

## Supplementary data

### GLP Statement

**Study title** : Acute oral toxicity study of *Bacillus subtilis* IDCC 1101 in rat

**Study number** : TGK-2021-000125

#### Sponsor

**Name** : ILDONG BIOSCIENCE Co., LTD  
**Address** : 17, Poseunggongdan-ro, Poseung-eup, Pyeongtaek-si, Gyeonggi-do, Republic of Korea  
**Representative** : LEE JANG HYEY  
**Person in charge** : Yo Hwan Kim  
**Department and position** : Quality Control Team Manager  
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#### Test facility

**Name** : Korea Testing and Research Institute, Hwasun  
**Address** : 12-63, Sandan-gil, Hwasun-eup, Hwasun-gun, Jeollanam-do, Republic of Korea  
**Test facility management** : Park Myeong-kyu  
**Contact Number** : Tel. +82-61-370-7700 Fax, +82-61-370-7777

This study was conducted under the supervision of the study director and is in compliance with the principles of Good Laboratory Practice.

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#### 1. Good Laboratory Practice

1.1. OECD "Principles of Good Laboratory Practice" ENV/MC/CHEM (98)17 (as revised in 1997)

#### 2. Test regulation

2.1. OECD Guideline for testing of chemicals, Section 4, TG 423 "Acute Oral Toxicity-Acute Toxic Class Method" (Adopted : 17<sup>th</sup> December 2001)

*(Signed in the original report, annex 5)*

**Study director** Jang Seong-yong, M.S.

Date

*(Signed in the original report, annex 5)*

**Test facility management** Hong Seung-guk, M.S.

Date

\* This report was translated a Korean sentence into English according to sponsor's request by study director. The original data had not been changed.

**Translator** Jang Seong-yong, M.S.

Date

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### Quality Assurance Statement

**Study title** : Acute oral toxicity study of *Bacillus subtilis* IDCC 1101 in rat

**Study title** : TGK-2021-000125

Study Phases	Inspections	Reports to study director and Test facility management
Draft study plan audit	2021-03-25	2021-03-25
Study plan audit	2021-03-26	2021-03-26
Animal receipt audit	2021-03-30	2021-03-30
Body weight audit	2021-03-30	2021-03-30
Group assignment audit	2021-04-05	2021-04-05
Preparation of test substance audit (1)	2021-04-06	2021-04-06
Preparation of test substance audit (2)	2021-04-14	2021-04-14
Test substance administration audit	2021-04-06	2021-04-06
Clinical signs audit (1)	2021-04-06	2021-04-06
Necropsy audit (1)	2021-04-20	2021-04-20
Necropsy audit (2)	2021-05-14	2021-05-14
Raw data audit	2021-05-25	2021-05-25
Draft final report audit	2021-05-25	2021-05-25
Final report audit	2021-05-27	2021-05-27

Inspections of the routine and repetitive procedures that constitute the study was carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Quality Assurance Unit of Korea Testing and Research Institute, Hwasun and is considered to be an accurate account of the raw data generated and of the procedures followed.

Inspections were accomplished as noted, and reported to the study director and management immediately following their completion. Based on these inspections and the review of the report, this study was conducted and reported in conformance with the Good Laboratory Practice regulations.

**Quality assurance personnel**

*(Signed in the original report, annex 6)*

Seo Mu-yeb, M.S.

Date

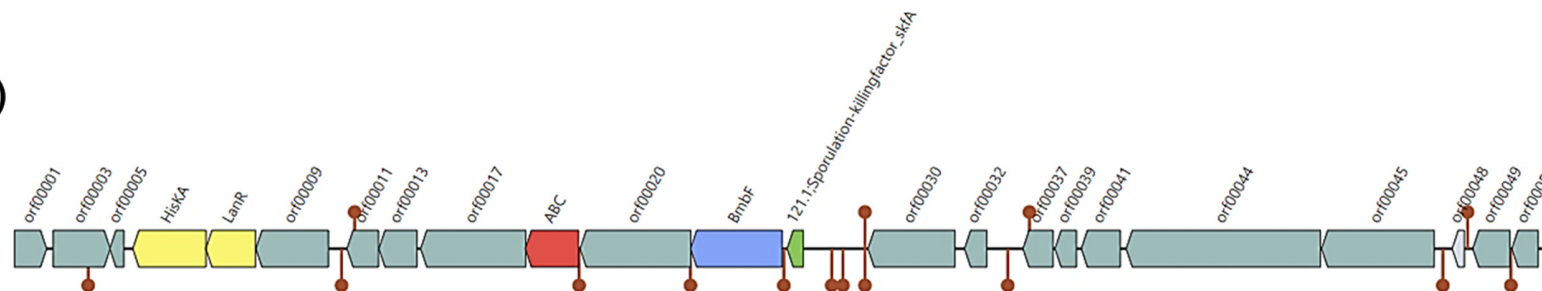
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**Figure S1.** The GLP and quality assurance statements for acute oral toxicity study of BS IDCC1101.

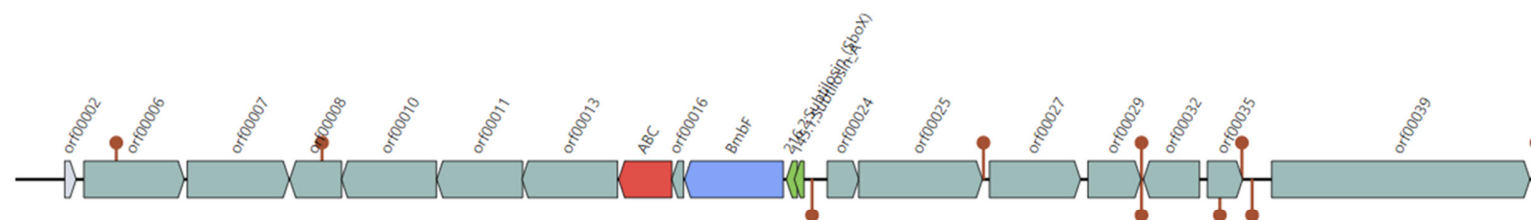
**Table S1.** Summary of BLASTn screening results of *B. subtilis* IDCC1101.

Description	Accession	Coverage	E-value	Identities (%)
<i>Bacillus subtilis</i> strain SRCM103971 chromosome, complete genome	CP035166.1	34.35	0.0	99
<i>Bacillus</i> sp. LM 4-2, complete genome	CP011101.1	34.7	0.0	99
<i>Bacillus subtilis</i> strain SRCM103551 chromosome, complete genome	CP035230.1	12.96	0.0	99
<i>Bacillus subtilis</i> strain QB61 chromosome, complete genome	CP029461.2	11.21	0.0	99
<i>Bacillus subtilis</i> strain SRCM103696 chromosome, complete genome	CP035394.1	11.07	0.0	99

(A)



(B)



■ Blast hit with UniRef90  
■ Core Peptide  
■ Modification  
■ Immunity / Transport  
■ Regulation

Figure S2. Organization of bacteriocin cluster genes.

**Table S2.** Biochemical properties of *Bacillus subtilis* reported for use as a commercial probiotic

<i>B. subtilis</i>	Source	Antibiotic resistance (MIC µg/mL)	Enzyme activity	L-/D- lactate	BA <sup>1)</sup>	Hemolytic activity	Cell cytotoxicity		Acute oral toxicity	Ref.
							Cell	Cytotoxicity		
BS IDCC1101	Cheonggukjang	Streptomycin (64)	ND	ND	ND	γ	HaCaT	Non-toxic	Non-adverse	
BS50	Soil	Streptomycin (125)	- <sup>2)</sup>	-	-	α	Caco-2	Non-toxic	-	[1]
CU1	Soil/water	ND	-	-	-	γ	Vero	Non-toxic	-	[2]
MB40	Soil	Streptomycin (>32)	-	-	-	-	-	-	Non-adverse	[3]
Natto	Natto	ND	-	-	-	γ	HT29-16E	Non-toxic	Non-adverse	[4]
PY79	Straw	-	-	-	-	-	HEp-2, Caco-2	Non-toxic	-	[5]
VKPM B2335	Straw	Oxacillin*	-	-	-	γ	Caco-2 and mucin	Low adhesion	Non-adverse	[6]
KATMIRA1933	Yogu Farm	Streptomycin, bacitracin*	-	-	-	β	-	-	-	[7]

<sup>1)</sup> BA: biogenic amine.

<sup>2)</sup> -: not tested; ND: not detected.

\*MIC was not investigated since they used paper disc method.

**Table S3.** Mortality and Clinical signs of female rats.

Group	Dose (mg/kg B.W.)	Mortality	Animal number	Hours after administration					Days after administration													
				0.5	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
G1	300	0% (0/3) <sup>a</sup>	2101	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			2102	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			2103	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
G2	300	0% (0/3)	2201	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			2202	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			2203	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
G3	2000	0% (0/3)	2301	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			2302	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			2303	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
G4	2000	0% (0/3)	2401	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			2402	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			2403	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

B.W.: Body weight, <sup>a</sup>: Number of dead animals/Number of tested animals, N: Normal

**Table S4.** Necropsy findings of female rats.

Group	Dose (mg/kg B.W.)	Animal number	Removal reason	Necropsy findings	
				External	Internal
G1	300	2101	Terminal sacrifice	No gross findings	No gross findings
		2102	Terminal sacrifice	No gross findings	No gross findings
		2103	Terminal sacrifice	No gross findings	No gross findings
G2	300	2201	Terminal sacrifice	No gross findings	No gross findings
		2202	Terminal sacrifice	No gross findings	No gross findings
		2203	Terminal sacrifice	No gross findings	No gross findings
G3	2000	2301	Terminal sacrifice	No gross findings	No gross findings
		2302	Terminal sacrifice	No gross findings	No gross findings
		2303	Terminal sacrifice	No gross findings	No gross findings
G4	2000	2401	Terminal sacrifice	No gross findings	No gross findings
		2402	Terminal sacrifice	No gross findings	No gross findings
		2403	Terminal sacrifice	No gross findings	No gross findings

B.W.: Body weight

## References

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