Antibiotic resistance, biochemical typing, and PFGE typing of *Bifidobacterium* strains commonly used in probiotic health foods

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Abstract This study firstly analyzed the antibiotic resistance, biochemical typing, and pulsed-field gel electrophoresis typing of 45 *Bifidobacterium* strains commonly used in health foods. Most strains were resistant to antibiotics but their antibiotic resistance rates were not high: Fos (56.52%), TET (43.48%), CRO (21.74%), AMC (15.22%), GEN (13.04%), RIF (10.87%), CHL (8.7%), CTX (6.52%), VAN (4.35%), and ERY (4.35%). The 45 strains could be divided into 14 pulsed-field gel electrophoresis types, of which the strain numbers of six pulsed-field gel electrophoresis types were more than one. All the *Bifidobacterium* strains could be divided into nine types by API50CHL biochemical identification. The same species displayed same biochemical typings, except for *B. animalis*. Furthermore, the results confirmed that the same pulsed-field gel electrophoresis-type strains had closer antibiotic resistance patterns, and the same biochemical-type strain also had similar antibiotic resistance patterns.

Keywords *Bifidobacterium* · Antibiotic resistance · Biochemical typing · Pulsed-field gel electrophoresis · Probiotic health foods

Introduction

Bifidobacteria are Gram-positive, strictly anaerobic, irregular or branched rod-shaped, and Y-shaped bacteria. They are often found in the gastrointestinal tracts of humans, animals, and insects and are one of the most important members in the microbiota [1]. In about 1900, scientists isolated and described *Bifidobacterium* from human feces. These bacteria have significant roles in keeping their human hosts healthy [2].

As an important probiotic, *Bifidobacterium* is widely used in health foods, such as yogurt and cheese, as well as an edible probiotics powder. Traditional probiotic strains have a long history of safe use. However, with the use of new bifidobacteria species, probiotic security issues have become a focus of scientific researchers worldwide. The antibiotic resistance of probiotics is a very important aspect of its safety [3]. Several studies have shown that probiotics are resistant to multiple antibiotics: antibiotic resistance test results on the Belgian market by Masco et al. [4] showed that *Bifidobacterium* bacteria were resistant to gentamicin and polymyxin B, whereas Milazzo et al. [5] confirmed that two *Bifidobacterium* isolates exhibited high antibiotic resistance to trimethoprim/sulfamethoxazole and fluoroquinolone. However, the antibiotics resistance of *Bifidobacterium* commonly used in health foods has not been systemically studied in previous reports.

Pulsed-field gel electrophoresis (PFGE) has been recognized as the “gold standard” of typing methods for many years. It is often used as the reference standard for judging the accuracy of other typing methods [6]. However, there is little information about which technology should be applied in typing the bifidobacteria strains commonly used in health foods.

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This study used an API50CHL biochemical identification kit to perform biochemical typing, and the broth microdilution method was used to test the antibiotic resistance of *Bifidobacterium* strains. PFGE was used to determine their electrophoretic type. Furthermore, the relationship among biochemical typing, antibiotic resistance, and genomic type was analyzed.

Materials and methods

Strain

Positive control strains as reference standards were *Streptococcus pneumoniae* (ATCC49619) and *Escherichia coli* (ATCC25922). The test strains were probiotic *Bifidobacterium* strains usually used at Chinese health care markets, and the strains were offered by the microbiology department of China National Center for Food Safety Risk Assessment. A total of 45 *Bifidobacterium* strains mainly derived from nutritional health food and dairy products had been tested by an API50CHL biochemical identification plate before conducting the experiments. Preliminary information of the strains is shown in Table 1. Because of economic interest, strains information has not been described in detail.

Methods

API50CHL identification

An API50CHL biochemical identification kit (bioMérieux) was used for identification. The biochemical reaction results were analyzed by an API software while referring to Berger's manual for identification of bacteria to obtain the biochemical identification results.

Determination of antibiotic resistance

Antibiotic resistance was tested using the broth dilution method recommended by the Clinical and Laboratory Standards (CLSI) [7]. Mueller–Hinton Broth with cation adjusted and 2.5–5% (V/V) lysed horse blood was used as the medium. Drug susceptibility was tested according to the operating instructions of microporous drug plates (Tianjin Jin Zhang Co., China). A total of 16 antibiotics were selected and the concentrations used are shown in Table 1.

PFGE determination

The experiment was based on the standard operating procedure manual of PNL04 regulations of the US Pulse Net

protocol 1 [8]. DNA was digested by the restriction enzyme *XbaI* (BioLabs, New England) at 30 U at 37 °C, after which electrophoretic separation was conducted using an electrophoresis buffer (0.5 × TBE) at 14 °C for 22 h, and the pulse time was 1–15 s. *S. braenderup* H9812 digested with *XbaI* was used as a unified molecular weight standard [9].

Relationship determination

Cluster analysis of the biochemical results was conducted using the BioNumerics (Version 5.0) database software (Applied Maths BVBA, Belgium).

The results were determined by the susceptibility standard of the National Committee for Clinical Laboratory Standards, including susceptible (S), intermediate susceptible (I), and resistant (R); when microbes were resistant to three or more antibiotics, this was called multi-drug antibiotic resistance (MDR). The results of PFGE were processed by the BioNumerics database software (AppliedMathsBVBA, Belgium) and graphics bands were identified. Similarity coefficient of electrophoretic bands between different strains was expressed by the Dice coefficient, and the number ranged between 0 and 100, where 0 indicates complete irrelevance and 100 represents the same. Different bands are judged as different types.

Results and discussion

Biochemical analysis

As can be seen in Fig. 1, all *Bifidobacterium* strains can be divided into nine types, named a–i. Among them, Type a (n = 6) included one species: *B. infantis*; Type b (n = 8) included one species: *B. breve*; Type c (n = 3), d (n = 5), e (n = 3), and f (n = 5) only included the same species: *B. animalis*; Type h (n = 13) included one species: *B. longum* ssp; and Types g and i included *B. adolescentis* and *B. bifidum*, respectively.

Antibiotic resistance analysis

The antibiotic resistance patterns of *Bifidobacterium* strains to 16 antibiotics are shown in Table 1 [7, 8]. As can be seen in Table 1, 45 *Bifidobacterium* strains were resistant to 10 antibiotics to varying degrees. Antibiotic resistance rates that ranged between 2.22% and 55.56% (from high to low) were FOS (55.56%), TET (44.44%), CRO (22.22%), AMC (13.33%), GEN (13.33%), GAT (11.11%), CHL (8.89%), CTX (6.67%), ERY (4.44%), and VAN (2.22%). Fourteen types of antibiotics displayed intermediary degrees, mainly between 2.22 and 22.22%. Among these,

Table 1 Antimicrobial resistance of the 45 *Bifidobacterium* to 16 antibiotics

Strain no.	Strain identity	Strain source	Antibiotics							
			AMP	PEN	IPM	GEN	VAN	ERY	CLI	SXT
			Range of MIC tested (µg/mL)							
			0.03–32	0.03–32	0.002–2	0.06–64	0.25–256	0.03–32	0.015–16	0.03–32
<i>a</i>										
B01	<i>B. longum</i>	China	S	S	S	S	S	S	S	S
B02	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S
B03	<i>B. animalis</i>	Korea	S	S	S	S	S	S	S	S
B04	<i>B. longum</i>	Japan	S	S	I	R	I	S	S	S
B05	<i>B. longum</i>	Japan	S	S	S	S	R	I	S	S
B06	<i>B. longum</i>	Japan	S	S	S	S	S	S	S	S
B07	<i>B. breve</i>	Japan	S	I	I	R	S	S	S	S
B08	<i>B. breve</i>	Japan	S	I	I	R	S	S	S	S
B09	<i>B. breve</i>	Japan	S	S	I	R	S	S	S	S
B10	<i>B. infantis</i>	Japan	S	S	S	S	S	S	S	S
B11	<i>B. infantis</i>	Japan	S	S	I	S	S	I	S	S
B12	<i>B. infantis</i>	Japan	S	S	I	I	S	S	S	S
B13	<i>B. longum</i>	French	S	S	S	S	S	S	S	S
B14	<i>B. bifidum</i>	French	S	S	S	R	S	S	S	S
B15	<i>B. animalis</i>	Korea	S	S	S	I	S	S	S	S
B16	<i>B. animalis</i>	Korea	S	S	S	S	S	S	S	S
B17	<i>B. breve</i>	Denmark	S	S	S	S	S	S	S	S
B18	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S
B19	<i>B. animalis</i>	IMCAS	S	S	S	S	S	S	S	S
B20	<i>B. longum</i>	Denmark	S	S	S	S	S	S	S	S
B21	<i>B. longum</i>	China	S	S	S	S	S	S	S	S
B22	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S
B23	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S
B25	<i>B. breve</i>	China	S	S	S	R	S	S	S	S
B26	<i>B. breve</i>	China	S	S	S	S	S	S	S	S
B27	<i>B. adolescentis</i>	China	S	S	S	S	S	S	S	S
B28	<i>B. animalis</i>	China	S	S	S	S	S	S	S	S
B29	<i>B. longum</i>	China	S	S	S	S	S	S	S	I
B30	<i>B. animalis</i>	French	S	S	S	S	S	S	S	S
B31	<i>B. animalis</i>	China	S	S	S	S	S	S	S	S
B32	<i>B. longum</i>	China	S	S	S	S	S	S	S	S
B33	<i>B. longum</i>	China	S	S	S	S	I	S	S	S
B34	<i>B. longum</i>	Canada	S	S	S	S	S	S	S	S
B35	<i>B. breve</i>	Japan	S	S	S	I	S	S	S	S
B36	<i>B. breve</i>	Japan	S	S	S	S	S	S	S	S
B37	<i>B. infantis</i>	Japan	S	S	S	S	S	S	S	S
B38	<i>B. animalis</i>	Japan	S	S	S	S	S	S	S	S
B39	<i>B. infantis</i>	Japan	S	S	S	S	S	S	S	S
B40	<i>B. longum</i>	China	S	S	S	S	S	R	S	S
B41	<i>B. longum</i>	Japan	S	S	S	S	S	S	S	S
B42	<i>B. animalis</i>	French	S	S	S	S	S	S	S	S
B43	<i>B. animalis</i>	Canada	S	S	S	S	S	S	S	S
B44	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S
B45	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S

Table 1 continued

Strain no.	Strain identity	Strain source	Antibiotics							
			AMP	PEN	IPM	GEN	VAN	ERY	CLI	SXT
			Range of MIC tested (µg/mL)							
			0.03–32	0.03–32	0.002–2	0.06–64	0.25–256	0.03–32	0.015–16	0.03–32
B46	<i>B. infantis</i>	Denmark	S	S	S	S	S	R	S	S
R(%)			0	0	0	13.04	4.35	4.35	0	0
I(%)			0	4.35	13.04	6.52	4.35	4.35	0	2.17
S(%)			100	95.65	86.96	80.43	91.3	91.3	100	97.83
Strain no.	Strain identity	Strain source	Antibiotics							
			AMC	GAT	CHL	TET	FOS	CRO	CTX	RIF
			Range of MIC tested (µg/mL)							
			0.03–32	0.015–16	0.015–16	0.06–64	0.25–256	0.125–128	0.125–128	0.015–16
<i>b</i>										
B01	<i>B. longum</i>	China	R	R	R	R	R	S	S	S
B02	<i>B. animalis</i>	Denmark	S	S	S	R	R	R	I	S
B03	<i>B. animalis</i>	Korea	S	S	S	R	R	R	I	S
B04	<i>B. longum</i>	Japan	S	R	I	S	R	S	S	S
B05	<i>B. longum</i>	Japan	R	R	R	R	R	S	S	S
B06	<i>B. longum</i>	Japan	S	S	S	S	I	S	S	S
B07	<i>B. breve</i>	Japan	S	I	I	R	R	I	S	S
B08	<i>B. breve</i>	Japan	S	I	I	R	S	I	S	S
B09	<i>B. breve</i>	Japan	S	R	I	R	R	I	S	S
B10	<i>B. infantis</i>	Japan	S	S	S	R	R	S	S	S
B11	<i>B. infantis</i>	Japan	S	S	S	R	I	I	S	S
B12	<i>B. infantis</i>	Japan	S	S	I	I	R	S	S	I
B13	<i>B. longum</i>	French	S	I	S	S	R	S	S	S
B14	<i>B. bifidum</i>	French	S	S	R	S	R	S	S	S
B15	<i>B. animalis</i>	Korea	S	S	S	S	I	R	S	S
B16	<i>B. animalis</i>	Korea	S	S	S	I	I	S	S	S
B17	<i>B. breve</i>	Denmark	S	S	S	S	S	S	S	S
B18	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S
B19	<i>B. animalis</i>	IMCAS	S	S	S	I	I	S	S	S
B20	<i>B. longum</i>	Denmark	R	I	S	S	R	S	S	S
B21	<i>B. longum</i>	China	S	I	S	S	R	S	S	S
B22	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S
B23	<i>B. animalis</i>	Denmark	S	S	S	R	R	R	I	S
B25	<i>B. breve</i>	China	S	I	S	S	S	I	S	S
B26	<i>B. breve</i>	China	S	S	S	R	R	R	S	S
B27	<i>B.adolescentis</i>	China	S	I	I	S	R	I	S	S
B28	<i>B. animalis</i>	China	S	S	S	S	S	S	S	S
B29	<i>B. longum</i>	China	R	I	I	I	R	S	S	S
B30	<i>B. animalis</i>	French	S	S	S	R	S	I	R	I
B31	<i>B. animalis</i>	China	S	S	S	R	R	I	S	S
B32	<i>B. longum</i>	China	S	S	I	R	R	R	I	S
B33	<i>B. longum</i>	China	I	S	R	S	R	S	S	S
B34	<i>B. longum</i>	Canada	R	R	S	S	I	S	S	S
B35	<i>B. breve</i>	Japan	S	S	S	R	R	I	S	S
B36	<i>B. breve</i>	Japan	S	I	S	R	R	R	R	S

Table 1 continued

Strain no.	Strain identity	Strain source	Antibiotics							
			AMC	GAT	CHL	TET	FOS	CRO	CTX	RIF
			Range of MIC tested (µg/mL)							
			0.03–32	0.015–16	0.015–16	0.06–64	0.25–256	0.125–128	0.125–128	0.015–16
B37	<i>B. infantis</i>	Japan	S	S	S	R	I	R	R	I
B38	<i>B. animalis</i>	Japan	R	S	S	R	S	R	I	S
B39	<i>B. infantis</i>	Japan	S	I	S	R	R	R	I	I
B40	<i>B. longum</i>	China	S	S	I	S	R	S	S	S
B41	<i>B. longum</i>	Japan	S	S	I	R	R	S	S	S
B42	<i>B. animalis</i>	French	S	S	S	S	S	S	S	S
B43	<i>B. animalis</i>	Canada	S	S	S	S	I	S	S	S
B44	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S
B45	<i>B. animalis</i>	Denmark	S	S	S	S	S	S	S	S
B46	<i>B. infantis</i>	Denmark	S	S	S	S	S	S	S	S
R(%)			15.22	10.87	8.7	43.48	56.52	21.74	6.52	0
I(%)			2.17	23.91	21.74	8.7	17.39	19.57	13.04	10.87
S(%)			82.61	65.22	69.57	47.83	26.09	58.7	80.43	89.13

MIC is expressed as the minimum inhibitory concentration (µg/mL) of the antimicrobial compound

R resistant; S susceptible; I intermediate susceptible; AMP ampicillin; IPM imipenem; PEN penicillin; VAN vancomycin; ERY erythromycin; CLI clindamycin; SXT trimethoprim/sulfamethoxazole; GEN gentamicin; GAT gatifloxacin; CHL chloramphenicol; AMC amoxicillin/clavulanic acid; FOS fosfomycin; CRO ceftriaxone; TET tetracycline; RIF rifampin; CTX cefotaxime

GAT and CHL showed the highest degree of intermediary antibiotic resistance at 22.22%, followed by FOS, CTX, and IPM with 17.78, 13.33, and 13.33%, respectively. All strains presented different sensitivity rates to all antibiotics, spread between 26.67 and 100%. The sensitivities of AMP and CLI were 100%, followed by SXT and PEN at sensitive rates of 97.78% and 95.56%, respectively.

A total of 11 strains, B06 (*B. longum*), B17 (*B. breve*), B16, B18, B19, B22, B28, B42, B43, B44, and B45 (all *B. animalis*) did not show antibiotic resistance to all the antibiotics. Among the resistant strains, 15 were multi-drug resistant strains, and the 19 remaining strains were only resistant to one or two types of antibiotics.

Probiotics antibiotic resistance is an important measurement for the risk assessment of *Bifidobacterium*. The approach for evaluating the safety of probiotics, which was created by the European safety certification agency (QPS), includes identifying the antibiotic resistance of strains and confirming it at the genetic level and then determining whether the antibiotic resistance will be passed on to the intestinal microbial flora [10]. The FDA has no special provisions for the antibiotic resistance of probiotics, and probiotic fermented foods are recognized as GRAS (generally recognized as safe) but the probiotic strain itself is not seen as GRAS [11, 12].

Considering the importance of probiotic safety, the FAO/WHO guidelines emphasize that antibiotic resistance spectrum, animal and human experiments, and other safety testing of probiotics should be conducted. Furthermore, the ability to produce toxins should be evaluated, a hemolytic activity assay should be conducted, and the use of clinical probiotics should be safety monitored [13–15]. In addition, antibiotic resistance of probiotic strains maybe related to the origin of the strains [16].

PFGE typing analysis

As can be seen in Fig. 2, clear PFGE patterns were obtained for all *Bifidobacterium* strains, with stripes distributed between 10 and 25. Forty-five *Bifidobacterium* strains can be divided into 14 PFGE types, which were labeled from A to N. Among these, PFP (the strains with same PFGE profile or the strain with PFGE profile) M (n = 14) belonged to one species (*B. animalis*); PFP J (n = 7) and PFP I (n = 1) belonged to the same species (*B. breve*). PFP G (n = 5) and PFP H (n = 1, AS1.1853) included the same species (*B. infantis*). PFP B (n = 4), PFP C (n = 4), PFP A (n = 2), PFP D (n = 1), PFP E (n = 1), and PFP F (n = 1) were classified into the same species (*B. longum*). PFP L (n = 2) belonged to *B.*

animalis. PFP K (n = 1) was *B. youth* and PFP N (n = 1) was *B. bifidum*.

As can be seen in Table 2, every antibiotic resistance had specific antibiotic resistance genes. Those resistance genes to antibiotics (PEN, AMC, and AMP) belonged to the lactam antibiotics had a same TEM gene.

Fig. 2 Dendrogram of cluster analysis of the 45 *Bifidobacterium* strains generated by the Bionumerics software. B01–B46 strain number; A–N PFGE profile

Fig. 1

Fig. 1 Dendrogram of *Bifidobacterium* strains studied by API50CHL. B01–B46 strain numbers (a–i) biochemical type

Fig. 2

Bacterial chromosomal DNA can be analyzed by the PFGE typing method, where the chromosome is cut into 10–800 kb fragments with 5–30 bars by restriction endonuclease with rare site. The electrophoresis direction of DNA molecule changes with the change in the electric field in the alternating electric field, and the DNA molecule moves forward in the gel, so different sized DNA fragments are isolated, with high resolution and good stability [17, 18].

Pulse Net standard operating methods were used in the test to PFGE type bifidobacteria. All strains exhibited about 10–20 bands and the 46 *Bifidobacterium* strains were divided into 14 PFGE types. It can be seen that the same type of bacteria were clustered together, suggesting that these bacteria evolved from a common ancestor.

Comparison among antibiotic resistance, biochemical type, and PFGE type

PFP A (n = 2) were *B. longum* strains, had the same biochemical types and similar antibiotic resistance patterns, and were all resistant to FOS.

PFP B (n = 4) were *B. longum* strains and had the same biochemical types and similar antibiotic resistance patterns. B13 and B21 had the same antibiotic resistance spectrum and were only resistant to one antibiotic (FOS). B01 and B32 were resistant to TET and FOS.

PFP C (n = 4) were *B. longum* and had the same biochemical types. B06 was sensitive to all antibiotics, and the antibiotic resistance patterns of the remaining three strains (B04, B05, and B33) were similar, mainly resistant to FOS, GAT, and CHL.

PFP G (n = 5) were *B. infantis* and had the same biochemical types. Their antibiotic resistance patterns had certain similarities. B11 and B12 were respectively resistant to TET and FOS, and the remaining three strains B10, B37, and B39 were mainly resistant to TET, FOS, and CRO.

PFP J (n = 7) were *B. breve* and had the same biochemical types. Their antibiotic resistance patterns had high similarity. B25 was only resistant to GEN, B07, B08, and B09 were mainly resistant to GEN, TET, FOS, and B26, B35, and B36 were resistant to TET, FOS, and CRO.

PFP M (n = 14) were *B. animalis* strains. The biochemical types included types c, d, e, and f, and their antibiotic resistance patterns were highly similar. Five strains (B22, B28, B42, B44, and B45) were sensitive to all the antibiotics, B15 was only resistant to CRO, and the other eight strains were mainly resistant to TET, FOS, CRO, and AMC. B02, B03, and B23 had the same antibiotic resistance spectrum.

PFP D, E, F, H, K, N had one bacterium. Among these types, PFP D, E, and F belonged to *B. longum*, and they had

the same biochemical types and were resistant to AMC. PFP E and F had the same antibiotic resistance spectrum. PFP H, K, and N belonged to three different strains, and their biochemical types were different, and their antibiotic resistance patterns were also different from each other. PFP L (n = 2) and I (n = 1) included three strains, and both of them were sensitive to all the antibiotics. PFP L had the same biochemical types.

The biochemical type was highly similar to PFGE genotype. Most strains had the same biochemical type and also had a similar resistant spectrum although rarely identical.

In this study, the antibiotic resistance, biochemical type, and PFGE type of *Bifidobacterium* strains were compared. Majority of the *Bifidobacterium* strains had different antibiotic resistances; some strains were multi-drug resistant to three or more antibiotics. This shows that the antibiotic resistance of bifidobacteria to antibiotics has become a relatively common phenomenon. Xiao et al. [19] found that the trial strains were susceptible to chloramphenicol, ampicillin, vancomycin, and linezolid and were intrinsically resistant to aminoglycoside group agents. Susceptibility to erythromycin, clindamycin, rifampicin, tetracycline, and trimethoprim varied among the strains. All strains of *Bifidobacterium animalis subsp. lactis* were resistant to tetracycline. No risk factor for safety was found for bifidobacterial strains distributed in the Japanese market in respect of their antimicrobial antibiotic resistance although the presence of the tet (W) gene in some strains stresses the need for future evaluation [19].

This result was similar with previous findings [20]. From a comparison of the antibiotic resistance, biochemical type, and PFGE type, it can be concluded that most strains that had the same genotype also had the same biochemical type and had highly similar antibiotic resistance patterns [21–23].

The security of bifidobacteria products has been proven in long-term use, wherein little infection or pathogenicity about these bacteria has been reported. However, standards and rules to guide the safe use of *Bifidobacterium* in health products and foods should still be established [24, 25].

In addition, from the results of this study we could observe there were some differences between the antibiotic resistance patterns of different bifidobacteria species but the same *Bifidobacterium* genus had certain similarities in their antibiotic resistance patterns.

From the comparison between the biochemical type, PFGE typing, and antibiotic resistance results, we could see that the strain antibiotic resistance patterns within the same type were similar among PFP A, B, C, G, J, M, and L, but the same exact patterns were not found. This was also similar for the biochemical type. Biochemical typing can be used as a reference tool for effective typing.

Table 2 Resistance spectrum biochemical type and PFGE profiles of 45 *Bifidobacterium* strains tested

Strain no.	Identification	Resistance pattern	PFGE profiles	Biochemical type
B14	<i>B. bifidum</i>	GEN-CHL-FOS	N	i
B46	<i>B. infantis</i>	ERY	H	a
B27	<i>B. adolescentis</i>	FOS	K	g
B34	<i>B. longum</i>	AMC-GAT	D	h
B20	<i>B. longum</i>	AMC-FOS	E	h
B29	<i>B. longum</i>	AMC-FOS	F	h
B40	<i>B. longum</i>	ERY-FOS	A	h
B41	<i>B. longum</i>	TET-FOS	A	h
B01	<i>B. longum</i>	AMC-GAT-CHL-TET-FOS	B	h
B32	<i>B. longum</i>	TET-FOS-CRO	B	h
B13	<i>B. longum</i>	FOS	B	h
B21	<i>B. longum</i>	FOS	B	h
B09	<i>B. breve</i>	GEN-GAT-TET-FOS	J	b
B07	<i>B. breve</i>	GEN-TET-FOS	J	b
B08	<i>B. breve</i>	GEN-TET	J	b
B25	<i>B. breve</i>	GEN	J	b
B36	<i>B. breve</i>	TET-FOS-CRO-CTX	J	b
B26	<i>B. breve</i>	TET-FOS-CRO	J	b
B35	<i>B. breve</i>	TET-FOS	J	b
B39	<i>B. infantis</i>	TET-FOS-CRO	G	a
B10	<i>B. infantis</i>	TET-FOS	G	a
B37	<i>B. infantis</i>	TET-CRO-CTX	G	a
B11	<i>B. infantis</i>	TET	G	a
B12	<i>B. infantis</i>	FOS	G	a
B05	<i>B. longum</i>	VAN-AMC-GAT-CHL-TET-FOS	C	h
B04	<i>B. longum</i>	GEN-GAT-FOS	C	h
B33	<i>B. longum</i>	CHL-FOS	C	h
B06	<i>B. longum</i>	/	C	h
B38	<i>B. animalis</i>	AMC-TET-CRO	M	f
B02	<i>B. animalis</i>	TET-FOS-CRO	M	d
B03	<i>B. animalis</i>	TET-FOS-CRO	M	c
B23	<i>B. animalis</i>	TET-FOS-CRO	M	d
B31	<i>B. animalis</i>	TET-FOS	M	e
B30	<i>B. animalis</i>	TET-CTX	M	c
B15	<i>B. animalis</i>	CRO	M	f
B16	<i>B. animalis</i>	/	M	d
B22	<i>B. animalis</i>	/	M	d
B28	<i>B. animalis</i>	/	M	f
B42	<i>B. animalis</i>	/	M	d
B43	<i>B. animalis</i>	/	M	f
B44	<i>B. animalis</i>	/	M	c
B45	<i>B. animalis</i>	/	M	e
B18	<i>B. animalis</i>	/	L	f
B19	<i>B. animalis</i>	/	L	e
B17	<i>B. breve</i>	/	I	b

Antibiotic resistance patterns of the strains among the different PFGE types had points of resemblance but were still slightly different. This suggests that strains with the

same PFGE type had similar antibiotic resistance patterns, and the strains that were a different PFGE type had greater differences in their antibiotic resistance patterns [11].

When the PFGE type was the same but antibiotic resistance patterns were not exactly the same, this could be due to mutations of the antibiotic resistance gene [26, 27]. As the mutation sites are not on the PFGE restriction enzyme sites, this could result in drug antibiotic resistances that could not be reflected using the PFGE typing [28–30].

In conclusion, we compared the biochemical type, drug antibiotic resistance, and genotyping of 45 *Bifidobacterium* strains and found that there was a certain correlation among them. Strains with the same PFPs had the same biochemical type and had mostly a similar drug spectrum. Biochemical typing methods could be used as a guideline for other typing methods. The PFPs groups were mostly related to the species/subspecies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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