Does the applicability of *Bacillus* strains in probiotics rely upon their taxonomy?

L. A. Safronova[1], L. B. Zelena[1], V. V. Klochko[1], O. N. Reva[2]

[1]Institute of Microbiology and Virology of the National Academy of Science of Ukraine, 154 Zabolotnogo Str., Kiev D-03680, Ukraine.

[2]University of Pretoria, Dep. Biochemistry, Lynnwood Rd., Hillcrest, Pretoria, South Africa.

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Correspondence

Oleg N. Reva, Bioinformatics and Computational Biology Unit, Biochemistry Department, University of Pretoria, FABI-II building, Lynnwood Rd., Hillcrest, Pretoria 0002, South Africa. E-mail: oleg.reva@up.ac.za

Abstracts

The taxonomic position and biological activities of two *Bacillus* strains used in veterinary probiotics were studied in this work. These microorganisms inhibit growth of a broad spectrum of pathogenic cultures. They synthesize proteolytic enzymes and other biologically active metabolites, and to some extent, supplement each other with probiotic activities. It is not clear whether these versatile activities are properties of individual strains or bacterial taxa as whole. 16S rRNA comparisons were conducted and illustrated the relatedness of these strains to *Bacillus amyloliquefaciens*. Their cell wall fatty acid contents were consequently analysed and specified a

relation to the '*B. velezensis*' ecomorph. On account of the previous observations, a simple method of 16S rRNA profiling by polymorphic nucleotides was proposed to determine a group of organisms closely related to '*B. velezensis*' and *B. amyloliquefaciens* ssp. *plantarum*, for they are biologically active strains suitable for use in biotechnology. The extreme genetic plasticity of these bacteria endowed each strain with a unique spectrum of antagonistic activity.

Probiotics are widely used for the treatment of infections and dysbacteriosis of different aetiology, as well as diseases associated with immune deficiency (Gareau *et al*., 2010). They may suppress pathogenic and conditionally pathogenic microflora and stimulate the host immune response of macroorganisms. The clinical efficiency of probiotics is complemented by the absolute safety of their use. These are also believed to be able to supplement or even replace antibiotics and their preparatory measures.

Majority of the widely used probiotics composes of representatives of the resident intestinal microflora i.e., *Escherichia coli*, *Lactobacillus* and *Bifidobacterium*. Probiotics of *Bacillus* which belong to the transitory intestinal microflora, have also been reported to be widely used as well (Casula & Cutting, 2002; Duc *et al*., 2004; Sorokulova *et al*., 2008). *Bacillus subtilis* and related organisms demonstrate active suppression and ousting of pathogenic bacteria, however, the exact mechanisms of this activity remain obscure. In contrast to *Lactobacillus* producing a substantial amount of lactic acid that inhibits growth of other micro-organisms, *B. subtilis* convert glucose and other carbonate sources to neutral acetoin, glycerol and 2,3-butanediol as the main products (Blackwood *et al*., 1947). It was reported that antibacterial function in *B. subtilis* and *B. amyloliquefaciens* is caused at least partly by nonribosomally produced lipopeptides acting against pathogenic viruses, bacteria and fungi (Koumoutsi *et al*., 2004).

According to the Guidelines for the Evaluation of Probiotics in Food published by the World Health Organization the precise species identification of candidate strains is required in line with

the practical efficacy and safety control. *Bacillus* species identification is rather complicated as there are many sibling species which have been proposed and yet cannot be distinguished by the phenotype. The latter applies to species which relate to *B. subtilis* vernacularly termed as *B. subtilis* group of microorganisms (Reva *et al*., 2004; Wang *et al*., 2007; Connor *et al*., 2010). This group composes of microorganisms such as *B. amyloliquefaciens*; *B. atrophaeus*, *B. axarquiensis*, *B. malacitensis*, *B. mojavensis* and *B. vallismortis* (Wang *et al*., 2007; Rooney *et al*., 2009). A broad industrial use of these organisms has attracted a scrupulous attention to their systematics and taxonomy. There is a general belief which bases both on practical evidences and common sense expectations that the taxonomic position of a strain predefines its applicability in biotechnology. However, it is not clear whether the probiotic activities are shared by members of a species or higher taxonomic units and also to what extent do these activities remain unique to each individual strain. The introduction of molecular methods into taxonomic studies improved our understanding towards the mechanisms of positive action of probiotics, and also allowed us to attend to the question mentioned in the previous statement. In this work two *Bacillus* sp. 39 and 51 strains (catalogued as UCB B-5139 and UCB B-5140, respectively) used in veterinarian probiotic Endosporin were investigated and compared to other related organisms. These microorganisms inhibit growth of many pathogenic bacteria. They synthesize proteolytic enzymes and other biologically active secondary metabolites, and to some extent, supplement each other with their probiotic activities.

The two *Bacillus* sp. 39 and 51 strains are widely used in probiotic Endosporin for prophylaxis and treatment of endometritis, intestinal infections, festering wounds and postpartum pyoinflammatory complications in cattle. Endosporin shows better efficiency and no side effect manifestations as compared to the traditional etiotropic drugs such as Tricyllinum and Furazolidone (patents UA76669 and UA14569C1; and Safronova *et al*., 2009). These active cultures were chosen from more than 300 candidate strains of *Bacillus*-antagonists tested in the laboratory and field conditions. Antagonistic activity was evaluated at least three times per a

strain by measuring diameters of growth inhibition zones. *Bacillus* cells were grown overnight and resuspended in 9 g/l NaCl water solution to reach 2×10^9 cells/ml. These were consequently inoculated into centres of Petri dishes of 90 mm in diameter with 20 ml of solid growth medium. The growth medium contained 30 ml/l of Hottinger broth (Medgamal LC-001732, a detailed composition of the Hottingen broth is provided at www.vkm.ru/catalog/text/media.txt); 5 g/l of NaCl; 5 g/l of Bacto Peptone powder; 10 g/l of glucose; 30 g/l of bacterial agar. The pH of the medium used in the experiment was adjusted to 7.2±0.2 but not buffered. Petri dishes with inoculated *Bacillus*-antagonists were incubated for 3 days at 28˚C. Test-cultures were grown overnight and resuspended in water to reach 5×10^8 cells/ml as measured by OD_{650} density. A loopful of bacterial suspension was streaked from the edge of the Petri dish to the centre, 2 mm away from the antagonist growth. Paired cultures were incubated overnight at 37˚C. Test-culture inhibition zones were measured and the average values of three repetitions were calculated (Table S1). The antagonistic spectra of the strains *Bacillus* sp. 39 and 51 supplement each other. Strain 39 is the most active against *Staphylococcus aureus*, *E. coli*, *Proteus morganii* and several fungal pathogens. Strain 51 also inhibits growth of these cultures moderately but it is highly active against *Pseudomonas aeruginosa*, *Acinetobacter* sp. and *Clavibacter michiganensis*. These strains showed to be the most active inhibitors of growth of pathogens in comparison to other 96 selected antagonists, belonging to the *B. subtilis – B. amyloliquefaciens* group (Fig. 1 and Table S1). A noteworthy fact is that strain 51 is highly active against *P. aeruginosa,* a bacterium which cannot be inhibited at all by the majority of *Bacillus* antagonists. It may be concluded from the analysis of spectra of antagonistic activity that strains 39 and 51 produce quite unique and dissimilar sets of antibacterial compounds. It thus became of practical and scientific interest to investigate the taxonomic position of these two organisms.

Species identification of *Bacillus* by phenotype was performed as described previously (Reva *et al*., 2001) and by the use of a API 50 CHB/E (Biomerieux, France) (Logan and Berkley, 1984). The two strains were therefore determined to be part of the *B. subtilis* group (data not shown).

Sequencing of 16S rRNA genes and comparisons of cellular fatty acids were performed for more accurate identification. Bacterial DNA was extracted from overnight cultures by the DNA-sorb B extracting KIT (Russia). 16S rRNA was amplified with primers 27f and 1492r according to the standard protocol (Lane, 1991). Amplified DNA fragments were sequenced with the same primers in two directions on ABI 310 (Applied Biosystems). Base calling quality control and DNA read alignment were performed using the Staden Package programs (Staden *et al*., 2000). Resulted partial sequences of 16S rRNA genes of strains 39 and 51 were submitted to GenBank under the accession numbers JF346868 and JF346869, respectively.

Despite the noticeable differences in the spectra of antagonistic activities of strains 39 and 51, their 16S rRNA sequences were identical. A BLASTN search of these sequences against the GenBank database resulted in 100 % matches with multiple fragments of *B. amyloliquefaciens* and '*B. velezensis*'. The latter organism is now considered as a synonym of *B. amyloliquefaciens* (Wang *et al*., 2008) and represents a detached group of strains within this species. A tree graph of phylogenetic relationships of strains 39 and 51 with the type strains of several other *Bacillus* species is shown in Fig. 2. It was concluded that the strains of interest belong to the *B. amyloliquefaciens* / '*B. velezensis*' lineage.

Species identification within the *B. subtilis* group by the use of 16S rRNA sequences is problematic, firstly because this gene is very conserved and the differences between species may count only for few nucleotides that are comparable with the level of sequencing errors. Secondly, the genomes of organisms forming part of this group usually contain 9 to 10 alleles of 16S rRNA genes which may be relatively variable (Kunst and Devin, 1991). To optimize the identification approach, special attention should be paid to profiling the most variable nucleotides instead of comparing the whole sequences of 16S rRNA against each other. This approach was used before by Goto *et al*. (2000) and in our previous work (Reva *et al*., 2004) and showed to be probable.

Polymorphic nucleotides were defined by positional entropy values above 0.3 as determined by the BioEdit Entropy plot tool from a given set of 33 aligned 16S rRNA sequences (see Table 1).

The determined polymorphic nucleotides were then mapped onto the secondary structure of the lower body of 16S rRNA small subunit of *B. subtilis* ssp. *subtilis* 168 obtained from the Comparative RNA Web portal (www.rna.ccbb.utexas.edu). In total 9 polymorphic nucleotides were identified, as they showed significant entropy values in the sequence alignment. The distribution of variable nucleotides is not random (Fig. 3). The polymorphic sites are either the counterparts in the 16S rRNA molecular structure linked to each other by hydrogen bonds, or they are solitary nucleotides adjacent to the structural loops. The profiles of polymorphic nucleotides constructed for the available 16S rRNA sequences of the studied probiotic strains and other microorganisms affiliated with the *B. subtilis* group (Reva *et al*., 2004) as well as different alleles of 16S rRNA in the genomes of *B. subtilis* ssp. *subtilis* 168, *B. subtilis* ssp. *spizizenii* W23, *B. amyloliquefaciens* ssp. *amyloliquefaciens* DSM7^T and *B. amyloliquefaciens* ssp. *plantarum* FZB42^T (see Table 1). As the obtained profiles were too short to be considered as real DNA sequences, their phylogenetic links were inferred by the basic parsimony algorithms implemented in Phylip *pars.exe*. The resulted cladogram is shown in Fig. 4.

Several interesting facts may be concluded from this analysis. Firstly, the species of this group form two clearly separated clusters – the one is for *B. subtilis* (*B. axarquiensis*, *B. malacitensis*, *B. mojavensis* and *B. sonorensis*) and the other is gravitated towards *B. amyloliquefaciens* (*B. atrophaeus*, *B. vallismortis* and '*B. velezensis*'). The next finding is that inside these clusters the species cannot be reliably distinguished basing on 16S rRNA comparisons as the level of variation between species is the same as between subspecies and even between different alleles of this gene in one genome (Tabel 4 and Fig. 3). This finding thus questions the taxonomic consistency of this group of microorganisms. However, the taxonomic position of the strains 39 and 51 was narrowed down by this approach to either *B. amyloliquefaciens* ssp. *plantarum* or '*B. velezensis*'.

To clarify the taxonomic position of strains 39 and 51, contents of the cell wall fatty acids were analysed. Cellular fatty acids have long been used in bacterial taxonomy (Athalye *et al*., 1985),

Bacillus taxonomy in particular (Kaneda, 1977; Rooney *et al*., 2009; Borriss *et al*., 2010; Connor *et al*., 2010). The fatty acid contents of bacterial cell walls were analysed using a Agilent 6890N gas chromatograph equipped with the Agilent 5973 inert column HP-5MS (J&W Scientific, USA). The analysis was carried out with the following specifications: Carrier gas – helium, starting temperature of the column – 150°C; final temperature of the column – 250°C; temperature gradient – 4°C degrees per min; interface temperature – 280°C; ionisation type – electron impact; ionisation energy – 70 eV. The obtained raw data was analysed using the software package ChemStation supplied with an integrated mass-spectrogram database NIST 02. The standard set of methyl ethers of bacterial fatty acids (Supelco, № 4708-U, USA) was used as a control. All measurements were done three times in a row followed by calculations of the average fatty acid content.

The results of the analysis are shown in Table 2. Both strains displayed similar fatty acid profiles and were clearly distinguishable from both *B. subtilis* and *B. amyloliquefaciens*. According to Borriss *et al*. (2010) measures for all *B. amyloliquefaciens* fatty acid profiles are characteristic when the high percentage of i-C15:0 is in range 26 to 40% and C17:0 in range 3 to 11%. Thus, strains 39 and 51 were found to possess 24-25% of i-C15:0 and 1% and below of C17:0. A percentage of i-C15:0 was the same as in *B. amyloliquefaciens* cells but significantly smaller than in *B. subtilis*. In general the fatty acid profiles of strains 39 and 51 resemble those reported for '*B. velezensis*' (Ruiz-García *et al*, 2005). A remarkably high percentage of C16:0 around 10% is specific for '*B. velezensis*' (see Table 2).

It was unexpected for two strains which were selected from a big collection of *Bacillus* isolates to show alternative spectra of strong antagonistic activity and belong to the same species or even subspecies. It has deliberately been observed that a number of strains chosen independently from different laboratories as the most effective inhibitors of phytopathogens all appear to be genetically related bacteria. All these strains have recently been defined as *B. amyloliquefaciens* ssp. *plantarum* (Reva *et al*., 2004; Borriss *et al*., 2010), a taxon that is closely related to

'*B. velezensis*' (Fig. 4) which the strains 39 and 51 belong to. The availability of the complete genome sequences of *B. amyloliquefaciens* ssp. *amyloliquefaciens* DSM7^T and *B. amyloliquefaciens* ssp. *plantarum* FZB42^T shed light on peculiarities of their genomes (Rückert *et al*., 2011). *B. amyloliquefaciens* ssp. *plantarum* contains more genes encoding polypeptide antibiotics than the type strain of this species. Genes of polypeptide and polyketide synthases make up 10% of the chromosome of FZB42^T (Chen *et al*., 2009). A comparative survey of polypeptide and polyketide biosynthesis (Borriss *et al*., 2010) and polypeptide synthases targeted genotyping (Reva, 2005) demonstrated that they are the most polymorphic and fast evolving genes which determine the antagonistic activity of *Bacillus*. Manufacturers of probiotics and biopesticides should draw close attention to groups of organisms which are related to *B. amyloliquefaciens*. However, it has to be taken into consideration that the extreme genetic plasticity of these bacteria endowed each strain with a unique spectrum of antagonistic activity. Apart from the fact that *Bacillus* species are problematic to identify, it has to be noted that the bacteria forming part of this group may easily be pinpointed in a large scale screening by characteristic profiling of 16S rRNA and fatty acid content.

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Figure legends

Fig. 1. Comparison of antagonistic activity of 98 strains of *B. subtilis – B. amyloliquefaciens* in relation to pathogenic cultures: 1) *Candida albicans* 690; 2) *Escherichia coli* 070; 3) *Proteus vulgaris* U-8; 4) *Salmonella typhi* 11; 5) *Staphylococcus aureus* 209; 6) *Pseudomonas aeruginosa* 4141; and 7) *Clavibacter michiganensis* ssp. *michiganensis* 7905. Gray boxes show the range of the activity from the minimal to maximal value registered in this group, and the white bars depict average values. Activities of the strains 39 and 51 are indicated by open cycles and diamonds, respectively.

Fig. 2. Phylogenetic tree calculated by NJ algorithm based on 16S rRNA sequence alignment. For the boot-strap analysis 100 replicas of the sequence alignment have been generated.

Fig. 3. Secondary structure of the low body of small subunit of 16S rRNA 5'-[1–575]-3'. Polymorphic nucleotides are numbered from 1 to 9 and depicted by bold typeface.

Fig. 4. The cladogram is a parsimony tree based on profiles of polymorphic nucleotides of 16S rRNA.

Table 1. Profiles of polymorphic nucleotides in DNA sequences of microorganisms of the *B. subtilis* group.

Species, strains and alleles	Positions of polymorphic nucleotides								
	180th	185th	202nd	234th	271st	285th	465th	472nd	483rd
G1. *B. amyloliquefaciens* ssp. *amyloliquefaciens* DSM7 alleles A and B; *B. amyloliquefaciens* ssp. *plantarum* FZB42 alleles A, B, G and J; and UCM B-5017, UCM B-5033, UCM B-5036, UCM B-5044, UCM B-5113, At1 and At4;	G	C	G	G	C	G	G	A	C
G2. *B. amyloliquefaciens* DSM7 alleles C, D, E, F, F, H, I and J;	C	T	A	G	C	G	G	A	C
G3. *B. amyloliquefaciens* FZB42 alleles C, D, E, H and I; '*B. velezensis*' C6-1 and 1-3; strains 39 and 51;	G	T	G	G	C	A	G	A	C
G4. '*B. velezensis*' CR-502^{T}	G	T	G	A	C	A	G	A	C
G5. *B. atrophaeus* DSM 7264^{T} and *B. vallismortis* BCRC 17183	C	T	A	G	C	A	G	A	C
G6. *B. atrophaeus* ATCC51189, GBSC56,	C	T	A	G	T	A	G	A	C

LSSC22; *B. vallismortis* DSM 11031^{T}; **G7**. *B. axarquiensis* LMG 22476 and LNXM37; *B.malacitensis* LMG 22477; *B. mojavensis* eela 2293 and 3EC4B2; *B. subtilis* subsp. *spizizenii* DSM 347^{T} and ATCC 6633 alleles A and B;	C	T	A	G	T	A	A	G	T
G8. *B. mojavensis* DSM 9205^{T} and *Bacillus* sp. UCM B-5051 and At3;	C	T	A	G	C	A	A	G	T
G9. *B. subtilis* ssp. *subtilis* DSM 10^{T} and 168 alleles B, D, G, H and W; *B. subtilis* UCM B-5184, At2 and At5;	G	T	A	G	C	A	A	G	T
G10. *B. subtilis* ssp. *subtilis* 168 alleles E and I; and *B. subtilis* UCM B-5049 and UCM B-5137	G	T	A	G	T	A	A	G	T
G11. *B. subtilis* 168 allele A	G	T	A	G	C	G	A	G	T
G12. *B. subtilis* 168 allele C	G	T	A	G	C	G	G	G	T
G13. *B. subtilis* 168 allele J	G	T	A	G	T	A	G	G	T
G14. *Bacillus sp.* UCM B-5008 and UCM B-5075	C	T	A	A	C	A	A	G	T
G15. *Bacillus sp.* UCM B-5014	C	T	A	A	T	A	A	G	T

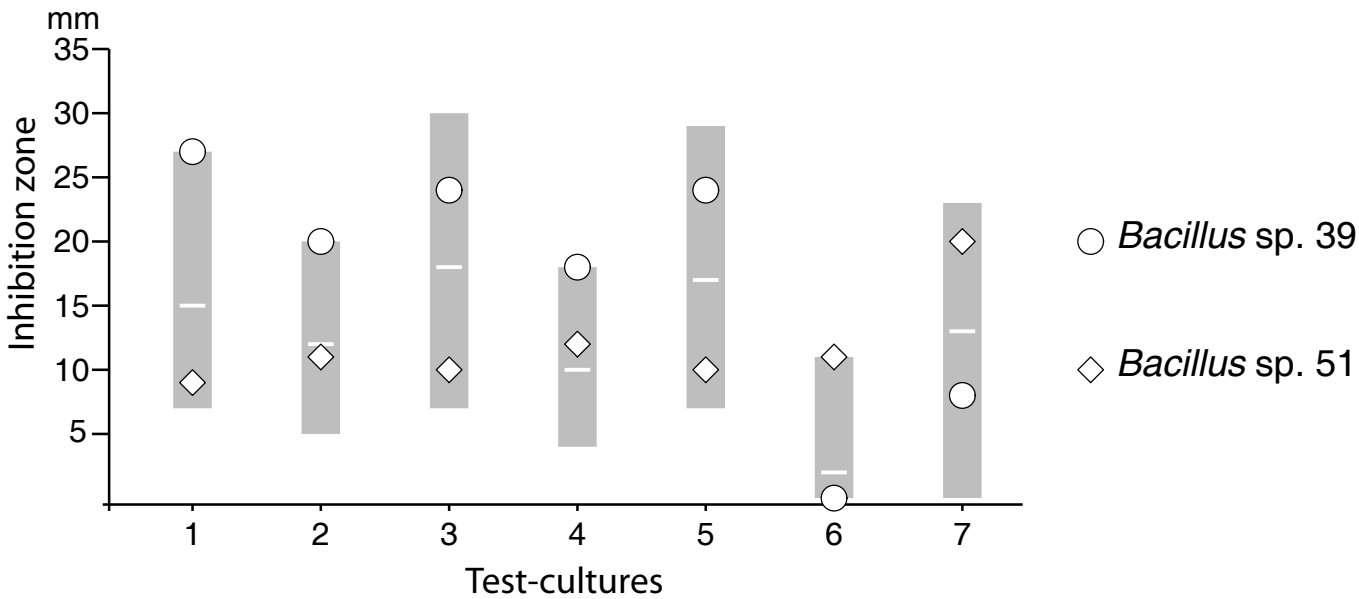
G16. *B. sonorensis* strain NRRL B-23154	C	T	A	G	G	G	A	G	A

Note: nucleotides are numbered as in Fig. 3 from 5'-end of the 16S rRNA gene. UCM and At strains are from the Ukrainian Collection of Microorganisms (see Reva *et al*., 2004).

Table 2. Cellular fatty acid content.

	Percentage of fatty acids					
	39	51	CCUG	DSM7	FZB42	CR502
i-C14:0	1,00	1,53	1,6	0,99	0,43	1,08
C14:0	0,99	0,83	–	0,36	1,21	2,96
i-C15:0	**24,75**	**24,35**	**20,6**	**40,29**	**31,0**	**29,86**
ai-C15:0	**29,38**	**31,96**	**44,1**	**28,32**	**31,73**	**32,7**
C15:0	2,24	2,36	–	–	–	–
i-C16:0	2,54	4,44	2,4	2,13	1,01	1,31
7-C16:1	0,37	0,45	–	0,42	0,19	–
C16:0	**10,15**	**7,40**	**5,7**	**–**	**–**	**13,41**
i-C17:0	15,23	13,63	10,9	13,14	12,11	7,67
ai-C17:0	11,15	11,95	15,8	–	–	4,27
C17:0	**0,62**	**0,56**	**–**	**6,46**	**7,70**	**–**
C18:2	0,24	0,00	–	–	–	–
cis-C18:1	0,44	0,00	–	–	–	–
C18:0	0,91	0,56	–	–	–	–

Note: 39 – *Bacillus* sp. 39; 51 – *Bacillus* sp., 51; CCUG – *B. subtilis* CCUG 10779 (as at www.ccug.se); DSM7 – *B. amyloliquefaciens* ssp. *amyloliquefaciens* DSM 7^T (Borriss *et al*., 2011); FZB42 – *B. amyloliquefaciens* ssp. *plantarum* FZB 42^T (Borriss *et al*., 2011); CR502 – '*B. velezensis*' CR 502^T (Ruiz-García *et al*, 2005); "–" – data not available. Descriptive fatty acids are shown in bold typeface.


mm
35
30
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Inhibition zone
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2
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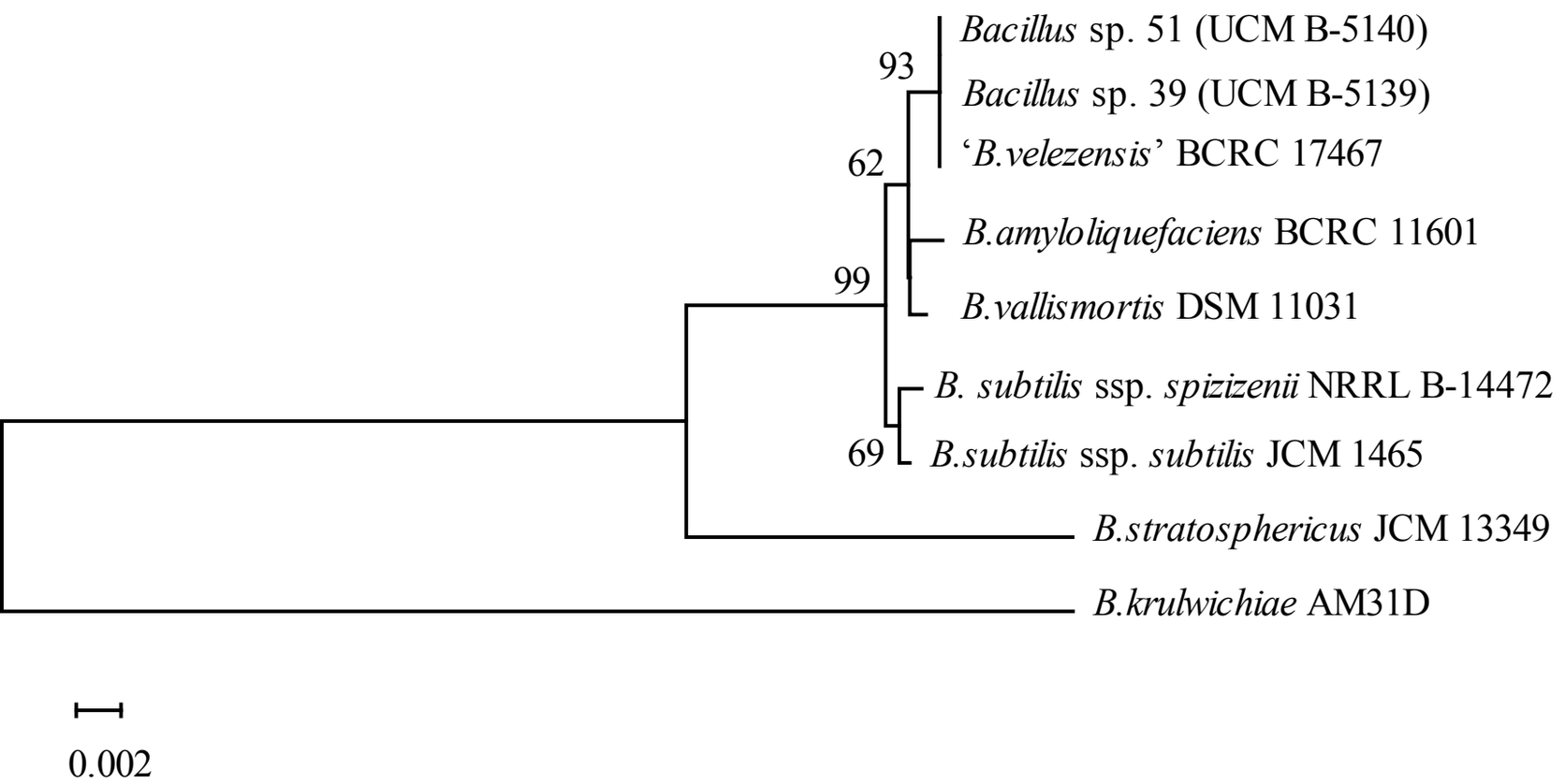
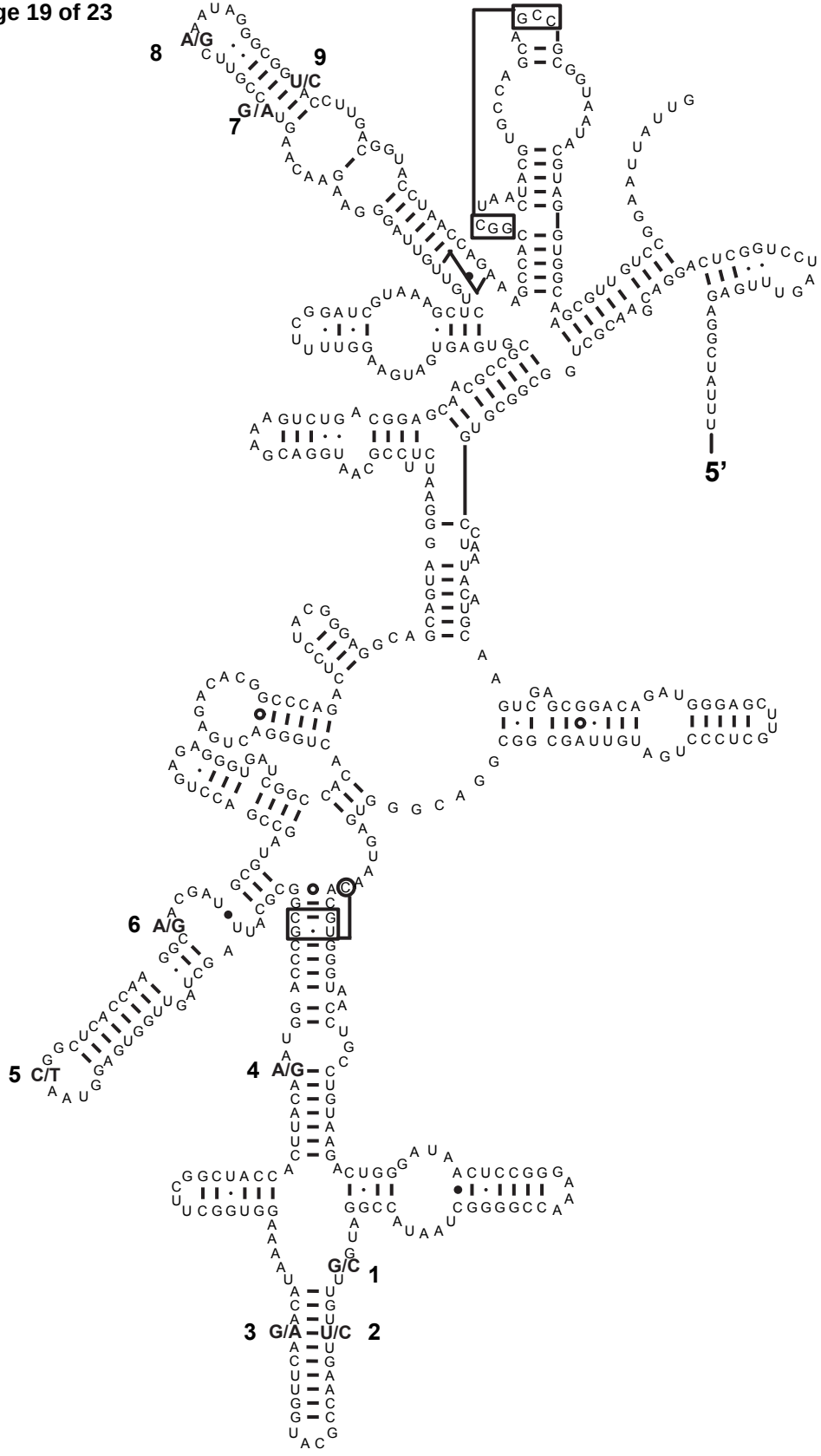
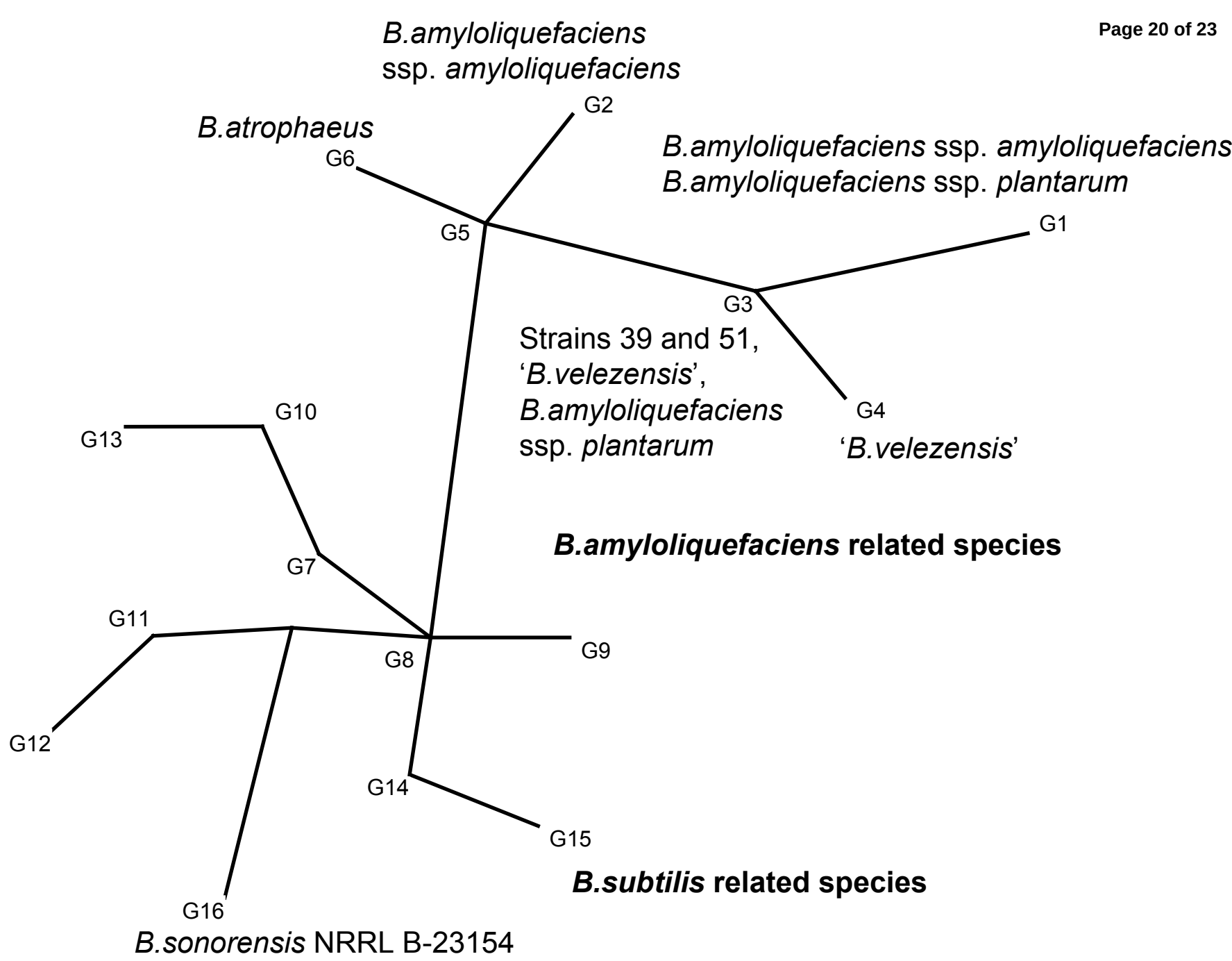
Test-cultures
Bacillus sp. 39
Bacillus sp. 51


Bacillus sp. 51 (UCM B-5140)
93
Bacillus sp. 39 (UCM B-5139)
'B.velezensis' BCRC 17467
62
B.amyloliquefaciens BCRC 11601
99
B.vallismortis DSM 11031
B. subtilis ssp. spizizenii NRRL B-14472
69
B.subtilis ssp. subtilis JCM 1465
B.stratosphericus JCM 13349
B.krulwichiae AM31D
0.002


8
A/G
9
U/C
7
G/A
5'
6
A/G
5
C/T
4
A/G
3
G/A
U/C
2
1
G/C


B.amyloliquefaciens
ssp. *amyloliquefaciens*
G2
B.atrophaeus
G6
B.amyloliquefaciens ssp. *amyloliquefaciens*
B.amyloliquefaciens ssp. *plantarum*
G5
G1
G3
Strains 39 and 51,
'*B.velezensis*',
B.amyloliquefaciens
ssp. *plantarum*
G4
'*B.velezensis*'
G10
G13
***B.amyloliquefaciens* related species**
G7
G11
G9
G8
G12
G14
G15
***B.subtilis* related species**
G16
B.sonorensis NRRL B-23154

Supplementary table 1. Antagonistic activity of strains of the *B. subtilis – B. amyloliquefaciens* group expressed in the inhibition zone diameters (mm).

Strains	***Candida albicans* 690**	***Escherichia coli* O70**	***Proteus vulgaris* U8**	***Salmonella tuphimurium* 11**	***Staphylococcus aureus* 209**	***Pseudomonas aeruginosa* 4141**	***Clavibacter michiganensis* ssp. *michiganensis* 7905**
UCM B-5001	19	16	22	13	22	3	16
UCM B-5002	23	19	28	16	28	4	21
UCM B-5007	16	14	19	12	19	2	14
UCM B-5008	15	13	17	11	17	2	12
UCM B-5009	16	14	20	12	19	2	13
UCM B-5010	15	12	18	11	17	2	12
UCM B-5011	19	16	23	13	23	3	17
UCM B-5012	12	11	13	9	13	1	8
UCM B-5014	11	9	12	8	13	1	8
UCM B-5015	15	12	17	10	17	2	12
UCM B-5016	16	13	18	11	18	2	13
UCM B-5017	16	13	19	11	20	4	15
UCM B-5019	17	14	20	12	19	2	14
UCM B-5021	19	16	23	14	23	4	18
UCM B-5022	13	11	16	10	16	1	10
UCM B-5024	21	18	26	15	25	4	20
UCM B-5025	19	16	23	13	23	3	17
UCM B-5027	11	10	12	8	13	1	7
UCM B-5028	11	8	11	7	12	1	7
UCM B-5029	13	11	15	10	15	1	10
UCM B-5030	18	16	22	13	21	3	16
UCM B-5031	15	13	17	11	17	1	12
UCM B-5032	13	10	15	9	15	2	10
UCM B-5033	15	12	17	10	17	2	12
UCM B-5034	15	13	18	11	18	2	12
UCM B-5035	22	18	26	15	26	4	20
UCM B-5036	14	11	17	10	17	3	12
UCM B-5037	12	10	14	8	14	1	9
UCM B-5038	16	13	19	11	19	3	15
UCM B-5039	22	17	26	15	26	4	20
UCM B-5040	14	11	18	10	17	2	13
UCM B-5041	14	11	16	9	16	2	11
UCM B-5042	20	16	24	13	23	4	19
UCM B-5043	14	13	16	10	16	1	11

UCM B-5044	13	11	16	9	15	1	11
UCM B-5045	13	11	16	9	16	1	11
UCM B-5047	19	15	23	13	22	3	16
UCM B-5049	14	13	17	10	16	2	12
UCM B-5051	19	16	24	13	23	4	18
UCM B-5052	17	13	20	11	20	3	15
UCM B-5054	18	15	22	12	21	3	16
UCM B-5055	18	15	22	13	22	3	16
UCM B-5058	12	10	14	8	14	1	9
UCM B-5062	15	12	18	10	17	2	12
UCM B-5063	10	8	11	7	12	1	7
UCM B-5064	14	13	18	11	17	2	13
UCM B-5065	12	10	13	8	13	1	9
UCM B-5070	23	18	28	16	28	4	22
UCM B-5071	11	9	13	8	13	1	8
UCM B-5074	20	16	24	13	24	4	18
UCM B-5076	11	9	11	8	12	1	7
UCM B-5077	22	18	27	15	27	4	21
UCM B-5095	14	13	17	11	16	1	12
UCM B-5096	17	14	20	12	20	3	15
UCM B-5097	16	14	20	11	19	4	16
UCM B-5098	20	16	24	14	24	4	19
UCM B-5099	16	14	20	12	20	3	15
UCM B-5100	20	16	24	13	23	3	18
UCM B-5110	15	12	17	10	17	2	13
UCM B-5111	20	16	25	14	24	4	19
UCM B-5113	9	8	9	7	9	0	5
UCM B-5114	19	15	24	13	23	3	18
UCM B-5115	12	12	15	10	14	1	10
UCM B-5116	20	16	24	13	23	4	18
UCM B-5117	18	14	22	12	21	3	16
UCM B-5118	23	19	28	16	28	4	22
UCM B-5119	21	17	25	14	25	4	19
UCM B-5120	18	15	22	12	22	3	16
UCM B-5121	18	14	21	12	21	3	16
UCM B-5122	16	11	18	10	18	2	14
UCM B-5123	10	8	12	7	12	1	8
UCM B-5124	8	7	8	6	9	0	5
UCM B-5125	11	11	13	9	13	0	9
UCM B-5126	25	19	30	16	29	4	23
UCM B-5127	14	11	18	10	17	2	12
UCM B-5128	14	13	18	11	17	2	12
UCM B-5129	9	10	11	8	11	1	8
UCM B-5130	22	18	27	15	26	3	20
UCM B-5131	9	6	9	5	10	1	5
UCM B-5132	10	8	12	7	12	1	8
UCM B-5133	7	6	8	6	9	4	8
UCM B-5134	11	8	12	7	12	2	9

UCM B-5136	8	6	9	5	9	1	6
UCM B-5139	**27**	**20**	**24**	**18**	**24**	**0**	**8**
UCM B-5140	**9**	**11**	**10**	**12**	**10**	**11**	**20**
UCM B-5601	9	8	10	7	10	1	7
UCM B-5602	8	5	8	4	8	0	4
UCM B-5610	12	9	15	8	14	2	10
UCM B-5610	8	8	10	6	9	0	6
UCM B-5617	7	6	7	5	7	0	4
UCM B-5756	12	9	15	8	15	3	11
UCM B-5757	7	5	8	4	8	0	3
UCM B-5758	13	11	16	9	16	2	12
UCM B-5759	7	7	9	5	8	0	6
UCM B-5800	14	11	18	9	17	2	13
UCM B-5844	7	5	10	4	9	2	7

UCM is acronym for the Ukrainian Collection of Microorganisms