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Comparative Analysis of an Integral Component of Bacterial Cell Division from the *Lactococcus* and *Bacillus* Genera

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Abstract

FtsZ proteins have been well-characterized to play a crucial role in cell division. Unfortunately, data are scarce on FtsZ proteins in the *Lactococcus* and *Bacillus* genera. The objective of this study was to analyze the features of FtsZ proteins in the *Bacillus* and *Lactococcus* genus groups. By exploring the available genomes, we identified and characterized FtsZ proteins in 19 *Bacillus* and 22 *Lactococcus* species. The sizes and weights of the FtsZ proteins ranged from 376 to 410 aa residues and 39.53 to 44.15 kDa in the *Bacillus* genus, respectively, and from 387 to 430 aa residues and 41.14 to 45.11 kDa in the *Lactococcus* genus, respectively. All the FtsZ proteins in the *Bacillus* and *Lactococcus* species were acidic and globular, and localized in the cytoplasm. Next, 3D modeling and multiple alignments were performed. We realized that the FtsZ proteins in the *Bacillus* and *Lactococcus* species exhibited five specific regions. Taken together, our study could provide a general background for further functional characterization of the FtsZ proteins in *Bacillus* and *Lactococcus* species.

Keywords

Lactococcus, *Bacillus*, FtsZ, conserved domain, protein

Introduction

Cell division is a crucial process for living organisms. Principally, cell division in bacteria is orchestrated by a divisome. In this step, FtsZ, a polymer-forming guanosine-5′-triphosphatease (GTPase), drives bacterial cell division (de Boer *et al.*, 1992; Löwe and Amos, 1998).

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Particularly, FtsZ proteins play the role as the pacemaker of the formation of the divisome and the cytokinesis process (Margolin, 2005) as it assembles into proto-filaments to construct a ring-like template (McQuillen and Xiao, 2020). The formation of this ring initiates and facilitates cellular division, enabling a single parent cell to give rise to two offspring cells, while the divisome plays a pivotal role in the constriction of the cell envelope and the generation of new cell wall segments at the division site. FtsZ also has an extended role beyond cellular division where it contributes to determining cell morphology and establishing polarity in certain bacterial species. In a nutshell, the FtsZ protein is indispensable in the orchestration of bacterial cell division, thereby significantly influencing the growth and structural dynamics of bacterial cells. The FtsZ proteins in bacterial species have been reported to share a similar manner with the tubulins in eukaryotic cells. Up until now, information about the FtsZ proteins in various prokaryotic cells, unfortunately, has been unclearly (Pal *et al.*, 2019).

The *Lactococcus* and *Bacillus* genus groups are two very common types of bacteria. Firstly, *Lactococcus* constitutes some of the genera forming the lactic acid bacteria family. This genus has been reportedly used in the food industry (Song *et al.*, 2017) such as in the production of dairy products (Li *et al.*, 2020). On the other hand, *Bacillus* species signify Gram-positive, spore-forming, rod-shaped, and aerobic bacteria (Miljaković *et al.*, 2020). This genus can be isolated from various sources, like soil (mostly), air, water, animal guts, vegetables, and other food items (Elshaghabee *et al.*, 2017). At the molecular scale, the cell division process, particularly the FtsZ proteins of these genus groups, has been not fully described (Pal *et al.*, 2019). Recently, a comprehensive search of the FtsZ proteins from approximately 70 bacterial families (belonging to 40 orders) was performed in order to find out the core set of codons in the coding region of the gene sequence (Pal *et al.*, 2019).

The aims of this study was to comprehensively describe the FtsZ proteins found in the *Lactococcus* and *Bacillus* genus groups.

Materials and Methods

In silico searches of FtsZ proteins in databases

The well-characterized *ftsZ* gene from *Escherichia coli* (strain ATCC 47076) (McQuillen and Xiao, 2020) was used as the seed sequence for comprehensively searching against the sequences of *Lactococcus* and *Bacillus* genera (Loman *et al.*, 2012; Tatusova *et al.*, 2014) available from the NCBI GenBank database (Wheeler *et al.*, 2008). The coding DNA sequences (CDSs) of the FtsZ proteins were then BlastP-ed to obtain the full-length amino acid (aa) sequences.

Analysis of features of the FtsZ proteins

The full-length aa sequences of the FtsZ proteins in the *Lactococcus* and *Bacillus* genus groups were applied in the Expasy Protparam portal (Gasteiger *et al.*, 2003; Gasteiger *et al.*, 2005). Particularly, six typical features of the molecules, namely protein sizes (aa residues), protein weights (kilo Dalton, kDa), iso-electric points (pI-s), instability indexes (II-s), aliphatic indexes (AI-s), and grand average of hydropathicity (GRAVY), were identified (Gasteiger *et al.* 2003). Basically, pI scores of > 7 and < 7 indicated basic and acidic proteins, respectively, while II values of > 41 and < 41 suggested unstable and stable proteins, respectively. GRAVY scores below 0 were more likely for hydrophilic proteins, while scores above 0 were more likely for hydrophobic proteins.

Prediction of subcellular localization of the FtsZ proteins

The subcellular localizations of the FtsZ proteins in the *Lactococcus* and *Bacillus* genus groups were predicted by using the YLoc portal (Briesemeister *et al.*, 2010a; 2010b). Particularly, the full-length aa sequence of each FtsZ protein was searched against the YLoc (Briesemeister *et al.*, 2010b) to suggest the putative localizations, like the nucleus, cytoplasm, mitochondrion, plasma membrane, extracellular space, endoplasmic reticulum, peroxisome, Golgi apparatus, and vacuole. The probability (%) and confidence scores were used to validate the predictions (Briesemeister *et al.*, 2010b).

Construction of 3D models of the FtsZ proteins

The full-length aa sequences of the FtsZ proteins in the *Lactococcus* and *Bacillus* species were used for the model predictions as previously described (La *et al.*, 2022). Particularly, the Phyre2 tool (Kelley *et al.*, 2015) was applied to analyze the secondary structures of the proteins, including the rates of alpha and beta subunits. Then, the 3D models of the FtsZ proteins in the *Lactococcus* and *Bacillus* species were constructed based on the available structures (Kelley *et al.*, 2015).

Analysis of conserved domains of the FtsZ proteins

The full-length aa sequences of the FtsZ proteins in the *Lactococcus* and *Bacillus* species were applied for alignment using the ClustalX software (Thompson *et al.*, 1997; Thompson *et al.*, 2002). The Pfam domain (Finn *et al.*, 2014; Mistry *et al.*, 2021) was then used to obtain the conserved domain of the bacterial FtsZ proteins (Löwe and Amos, 1998; Silber *et al.*, 2020). The conserved regions found in the FtsZ proteins in the *Lactococcus* and *Bacillus* species were graphically viewed by the BioEDIT software (Hall, 1999).

Results and Discussion

Identification and annotation of the FtsZ proteins in the *Lactococcus* and *Bacillus* genera

In order to screen the FtsZ proteins from the *Lactococcus* and *Bacillus* genera, we selected the well-annotated FtsZ protein in *E. coli* from a previous study (McQuillen and Xiao, 2020) for a comprehensive search against the assemblies of *Lactococcus* and *Bacillus* genera (Loman *et al.*, 2012; Tatusova *et al.*, 2014). As shown in **Tables 1 and 2**, a total of 19 and 22 FtsZ proteins were found in these genus groups, respectively. Particularly, we reported the occurrences of FtsZ proteins in 13 *Bacillus* species, namely *B. anthracis*, *B. benzoevorans*, *B. aquiflavi*, *B. amyloliquefaciens*, *B. massiliigabonensis*, *B. carboniphilus*, *B. vallismortis*, *B. dakarensis*, *B. solitudinis*, *B. paralicheniformis*, *B. infantis*, *B. mediterraneensis*, and *B. methanolicus* (**Table 1**).

Next, we also found information of the FtsZ proteins in *Lactococcus* sp. For instance, FtsZ proteins were identified and annotated in a variety of *Lactococcus* species, namely *L. allomyrinae*, *L. chungangensis*, *L. cremoris*, *L. formosensis*, *L. fujiensis*, *L. garvieae*, *L. hircilactis*, *L. hodotermopsidis*, *L. insecticola*, *L. lactis*, *L. nasutitermitis*, *L. petauri*, *L. piscium*, *L. plantarum*, *L. protaetiae*, L. *raffinolactis*, *L. reticulitermitis*, *L. taiwanensis*, and *L. termiticola*. The detailed information of the FtsZ proteins in *Lactococcus* sp. has been provided in **Table 2**. In this study, the CDS and full-length aa sequences from all the FtsZ proteins in the *Bacillus* and *Lactococcus* genus groups were then collected for further in silico analyses.

Analysis of conserved domains of the FtsZ proteins

In this study, we analyzed the general characteristics of the FtsZ proteins in the two genus groups using a web-based tool (Gasteiger *et al.*, 2003; Gasteiger *et al.*, 2005). Six features of the proteins, namely molecular lengths and weights, pI, II and AI scores, and GRAVY from the *Bacillus* and *Lactococcus* species, are subsequently provided in **Tables 3 and 4**, respectively.

We found that the majority (18 out of 19) of the FtsZ proteins in the *Bacillus* genus exhibited sizes of less than 400 aa residues (**Table 3**). Particularly, the sizes of the FtsZ proteins varied from 376 (in *B. carboniphilus*) to 410 aa residues (in *B. anthracis*) (**Table 3**). The molecular masses of the FtsZ proteins in the *Bacillus* genus ranged from 39.53 (in *B. carboniphilus*) to 44.15 kDa (in *B. anthracis*) (**Table 3**). Next, the pI scores of the FtsZ proteins in the *Bacillus* genus were all less than 7.0 (**Table 3**), which suggested that these proteins were acidic. The II scores of all the FtsZ proteins in the *Bacillus* species were less than 40, ranging from 27.2 (in *B. amyloliquefaciens*) to 38.7 (in *B. infantis*) (**Table 3**). These findings predicted that the FtsZ proteins in the *Bacillus* genus were stable. Additionally, the AI values of these FtsZ proteins varied from 82.3 (in *B. amyloliquefaciens*) to 95.5 (in *B. benzoevorans*) (**Table 3**). Interestingly, the GRAVY values of the FtsZ

Table 1. Information of the FtsZ proteins found in the *Bacillus* genus

#	Organism	Strain	ProteinID
1	*Bacillus anthracis*	V583	GEU27941.1
2	*Bacillus* sp.	Man26	WP_233314489.1
3	*Bacillus* sp.	B-jedd	WP_048824400.1
4	*Bacillus* sp.	-	MBO8176442.1
5	*Bacillus benzoevorans*	DSM 5391	WP_184522020.1
6	*Bacillus* sp.	CBEL-1	TDB50557.1
7	*Bacillus aquiflavi*	3H-10	WP_163242253.1
8	*Bacillus amyloliquefaciens*	N315	POO69966.1
9	*Bacillus massiliigabonensis*	Marseille-P2639	WP_102271539.1
10	*Bacillus* sp.	T33-2	WP_101581273.1
11	*Bacillus carboniphilus*	SaN35-3	WP_226538420.1
12	*Bacillus vallismortis*	DSM 11031	WP_010328070.1
13	*Bacillus* sp.	FJAT-47783	WP_243289962.1
14	*Bacillus dakarensis*	Marseille- P3515T	WP_077211442.1
15	*Bacillus solitudinis*	FJAT-45086	WP_100404556.1
16	*Bacillus paralicheniformis*	Bac48	WP_105978931.1
17	*Bacillus infantis*	2933tsa1	WP_148950233.1
18	*Bacillus mediterraneensis*	Marseille-P2366	WP_071459365.1
19	*Bacillus methanolicus*	PB1	WP_003350300.1

Note: -: No information.

proteins were less than 0, ranging from -0.35 (in *B. anthracis*) to -0.09 (**Table 3**). The hydrophobicity scores of the FtsZ proteins in the *Bacillus* species suggested that these proteins were more likely globular.

As compared to the FtsZ proteins in the *Bacillus* genus, the FtsZ proteins found in the *Lactococcus* species were also investigated and found to share similar phenomena (**Tables 3 and 4**). Briefly, the protein sizes and masses of the FtsZ proteins in the *Lactococcus* genus varied from 387 (in *L. lactis* subsp. *cremoris* TIFN1) to 430 aa residues (in *L. hodotermopsidis*), and 41.14 (in *L. lactis* subsp. *cremoris* TIFN1) to 45.11 kDa (in *L. hodotermopsidis*), respectively (**Table 4**). Next, all the identified FtsZ proteins in the 22 *Lactococcus* species were demonstrated to be acidic (pI scores were less than 7.0) and hydrophilic (GRAVY scores were negative) (**Table 4**). A variety (eight out of 22) of the FtsZ proteins in the *Lactococcus* genus were less than 40, which suggested that these proteins were stable, whereas the remaining (14 out of 22) proteins were unstable (**Table 4**). Furthermore, the AI scores of these proteins were found to range from 85.56 (in *L. hircilactis*) to 91.54 (in *L. insecticola*) (**Table 4**).

Previously, the typical characteristics of FtsZ proteins in other bacterial genus groups have also been reported. For example, the sizes and molecular weights of the FtsZ protein found in *Bartonella bacilliformis* (strain KC583) were recorded to be 593 aa residues and 63.61 kDa, respectively, while the FtsZ protein in *Geobacter sulfurreducens* (strain PCA) exhibited a length of 384 aa residues and a mass of 40.83 kDa (Pal *et al.*, 2019). Based on a recent report, the protein size and weight of the FtsZ molecule in *E. coli* (strain ATCC 47076) were found to be 383 aa residues and 40.32 kDa, respectively (McQuillen and Xiao, 2020). In *Alcaligenes faecalis* subsp. *faecalis* NCIB 8687, the FtsZ protein exhibited a size of 387 aa residues and a weight of 40.60 kDa(Pal *et al.*, 2019). Interestingly, all the FtsZ

Table 2. Information of the FtsZ proteins found in the *Lactococcus* genus

#	Organism	Strain	ProteinID
1	*Lactococcus*	SK11	WP_011676880.1
2	*Lactococcus allomyrinae*	1JSPR-7	WP_120772315.1
3	*Lactococcus chungangensis*	DSM 22330	WP_031366144.1
4	*Lactococcus cremoris*	MG1363	WP_011835774.1
5	*Lactococcus formosensis*	NBRC 109475	WP_213496889.1
6	*Lactococcus fujiensis*	JCM 16395	WP_054639583.1
7	*Lactococcus garvieae*	IPLA 31405	WP_003133462.1
8	*Lactococcus hircilactis*	DSM 28960	WP_153496745.1
9	*Lactococcus hodotermopsidis*	Hs30E4-3	WP_172207956.1
10	*Lactococcus insecticola*	Hs20B0-1	WP_172357477.1
11	*Lactococcus lactis*	-	WP_101961763.1
12	*Lactococcus lactis* subsp. *cremoris*	MG1363	CAA75616.1
13	*Lactococcus lactis* subsp. *cremoris*	TIFN1	EQC86115.1
14	*Lactococcus nasutitermitis*	NBRC 111537	WP_213534626.1
15	*Lactococcus petauri*	-	WP_242359400.1
16	*Lactococcus piscium*	BF1	WP_218724185.1
17	*Lactococcus plantarum*	NBRC 100936	WP_068164022.1
18	*Lactococcus protaetiae*	KACC 19320	WP_142767375.1
19	*Lactococcus raffinolactis*	NBRC 100932	WP_061775091.1
20	*Lactococcus reticulitermitis*	Rs-Y01	WP_094784078.1
21	*Lactococcus taiwanensis*	K_LL001	WP_205272043.1
22	*Lactococcus termiticola*	NtB2	WP_109245985.1

Note: -: No information.

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proteins found in these organisms were realized to be acidic and hydrophilic because the pI values were less than 7.0 and the GRAVY values were negative, respectively.

Predictions of subcellular localization and construction of 3D models of the FtsZ proteins in the *Lactococcus* and *Bacillus* genus groups

The FtsZ proteins in *Bacillus* were predicted to be distributed in the cytoplasm (**Table 3**). The probability scores were more than 90%, except for the prediction of the FtsZ protein found in *B. amyloliquefaciens* (**Table 3**). The confidence was strong (0.91) to very strong (0.99) (**Table 3**). Similarly, we demonstrated that all the FtsZ proteins found in the *Lactococcus* genus were localized in the cytoplasm with high confidence (**Table 4**). The percentages of probability and confidence scores of the prediction algorithms varied from 96.25 to 98.99%, and from 0.81 (strong confidence) to 0.99 (very strong confidence), respectively (**Table 4**).

Previously, the subcellular localization of the FtsZ proteins in several bacterial species has been reported. Briefly, the FtsZ proteins found in *E. coli* (strain K12) (McQuillen and Xiao, 2020), *Blautia* sp. (strain MCC269), *A. faecalis* (strain NCIB 8687), *B. bacilliformis* (strain KC583), and *Geobacter sulfurreducens* (Pal *et al.*, 2019) were predicted to be localized in the cytoplasm with high confidence levels. Taken together, these findings strongly suggest that the FtsZ proteins in the *Bacillus* and *Lactococcus* species, and perhaps in other bacterial genera as well, are distributed in the cytoplasm.

Next, we analyzed the secondary structures and simulated 3D models of the FtsZ proteins in

Table 3. Characteristics of the FtsZ proteins in the *Bacillus* genus

FtsZ proteins in *Bacillus* genus	Protein sizes	Protein weights	pI values	II values	AI values	GRAVY values	Predicted location	Probability	Confidence
GEU27941.1	410	44.15	4.8	32.8	85.9	-0.35	Cytoplasm	95.43	0.98
WP_233314489.1	382	39.99	5.1	28.4	95.0	-0.09	Cytoplasm	98.75	0.91
WP_048824400.1	378	39.90	5.1	33.3	94.0	-0.10	Cytoplasm	97.86	0.98
MBO8176442.1	380	40.39	5.0	32.1	93.0	-0.17	Cytoplasm	94.03	0.96
WP_184522020.1	379	39.87	5.1	30.9	95.5	-0.09	Cytoplasm	98.45	0.98
TDB50557.1	387	40.81	5.1	33.9	91.5	-0.17	Cytoplasm	98.45	0.98
WP_163242253.1	381	40.17	5.0	29.5	93.5	-0.12	Cytoplasm	98.80	0.97
POO69966.1	390	41.04	4.9	27.2	82.3	-0.23	Cytoplasm	86.51	0.96
WP_102271539.1	381	40.31	5.0	33.8	93.2	-0.15	Cytoplasm	98.58	0.98
WP_101581273.1	385	40.54	4.9	29.4	92.5	-0.16	Cytoplasm	97.86	0.98
WP_226538420.1	376	39.53	4.8	28.6	92.4	-0.12	Cytoplasm	98.41	0.98
WP_010328070.1	382	40.40	5.0	34.2	93.5	-0.19	Cytoplasm	97.52	0.98
WP_243289962.1	380	40.14	5.0	28.1	92.2	-0.12	Cytoplasm	98.40	0.99
WP_077211442.1	380	40.16	5.1	32.0	92.4	-0.15	Cytoplasm	98.40	0.99
WP_100404556.1	380	40.25	4.9	35.0	93.0	-0.18	Cytoplasm	90.44	0.95
WP_105978931.1	377	39.86	5.1	35.8	93.0	-0.20	Cytoplasm	99.39	0.99
WP_148950233.1	388	40.80	5.1	38.7	88.0	-0.22	Cytoplasm	96.40	0.95
WP_071459365.1	385	41.00	5.1	31.8	93.0	-0.17	Cytoplasm	98.80	0.97
WP_003350300.1	379	39.93	5.0	32.6	94.2	-0.11	Cytoplasm	97.86	0.98

Note: Protein size (aa residues), protein weight (kDa), pI - Iso-electric point, II - Instability index, AI - Aliphatic index, GRAVY - Grand average of hydropathicity.

the *Lactococcus* and *Bacillus* species by the Phyre2 web-based platform (Kelley *et al.*, 2015) as previously reported (La *et al.*, 2022). Here, two elements of the secondary structure, namely the alpha-helix and beta-pleated sheets, were the focus. The alpha-helices and beta-pleated sheets of the FtsZ proteins in the *Bacillus* species varied from 0.35 to 0.39, and from 0.18 to 0.20, respectively (**Figure 1A**). This phenomenon was also reported in the FtsZ proteins in the *Lactococcus* genus. Particularly, the alpha-helices of the FtsZ proteins in the *Lactococcus* species ranged from 0.32 to 0.36, whereas the beta-pleated sheets varied from 0.17 to 0.18 (**Figure 1B**).

We found two 3D models, namely 'c2vxyA' (**Figure 2A**) and 'c4dxdA' (**Figure 2B**), representative of the FtsZ proteins in the *Bacillus* species. Particularly, out of the 19 FtsZ proteins found in the *Bacillus* species, 17 had the 'c2vxyA' model and two had the 'c4dxdA' model. Meanwhile, the FtsZ proteins found in the 22 *Lactococcus* species were predicted to exhibit the 'c2vxyA' model (Figure 2A). Taken together, the construction of the 3D models of the FtsZ molecules could provide a solid foundation for further functional characterization of these proteins in the *Bacillus* and *Lactococcus* genus groups.

Investigation of the core set of conserved domains in the structure of the FtsZ proteins in the Bacillus and Lactococcus genus groups

We analyzed the conserved domains of the FtsZ proteins in the *Lactococcus* and *Bacillus* species by using various software tools (Thompson *et al.*, 1997; Thompson *et al.*, 2002; Finn *et al.*, 2014; Mistry *et al.*, 2021). The multiple alignments of the FtsZ proteins found in the 19 and 22 *Bacillus* and *Lactococcus* species, respectively, and six other bacterial strains were consequently well-described (**Figures 3 and 4).**

Table 4. Characteristics of the FtsZ proteins in the *Lactococcus* genus

FtsZ proteins in *Lactococcus* genus	Protein sizes	Protein weights	pI values	II values	AI values	GRAVY values	Predicted location	Probability	Confidence
WP_011676880.1	417	44.03	4.54	40.79	86.09	-0.23	Cytoplasm	97.57	0.98
WP_120772315.1	411	43.40	4.58	44.40	88.05	-0.17	Cytoplasm	98.21	0.85
WP_031366144.1	421	44.04	4.49	34.81	89.93	-0.15	Cytoplasm	97.71	0.97
WP_011835774.1	419	44.26	4.54	40.44	87.54	-0.21	Cytoplasm	98.39	0.97
WP_213496889.1	424	44.58	4.51	42.93	85.83	-0.18	Cytoplasm	97.93	0.95
WP_054639583.1	419	44.51	4.64	38.03	88.45	-0.25	Cytoplasm	97.71	0.97
WP_003133462.1	424	44.56	4.50	44.96	85.61	-0.19	Cytoplasm	98.35	0.97
WP_153496745.1	423	44.82	4.65	36.29	85.56	-0.24	Cytoplasm	98.26	0.97
WP_172207956.1	430	45.11	4.52	40.76	89.63	-0.16	Cytoplasm	97.36	0.97
WP_172357477.1	421	43.93	4.42	37.40	91.54	-0.13	Cytoplasm	96.25	0.94
WP_101961763.1	417	44.05	4.55	42.03	86.09	-0.23	Cytoplasm	97.57	0.98
CAA75616.1	419	44.36	4.65	40.35	88.23	-0.22	Cytoplasm	98.99	0.97
EQC86115.1	387	41.14	4.58	41.93	85.97	-0.28	Cytoplasm	98.52	0.99
WP_213534626.1	426	44.94	4.57	36.04	86.08	-0.22	Cytoplasm	98.78	0.81
WP_242359400.1	424	44.57	4.46	40.86	86.06	-0.18	Cytoplasm	97.93	0.95
WP_218724185.1	420	43.91	4.52	40.09	88.71	-0.15	Cytoplasm	97.85	0.95
WP_068164022.1	420	44.13	4.51	34.63	90.98	-0.13	Cytoplasm	97.72	0.93
WP_142767375.1	411	43.42	4.58	41.14	88.30	-0.17	Cytoplasm	98.21	0.85
WP_061775091.1	421	44.01	4.55	35.34	90.40	-0.14	Cytoplasm	96.40	0.94
WP_094784078.1	421	43.91	4.52	34.44	89.48	-0.13	Cytoplasm	96.40	0.94
WP_205272043.1	415	43.85	4.53	41.23	87.69	-0.23	Cytoplasm	98.60	0.92
WP_109245985.1	417	44.05	4.47	40.48	86.09	-0.20	Cytoplasm	98.60	0.96

Note: Protein size (aa residues), protein weight (kDa), pI - Iso-electric point, II - Instability index, AI - Aliphatic index, GRAVY - Grand average of hydropathicity.

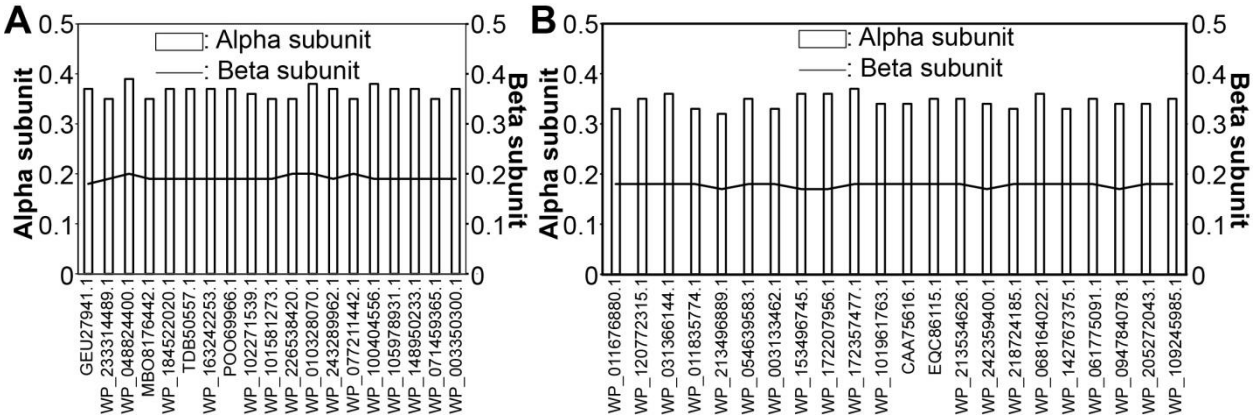

Figure 1. The ratios of alpha and beta subunits in the FtsZ proteins in (A) *Bacillus* and (B) *Lactococcus* species

As compared with the typical domains of FtsZ proteins (Löwe and Amos, 1998; Silber *et al.*, 2020) found in the Pfam domain (Finn *et al.*, 2014; Mistry *et al.*, 2021), the FtsZ proteins in the *Lactococcus* and *Bacillus* species clearly exhibited the existence of five distinct functional regions, namely the N-terminal peptide (NTP) region, a GTP-binding pocket, a C-terminal

linker (CTL), a C-terminal tail (CTT), and a C-terminal variable region (CTV) (**Figures 3 and 4**).

Particularly, the NTP regions identified in the FtsZ proteins from the *Lactococcus* and *Bacillus* genus groups were both reported to contain 25 aa residues (**Figures 3 and 4**). The NTP regions of these FtsZ proteins were recognized to end with the highly conserved isoleucine (I) residue (**Figures 3 and 4).** Of interest, the GTP-binding site was the conserved core domain of the FtsZ proteins. This region was specific with the appearance of the seven-aa-motif GGGTGTG (G and T meaning glycine and threonine, respectively) (**Figures 3 and 4**). The CTT region of the FtsZ proteins in the *Lactococcus* and *Bacillus* genus groups were recognized to contain approximately 10 aa residues (**Figures 3 and 4**). Among them, proline (P) and phenylalanine (F) were two highly-conserved aa found in the CTT region of the FtsZ proteins (Figures 3 and 4). Finally, the CTV region was defined as highly variable and harbored several aa residues. We found that two aa residues, arginine-lysine (RK), were highly conserved in the CTV region from the *Lactococcus* species (**Figure 3**), while three typical aa residues, leucine-arginine-asparagine (LRN), were specific in the CTV region from the *Bacillus* species (**Figure 4**).

Previously, the functional regions of the FtsZ proteins in various microorganism species have also been summarized (Silber *et al.*, 2020). For example, the NTP domain was poorly conserved among various bacterial genera and could contain a variety of dozens of aa residues (Silber *et al.*, 2020). Recent studies also confirmed the ending point of the NTP region with an I residue (Rossmann *et al.*, 1974; Silber *et al.*, 2020). For example, the NTP regions of the FtsZ proteins found in *Methanococcus jannaschii* and *B. subtilis* harbored 39 and 13 aa residues, respectively (Löwe, 1998; Raymond *et al.*, 2009). Up until now, the function of this part has been not assigned (Silber *et al.*, 2020). Interestingly, the GTP-binding region of the FtsZ proteins was reported to provide the interface for a head-to-tail polymerization of FtsZ proteins into proto-filaments (Scheffers *et al.*, 2002). Next, the CTV region of the FtsZ protein found in *B. subtilis* was reported to be highly positively charged with six conserved aa residues (NRNKRG) (Raymond *et al.*, 2009). This region plays an important role in the lateral interaction between FtsZ proteins and proto-filaments. The CTL region is an unstructured domain localized between the GTP-binding site and the CTT/CTV regions. The size of this region has been reported to be highly variable, up to 330 aa residues (Vaughan *et al.*, 2004). Recently, two substrate

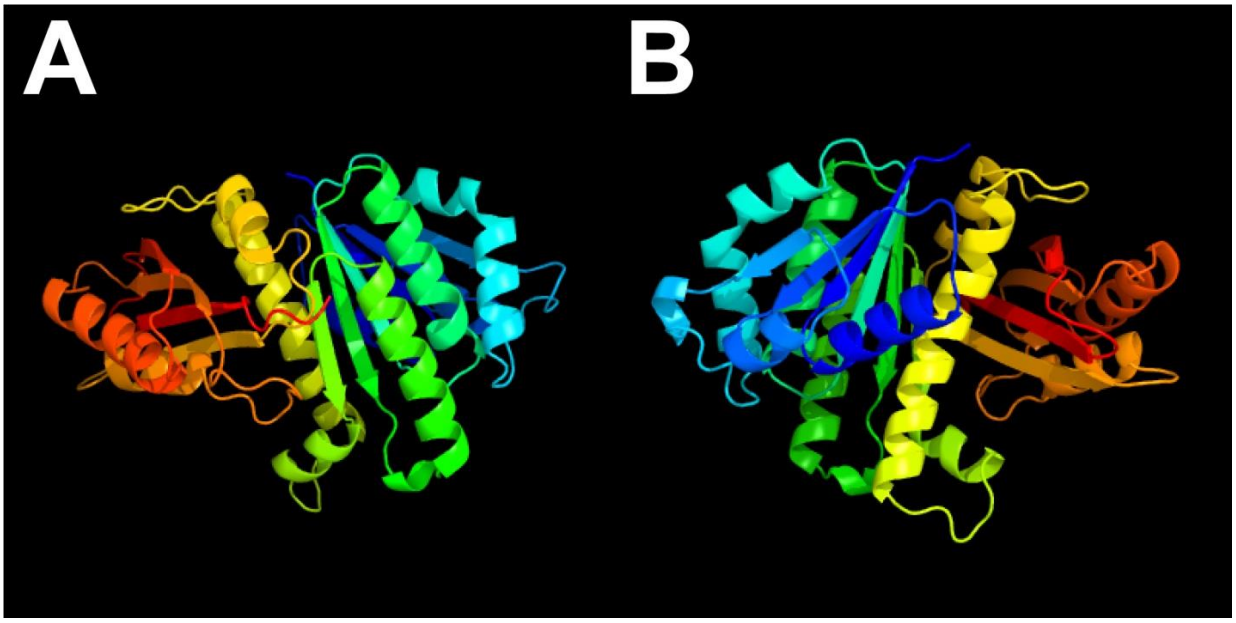

Figure 2. 3D models of the FtsZ proteins in the *Bacillus* and *Lactococcus* genus groups, namely (A) 'c2vxyA' and (B) 'c4dxdA'

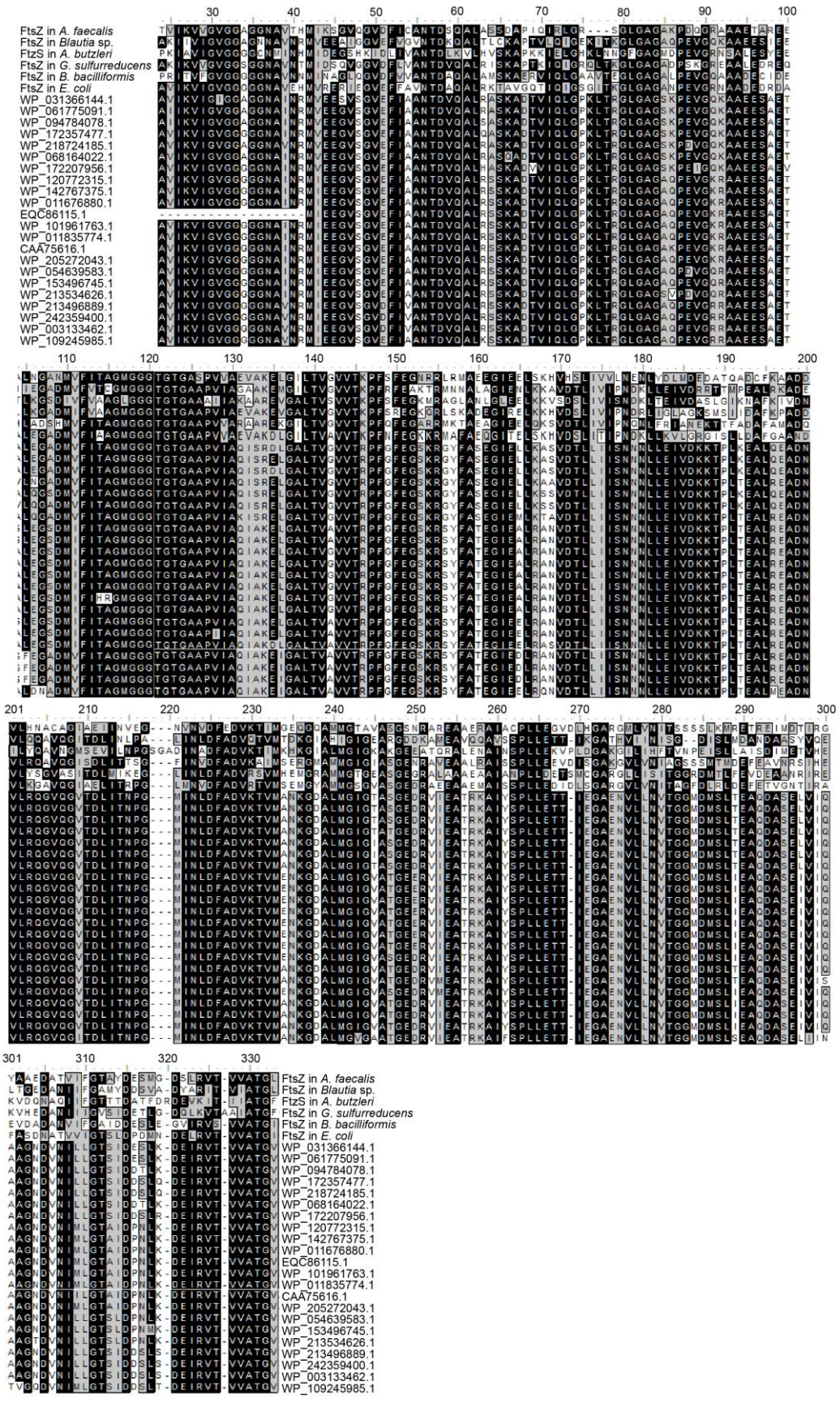

Figure 3. Functional regions of the the FtsZ proteins found in the *Lactococcus* genus group

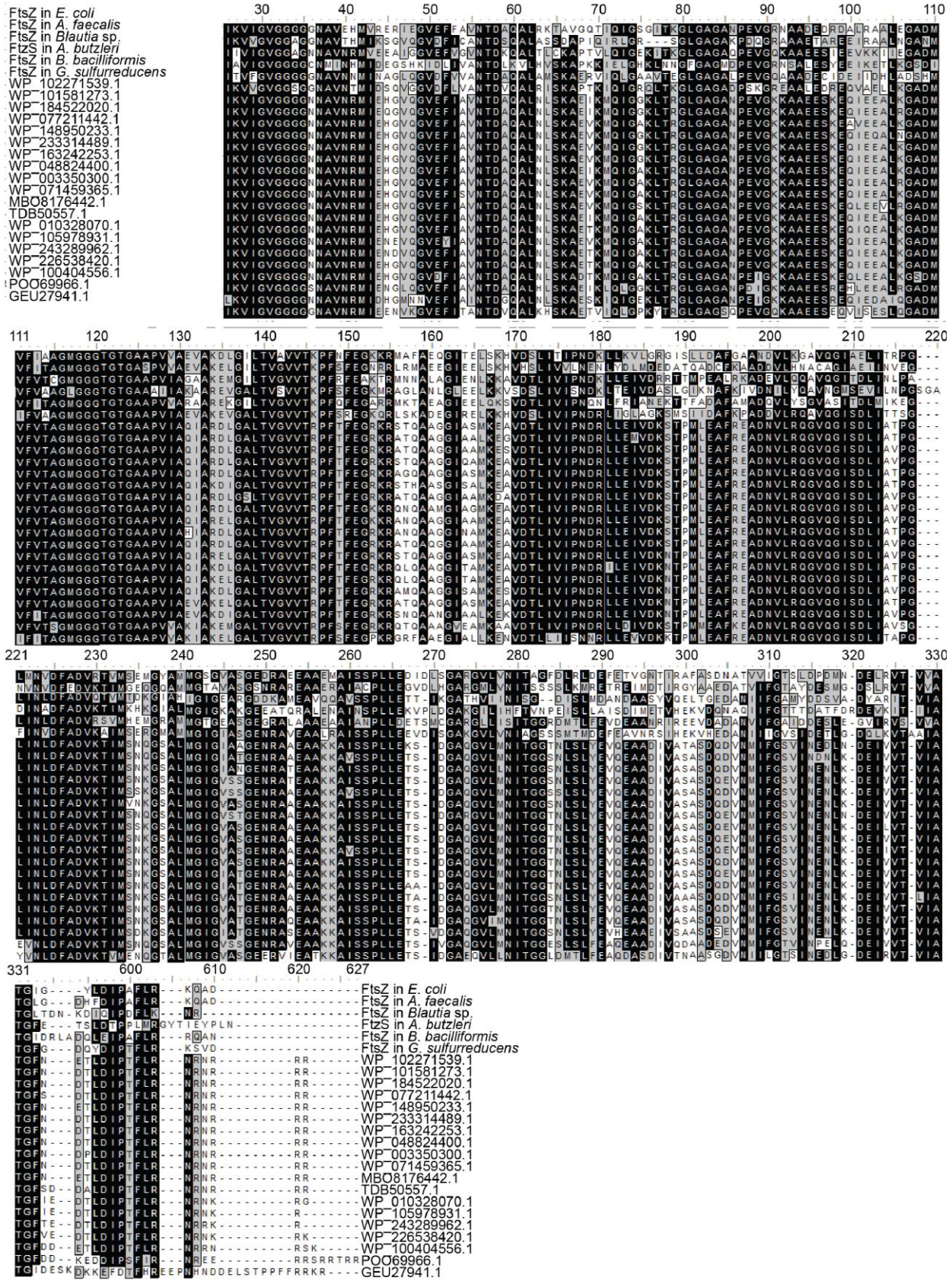

Figure 4. Functional regions of the FtsZ proteins found in the *Bacillus* genus group

binding sites, namely the nucleotide-binding domain and the inter-domain binding sites, on the FtsZ proteins were well-characterized in *Staphylococcus epidermidis* (Vemula *et al.*, 2023). The presence of these domains in the FtsZ proteins significantly made them perfect candidates for the development of broad-spectrum inhibitors (Battaje *et al.*, 2023; Di Somma *et al.*, 2023). Next, a comprehensive search revealed a total of eight and 113 FtsZ proteins in diverse archaea and bacteria, respectively (Makarova and Koonin 2010). Among them, the signature GTP-binding loop sequence, as well-characterized as GGGTGTG in the *Bacillus* and *Lactococcus* genera (**Figures 3 and 4**), were recognized to be the GTPase loop, which plays a crucial role in the hydrolysis of GTP and the subsequent disassembly of the protein (Makarova and Koonin, 2010).

Conclusions

In this study, a total of 19 and 22 FtsZ proteins were reported in the *Bacillus* and *Lactococcus* genus groups, respectively. Our analysis indicated that the FtsZ proteins in the *Bacillus* and *Lactococcus* species were slightly variable in size, mass, and II and AI scores, while these proteins were acidic and hydrophilic. All the FtsZ proteins in the *Bacillus* and *Lactococcus* genus groups were predicted to be localized in the cytoplasm. The multiple alignments obviously indicated that the conserved domain of the FtsZ proteins contained five distinct regions.

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