM 20231<sup>T</sup> was used as an outgroup. The scale bar indicates the sequence divergence.

Figure 2. A) Phylogenetic tree using the *tarABD* MLSA with the neighbor-joining method and B) heatmap based on the nucleotides similarities of the *tarABD* from *B. subtilis* genome to the reference *tar* or *tag* genes. *B. subtilis* with three subspecies contains 24 genomes, and *S. aureus* subsp. *aureus* DSM 20231<sup>T</sup> was used as an outgroup. Bootstrap values (>70 %) based on 1000 replicates are shown at the branch nodes. The branch nodes also recovered in maximum-likelihood and maximum-parsimony trees are marked by the filled black circles. The bar indicates the nucleotide substitution rate at the given length of the scale. The color indicates the tblastn nucleotide similarities of the *tarABD* from *B. subtilis* genome to the reference *tar* (red) or *tag* (green) genes.

Figure 3. Bacterial cell wall of the strains *B. s. subtilis* KCTC 3135<sup>T</sup> and *B. s. spizizenii* KCTC 3705<sup>T</sup> were examined by TEM. In Figures 4D and 4H, the x-axis and y-axis of the plot profile refer to the distance from the outer space to the bacterial cytoplasm and the average pixel intensity, respectively.
### Tables

Table 1. Genomic features of *Bacillus subtilis* subsp. *subtilis* KCTC 3135\textsuperscript{T} and *B. subtilis* subsp. *spizizenii* TU-B-10\textsuperscript{T}

<table>
<thead>
<tr>
<th>Features</th>
<th><em>B. subtilis</em> subsp. <em>subtilis</em> KCTC 3135\textsuperscript{T} (CP015375)</th>
<th><em>B. subtilis</em> subsp. <em>spizizenii</em> TU-B-10\textsuperscript{T} (CP002905)</th>
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<tr>
<td>Genomic size (bp)</td>
<td>4,211,343</td>
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<td>G + C content (%)</td>
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<tr>
<td>Total number of genes</td>
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<td>4,315</td>
</tr>
<tr>
<td>Protein-coding genes (CDS)</td>
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<td>4,188</td>
</tr>
<tr>
<td>rRNA operons</td>
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<td>10</td>
</tr>
<tr>
<td>tRNA genes</td>
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<td>92</td>
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Table 2. Antibiotic susceptibility

<table>
<thead>
<tr>
<th></th>
<th>KCTC 3135 (^T) (mm)(^b)</th>
<th>KCTC 3705 (^T) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD (5)(^a)</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>S (10)</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>AMP (10)</td>
<td>10</td>
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<td>K (30)</td>
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<td>NA (30)</td>
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<td>NV (30)</td>
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<td>C (30)</td>
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<td>TE (30)</td>
<td>10</td>
<td>24</td>
</tr>
</tbody>
</table>

\(^a\) Amount of antibiotics (µg)

\(^b\) Diameter of clear zone (mm)


R: Resistance
Figure 1.

- *B. subtilis* subsp. *subtilis* KCTC 3135\(^T\) (GCF_001697265.1)
- *B. subtilis* subsp. *subtilis* 168 (GCF_000009045.1)
- *B. subtilis* subsp. *subtilis* BSP1 (GCF_000321395.1)
- *B. subtilis* subsp. *subtilis* SG6 (GCF_000782835.1)
- *B. subtilis* subsp. *subtilis* BSa5 (GCF_000186745.1)
- *B. subtilis* subsp. *subtilis* OH 131.\(^T\) (GCF_000706705.1)
- *B. subtilis* subsp. *subtilis* YP1 (GCF_000877815.1)
- *B. subtilis* subsp. *subtilis* VV2 (GCF_00180235.1)
- *B. subtilis* subsp. *subtilis* BEST195 (GCF_000209795.2)
- *B. subtilis* subsp. *subtilis* KH2 (GCF_001890405.1)
- *B. subtilis* subsp. *subtilis* BAB-1 (GCF_000349795.1)
- *B. subtilis* subsp. *subtilis* UD1022 (GCF_001015095.1)
- *B. subtilis* subsp. *subtilis* RO-NN-1 (GCF_000227485.1)
- *B. subtilis* subsp. *subtilis* JS (GCF_000259365.1)
- *B. subtilis* subsp. *inaquosorum* KCTC 13429\(^T\) (GCF_000332645.1)
- *B. subtilis* subsp. *inaquosorum* DE111 (GCF_001534785.1)
- *B. subtilis* subsp. *spizizenii* TU-B-10\(^T\) (GCF_000227465.1)
- *B. subtilis* subsp. *spizizenii* HUK15 (GCF_001566945.1)
- *B. subtilis* subsp. *spizizenii* MJ01 (GCF_001889625.1)
- *B. subtilis* subsp. *spizizenii* DV1-B-1 (GCF_000245035.1)
- *B. subtilis* subsp. *spizizenii* W23 (GCF_000146565.1)
- *B. subtilis* subsp. *spizizenii* RFWG1A3 (GCF_000931815.1)
- *B. subtilis* subsp. *spizizenii* RFWG5B15 (GCF_000931845.1)
- *B. subtilis* subsp. *inaquosorum* J-5 (GCF_001889385.1)
- *S. aureus* subsp. *aureus* DSM 20231\(^T\) (GCF_001027105.1)
Figure 2.

A

B

Color Key
and Histogram

Value

tag

100

98

90

90

99

92

97

97

93

93

98

99

99

0

S. aureus subsp. aureus DSM 20231T

B. subtilis subsp. subtilis KCTC 3135T

B. subtilis subsp. subtilis SG6 (GCF_000782835.1)

B. subtilis subsp. subtilis BAB-1 (GCF_000349795.1)

B. subtilis subsp. subtilis JS (GCF_000259365.1)

B. subtilis subsp. subtilis OH 131.1 (GCF_00076705.1)

B. subtilis subsp. subtilis HF1 (GCF_000031195.1)

B. subtilis subsp. subtilis BEST195 (GCF_000209795.2)

B. subtilis subsp. subtilis YP1 (GCF_000877815.1)

B. subtilis subsp. subtilis BAB-1 (GCF_000349795.1)

B. subtilis subsp. subtilis MJ01 (GCF_001889625.1)

B. subtilis subsp. subtilis HUK15 (GCF_001566945.1)

B. subtilis subsp. subtilis KCTC 13429T (GCF_000332645.1)

B. subtilis subsp. subtilis W23 (GCF_000146565.1)

B. subtilis subsp. subtilis JS (GCF_000259365.1)

B. subtilis subsp. subtilis OH 131.1 (GCF_00076705.1)

B. subtilis subsp. subtilis BEST195 (GCF_000209795.2)

B. subtilis subsp. subtilis YP1 (GCF_000877815.1)

B. subtilis subsp. subtilis BAB-1 (GCF_000349795.1)

B. subtilis subsp. subtilis MJ01 (GCF_001889625.1)

B. subtilis subsp. subtilis HUK15 (GCF_001566945.1)

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B. subtilis subsp. subtilis HUK15 (GCF_001566945.1)

B. subtilis subsp. inaquosorum DE111 (GCF_001534785.1)

B. subtilis subsp. spizizenii TU-B-10T (GCF_000227465.1)

B. subtilis subsp. spizizenii MI01 (GCF_001889625.1)

B. subtilis subsp. spizizenii HUK15 (GCF_001566945.1)

B. subtilis subsp. inaquosorum DE111 (GCF_001534785.1)

B. subtilis subsp. spizizenii TU-B-10T (GCF_000227465.1)

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B. subtilis subsp. spizizenii TU-B-10T (GCF_000227465.1)

B. subtilis subsp. spizizenii MI01 (GCF_001889625.1)

B. subtilis subsp. spizizenii HUK15 (GCF_001566945.1)
Figure 3.

A 3135

B

C

D

E 3705

F

G

H

peptidoglycan

cytoplasmic membrane

Spikes

peptidoglycan

cytoplasmic membrane