Research Article

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Identification of *Bifidobacterium Animalis Ssp. Lactis* from Egyptian Women Breast Milk and Feces of Breast Fed Infant Based On 16S-23S rRNA Gene

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Abstract

Bifidobacterium represent one of the major genera of the intestinal tract of human and animals used as probiotics in dairy and nondairy foods for restore the intestinal microflora which confers a health benefit. The identification of Bifidobacterium by phenotypic features is commonly unreliable, time, money, and effort consuming. We sought to improve the Bifidobacterium identification method based on molecular level to identify probiotic bacteria in complex microbial communities. The application of 16S-23S rRNA oligonucleotide primers is the best and most reliable, rapid, and precise species and sub species identification approach. The ribosomal intergenic spacer region (ISR) located between the highly conserved 16S rRNA and 23S rRNA shows a high degree of variation in length and sequence and potential for intra species discrimination and providing the phylogenetic Relationship of the Genus Bifidobacterium spp. Results showed that one of the two primer sets Bflac2-Bflac5 species specific gives positive results differentiating between B. animalis ssp. Lactis isolated from breast fed infants milk of human and that isolated from feces of breast fed infant and detecting reference strain for B. animalis ssp. Lactis DSM10140. DNA sequences of the two strains were submitted to the Genbank NCBI under accession number (KT758845) named as B. animalis ssp. Lactis Egm1 (Egyptian milk) and accession number (KT758846) named as Egf1 Egyptian feces while the second primer give false positive result. Also, we aim to obtain patent protection under Intellectual property rights (IPRs) for B. animalis ssp. Lactis which was isolated from Egyptian resources to be used for a better and healthier food and dairy products.

Introduction

Bifidobacterium are non-motile, non-spore forming, non-gas producing, Gram-positive, and anaerobic, and catalase negative bacteria with a high G-C content (55 to 67%) *Bifidobacterium spp* constitute an important class of organisms in the intestinal microflora of healthy children and adults [1]. Probiotics are live microorganisms which confer a health benefit on the host when administered in adequate amounts [2]. The action of probiotics on intestinal flora results in vital benefits, including protection against pathogens, development of the immune system and positive effects on colonic health and host nutrition [3]. *B. animalis subsp. lactis* was selected as probiotic strain since it has been widely used in infant formulas and baby foods, dietary supplements and cultured milk products for more than 10 years ago in many countries all over the world. *Bifidobacterium animalis subsp. lactis* is the most common Bifidobacterium utilized as a probiotic in commercial dairy products in North America and Europe [4]. Differentiation and identification of *Bifidobacterium spp* are important tools for industry and quality assurance, safety, protecting rights of strains ownership and research, and identifying the strain of interest from among others. Identification of *Bifidobacterium spp* based on phenotypic using carbohydrate fermentation, cell wall analysis and detection of specific enzymes are generally used but these methods require very long time and do not always give clear results [5]. The distinction between some species currently used in commercial products such as *B. infantis* and *B. longum*, or *B. animalis* and *B. lactis* is difficult when phenotypic tests are in use [6]. Molecular identification of *Bifidobacterium* isolates can be carried out using very conservative sequences universally present in bacterial genome L-lactate dehydrogenase gene ldh, recA gene, 60 kDa heat-shock protein (HSP60) gene and rRNA polymorphisms are useful for characterizing *Bifidobacteria* found in the human colonic flora [7-10]. The rRNA genes (16S, 23S, and 5S) are ideal candidates for bacterial identification and evolutionary studies. The ribosomal intergenic spacer region (ISR) located between the 16S rRNA and 23S rRNA shows a high degree of variation in length and sequence and holds potential for intra species discrimination because they are highly conserved within the species [11]. 16S rRNA genes and the 16S-23S-spacer region and their sequences variability are widely used in studies of the bacterial diversity of dairy products and they have been successfully exploited primers allowing species or subspecies discrimination when applied on environmental isolates [12]. The ribosomal intergenic spacer region (ISR) located between the16S rRNA and 23S rRNA shows

a high degree of variation in length and sequence and holds potential for intra species discrimination because they are highly conserved within the species [11]. Species specific primers is a powerful, rapid, accurate, and sensitive method for the detection of target bacteria within complex ecosystems making it frequently used in the detection and identification of pathogenic bacteria [13]. The internal transcribed spacer (ITS) sequence is complementary to the 16S rRNA gene for phylogenetic analysis and was used to analyze the phylogenetic relationship [14].

Materials and Methods

Bacterial Samples

Three samples were collected, first sample was collected from healthy mother breast while the second and third samples were collected form fresh feces of two breast fed infants of the same mother at ages 6 and 21 months.

The samples were stored in siring and kept in icebox to transfer at laboratory. All samples have been collected from Meat Fadala village - Aga -Daqahlia Governorate - Egypt. We have been taken ethical consent parental to do this study.

Starter culture purchased from Daynsico Company was used as acontrol (YO-MIXTM260LYO500 DUC Yogurt cultures).

Bacterial isolation

- Samples were diluted using dilution technique up to 10^{-8}, where saline solution (0.9% Na Cl) used in diluting process.
- 0.1 ml from previous dilutions has been plated on MRS agar (Man, Rogosa Sharpe-Oxid) with pH 6.2 was supplemented added LiCl (0.03%) and Lcestein (0.05%) [15].
- The plates were incubated at 37 °C for 48 hours under anaerobic conditions (in anaerobe jar using Oxoid anaerogen compact).
- Single colony has been selected and grown in 5 ml broth media and slant agar were incubated under previous condition at 37 °C for 48 hours under anaerobic conditions in anaerobe jar using (Oxoid anaerogen compact).
- The isolates were examined according to their colony phenotype using gram reaction and catalase reaction. Gram stain test was performed according to while Catalase test was performed according to [16,17].
- The bacterial pellet was resuspended in fresh medium containing 45% (v/v) sterile glycerol and kept at -20 °C.

Molecular biology studies

Genomic DNA isolation and quantification

A-Total genomic DNA was extracted from three isolates according to manufacture instruction using. (Gene JET Genomic DNA Purification Protocol Kit#K0721-Thermo Fisher Scientific Inc.). B-Total DNA was migrated on 1% agarose gel and stained with Ethidium bromide (0.5 µg/ml) DNA was visualized using a UV Dual–Intesity Trans Illuminator and quantified using spectrophotometer (GENWAY630).

Selection of Primers for Bifidobacterium identification

Bifidobacterium species and group specific primers has been selected based on 16S rRNA, 16S-23S rRNA sequences according to as shown in table 1 [5,18-20].

Species	Primer	Nucleotide sequence	Annealing temp. (°C)	PCR product (bp)	Target Sequence	References
B. Bifidum	BiBI1 BiBI2	F CCACATGATCGCATGTGATTG R CCGAAGGCTTGCTCCCAAA	59	278	16S rDNA	[19]
B. breve	BiBR1 BiBR2	F CCGGATGCTCCATCACAC R ACAAAGTGCCTTGCTCCCT	57	288	16S rDNA	[19]
B. longum ssp. Longum	BiLO1 BiLO2	F TTCCAGTTGATCGCATGGTC R GGGAAGCCGTATCTCTACGA	59	831	16S rDNA	[20]
B. longum ssp. Infantis	BiIN1 BiIN2	F TTCCAGTTGATCGCATGGTC R GGAAACCCCATCTCTGGGAT	59	828	16S rDNA	[20]
B. animalis ssp. Lactis	Bflac2 Bflac5	F GTGGAGACACGGTTTCCC R CACACCACACAATCCAATAC	64	680	16S rDNA 16S-23S ITS	[23]
B. animalis ssp. Lactis	LW-1 LW-2	F GCACGGTTTCGGCCGTG R GGGAAACCGTGTCTCCAC	55	567	16S rDNA	[18]

Table 1: The Six primer sets which used in this study.

PCR amplification for 16S and16S–23S rRNA spacer region

Amplification reactions were prepared in the total volumes of 25 µl containing 12.5 µl GoTaq® promega, a pair of specific primers at the concentration of 0.25µmol each primer, 100 ng of Template DNA and water nuclease free Up to 25µl. PCR amplifications were carried out in Eppendorf Master Cycler Gradient applying the following PCR temperature profile: denaturation cycle of 94˚C for 5 min, followed by 35 cycles of 94˚C for 30 s, we test annealing temperature (50˚C, 55˚C, 58˚C, 59˚C, 60˚C) for 30 s for obtaining optimum annealing temperature, DNA extension 72˚C for 45 s and the final cycle of 72˚C for 4 min.

Amplified fragments visualization

Agarose gel electrophoresis 1.5% (wt/vol) was migrated the amplified DNA fragments, gels were stained with ethidium bromide (0.5 µg/ml) was directly loaded on the gel, DNA ladder 3Kb DNA marker from Thermo Scientific Gene was also loaded on the gel for fragment size comparison and visualized under UV light (Dual–Intesity Trans Illuminator) and quantified using spectrophotometer (GENWAY630).

16S-23S spacer sequencing

The species specificity of the primers fragments of PCR products as shown in figure 2 for Bif162- Bif662 and Bflac2-Bflac5 primers were sent to University of Potsdam, Institute of Biochemistry and Biology, Germany for sequencing using an ABI sequencer. The nucleotide sequences for resulted fragment were deposited at the Gen bank Database in September 13th, 2015 and it has been named as Egyptian milk and feces using Bank it tool.

Bioinformatics work flow

Primer selectivity based on in silico studies

In silico be validated before proceeding in the wet lab. The report described the properties of each primer, including melting temperature, percent G-C content, and PCR suitability Table 3. It helps us in evaluating potential PCR primers from the list of primers selected from Previous research for all the species under study *Bifidobacterium species B. bifidum, B. breve, B. longum ssp. Longum, B. longum ssp. Infantis, B. animalis ssp. Lactis* based on (NCBI) blast primer tool http://www.ncbi.nlm.nih.gov/tools/primer was used for test the primers as it has a wide range of parameters in this process include, nr database and *Bifidobacteriales*.

Sequences submissions and accession Numbers

Sequences of this study have been submitted to the NCBI using Bankit tool http://www.ncbi.nlm.nih.gov/BankIt/.

Sequences analysis

Sequence Similarity search was performed using the NCBI BLASTn online tool http://ncbi.nlm.nih.gov/BLAST/ against the nucleotide collection (nr/nt) database. BLASTN default parameters were used.

Phylogenetic tree construction

Phylogenetic tree was constructed based on the 16S-23S rRNA intergenic spacers (ITS) sequence comparisons length polymorphism of the PCR-amplified and sequences from Database using blast tree construct in www.clcbio.com using ClC workbench 7.5 based on Neighbor Joining method.

Results and Discussion

Phenotypic identification of bacteria isolates

As shown in table 2 the isolates (A, B, C) in figure 1 were examined using light microscopy after gram staining. The isolates (A) and (B) are gram positive and catalase negative while isolate (C) is gram negative so we excluded this isolate because it is impossible existence of Bifidobacterium [1].

In this sample which was collected from feces of breast fed infant age 21 months which confirms that the density of Bifidobacterium was decreased in the gastrointestinal along with age [21]. Bifidobacterium selective medium LiCl and L. cestein was found to be inhibitory to a wide range of non-Bifidobacteria strains commonly included in probiotic animal feed. *Bacilli, lactobacilli, lactococci* and *streptococci* failed to form colonies, *enterococci, pediococci* and the addition further inhibited *Saccharomyces cerevisiae* [22].

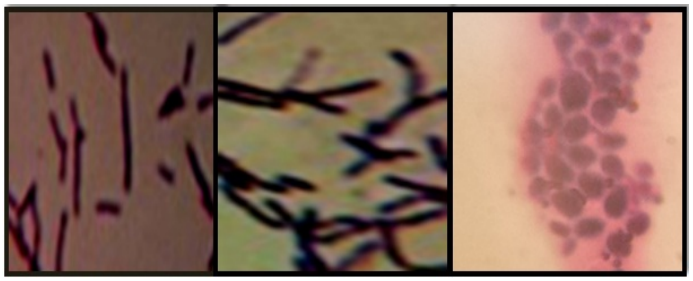

Figure 1: Light micrograph of bacterial isolates from breast milk (A), feces (B) and (C) isolates. Our results showed that breast milk (A), and feces (B) various shapes, oftenY or V branched baclli while (C) cocci so exclusion this isolate according [35].

	Strain Code	Gram staining	Shapes	Cata-lase test
A	Egm1	+Ve	Short bacilli	Ve -
B	Egf1	+Ve	Shortbaclli in Chans	ve-
C	Fb1	-Ve	Cocci	--

Table 2: Examined of bacterial after gram staining and Catalase test.

Molecular studies

Amplification of 16S and16S–23S rRNA spacer region and visualization

Results as shown in (figure 2) of PCR using 16S-23S rRNA specific primer (Bflac2-Bflac5) amplified two fragment 680bp isolated from feces and breast milk differentiation *B. animalis ssp. Lactis* in lan1 and lan2 this result agree with [23]. So PCR amplification of the 16S-23S rRNA ISRs showed to be a useful tool for bacterial species-specific typing because of the considerable variability in size and sequence among organisms.

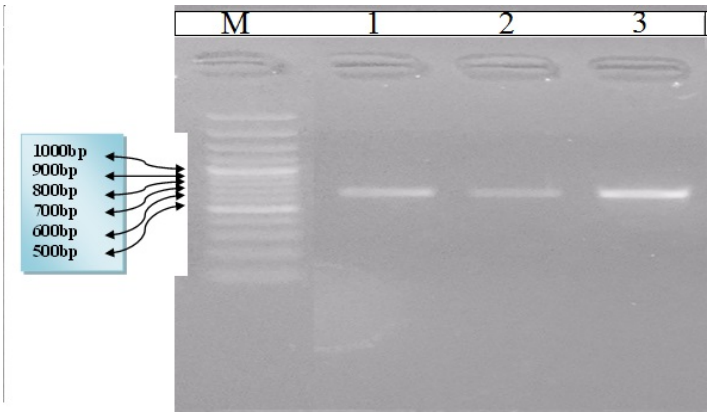

Figure 2: PCR amplified fragments using 16S and16S-23S rRNA, M = 3kb DNA ladder. Lane1 primer (Bflac2 -Bflac5): amplified fragment 680 bp from breast milk. Lane2 primer: amplified fragment 680 bp from feces. Lane3 Primer (Bflac2 -Bflac5) amplified fragment 680 bp for primeBflac2 &Bflac5 from reference strain (*B. animalis ssp. Lactis* DSM10140).

Sensitivity and Specificity of the primer

Results from (Table 3) showed that after systematically testing each of the seven primer sets, we found that Bflac2 5′-CACACCACACAATCCAATAC 3′ (20nt with G+C content of 45%, Tm=54.59°C) and Bflac5:5′GTGGAGACACGGTTTCCC3′ (18nt with G+C content of 61%, Tm= 57.63°C) were highly

specific for identification of implemented *B. animalis ssp. Lactis*. Results showed that the amplified fragments were the same DNA size compared to the reference strain *B. animalis ssp. Lactis* DSM10140. No PCR products were detected in other *Bifidobacterium species*, including *B. bifidum*, and *B. breve B. longum ssp. Longum* and *B. longum ssp. Infantis* bacterial strains.

Primer	Nucleotide sequence	Length NT	GC%	Tm	AT	product length	Speaces
BiBIF-1 *BiBIF-2*	F:CCACATGATCGCATGTGATTG R:CCGAAGGCTTGCTCCCAAA	21 19	47.62 57.89	58.01 60.60	59	278	*B. Bifidum*
BiBRE-1 *BiBRE-2*	F:CCGGATGCTCCATCACAC R:ACAAAGTGCCTTGCTCCCT	18 19	61.11 52.63	57.87 59.46	57	288	*B. breve*
BiLON-1 *BiLON-2*	F:TTCCAGTTGATCGCATGGTC R:GGGAAGCCGTATCTCTACGA	20 20	50.00 55.0	58.26 58.40	59	829	*B. longum ssp. Longum*
BiINF-1 *BiINF-2*	F:TTCCAGTTGATCGCATGGTC R:GGAAACCCCATCTCTGGGAT	20 20	50.00 55.0	58.26 58.77	59	828	*B. longum ssp. Infantis*
Bflac2 *Bflac5*	F:GTGGAGACACGGTTTCCC R:CACACCACACAATCCAATAC	18 20	61.11 45.00	57.63 54.59	64	666	*B. animalis ssp. Lactis*
LW-1 *LW-2*	F:GCACGGTTTCGGCCGTG R:GGGAAACCGTGTCTCCAC	17 18	70.59 61.11	62.15 57.63	55	569	*Bifidobacterium spp*

Table 3: Insilico analysis of selection primer sets.

PCR Primer Stats accepts a list of PCR primer sequences and returns a report describing the properties of each primer, including melting temperature, percent GC content, and PCR suitability (Table 3). It helps us in evaluating potential PCR primers from the list of primers selected by [5,18-20]. In this study six species specific primer pairs were used insilico test the results showed that five primers (BiBIF-1- BiBIF-2)-(BiBRE-1- BiBRE-2) (BiLON-1- BiLON-2) (BiINF-1- BiINF-1) (Bflac2- Bflac5) exhibited sensitivity and specificity of primers for those strains (*B. bifidum, B. breve, B. longum ssp. Longum, B. longum ssp. Infantis and B. animalis ssp. Lactis*. While (LW-1- LW-2) primer exhibited low sensitivity and specificity for *B. animalis ssp. Lactis* as shown in Table 4.

Primer	Nucleotide sequence	Strains
LW-1 LW-2	F GCACGGTTTC-GGCCGTG R GGGAAACCGT-GTCTCCAC	B. infantis Bifidobacterium sp B. animalis subsp.Lactis, B. animalis

Table 4: Insilico analysis of selection primers LW-1- LW-2.

Database submissions and accession Numbers

This is the first report that describes the identification of B. animalis ssp. Lactis from different locations in Egypt using molecular identification from breast milk and feces then sequenced and submitted to the Genbank Database in the NCBI with two new accession numbers Table 5.

No	Source	Released date	Locus	Length	Strain	Reference strain	Accession No
1	Breast milk" from Egyptian women	28-OCT-2015	16S rDNA 16S-23S ITS	602 bp	Egm1	*Bifidobacterium animalis subsp. Lactis*	KT758845
2	Feces of breast-fed infant which has been feed from same mother as INSD accession KT758845	28-OCT-2015	16S rDNA 16S-23S ITS	615 bp	Egf1	*Bifidobacterium animalis sub sp. Lactis*	KT758846

Table 5: Sequence features and accession numbers.

We have investigated the maternal influence on mother-to-child transmission *Bifidobacterium animalis subsp. Lactis* using 16S-23S rRNA spacer region (ITS) in various samples [24]. We found this strain from feces of breast-fed infant under accession (KT758846) under name Egf1 had been fed breast milk from the same mother as (INSD) accession (KT758845) under name Egm1. This confirms that *Bifidobacterium animalis subsp. lactis* has an excellent ability to survive passage through the gastrointestinal tract to agree with the results showed in we suggest that this strain possesses a superior ecological fitness to adapt in different hosts and possibly various ecological niches [25].

We found that the 16S-23S rRNA gene sequences, allowed the assessment of the biodiversity of Bifidobacterial populations present in human intestinal samples and breast milk. The ITS sequences provide insights about the precise identity and distribution of the Bifidobacterial isolates present in different human subjects, inter subject variability, and differences between two intestinal regions of the same individual. As shown in (Figure 3) the sequence length of 602 bp existing between (1,474,646 bp to 1,475,247 bp) in strain *Bifidobacterium animalis subsp. lactis* strain BF052 complete genome located in 16S-23S ribosomal RNA intergenic spacer, partial sequence according to [23].

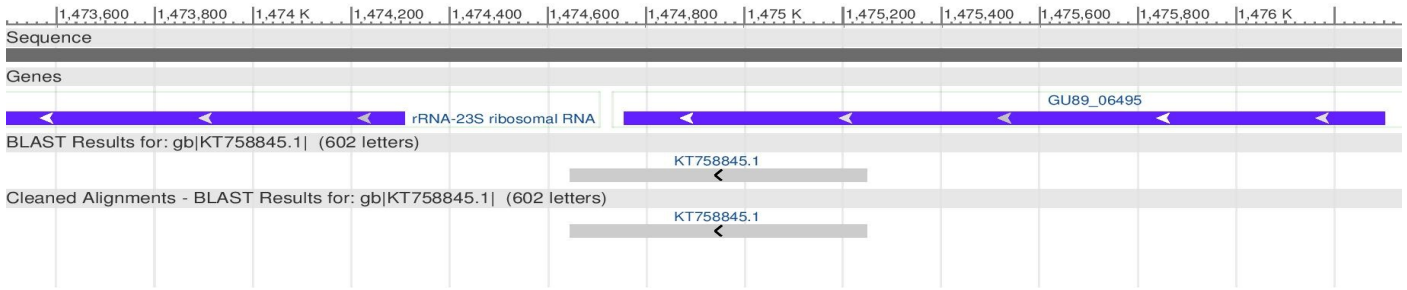

Figure 3: graphics explain the location of Sequences for *Bifidobacterium animalis subsp. lactis* strain Egm1partial sequence on complete genome for *Bifidobacterium animalis subsp. lactis strain* BF052.

S.No	Genbank Access NO.	Species of Bifidobacterium	Max scor	Total scor	QUERY COVER	E value	Identity
1	KT758845	B. animalis subsp. lactis strain Egm1, partial sequence	1112	1112	100%	0.0	100%
2	KT758846	B. animalis subsp. lactis strain Egf1, partial sequence	1112	1112	100%	0.0	100%
3	CP009045.1	B. animalis subsp. lactis strain BF052, complete genome	1112	4451	100%	0.0	100%
4	CP010433.1	B. animalis strain A6, complete genome	2093	2093	100%	0.0	100%
5	X89111.1	Bifidobacterium sp. 16S and 23S rRNA genes and ITS	1105	1105	100%	0.0	99%
6	X89513.1	B .lactis DNA 16S and 23S ribosomal RNA	1099	1099	100%	0.0	99%
7	CP002567.1	B. animalis subsp. animalis ATCC:25527, complete genome	1053	4198	100%	0.0	98%

Table 6: Percentages of similarity of *Bifidobacterium animalis subsp. Lactis* Egm1 and Egf1 and other *Bifidobacterium spp*, as extracted from the Databases using BLASTn.

Our results (table 6) showed significant similarity between 16S-23S rRNA sequences of different *Bifidobacterium spp* present in the GenBank database. BLASTn search of the non-redundant GenBank database yielded 100 hits for sequences similar to the strains (KT758845, KT758846), results showed the relatively high similarity percentage of 100% with *Bifidobacterium animalis subsp. lactis* strain BF052CP009045.1 and *Bifidobacterium animalis* strain A6, complete genome CP010433.1 , 99% similarity with *Bifidobacterium spp* (X89111.1) and *B. lactis*, (X89513), and 98% similarity with *Bifidobacterium animalis subsp. animalis* ATCC 25527CP002567.1, complete genome.

Phylogenetic relationship of the Genus Bifidobacterium spp

There were 34 strains of *Bifidobacteria spp* classify into four clads, sub clad and taxa (Figure 4).

Clad1: Exhibit most closely related for *Bifidobacterium animalis subsp. Lactis* are related to each other contained all of the Bifidobacterium animalis subsp. Lactis, *Bifidobacterium animalis subsp. Lactis* Egm1 KT758845, *Bifidobacterium animalis subsp. Lactis* Egf1 KT758846 in our study and *Bifidobacterium animalis strain* A6 CP010433 and *Bifidobacterium animalis* strain RH CP007755 are related to each other in the same clad and same node.

Clad2: Exhibit most closely related included *B. breve* strains, *B. breve* 12L CP006711-1, *B. breve* 689b CP006715-1, *B. breve* JCM 7017 CP006712-1 this strains are related to each other and exist in the same node.

Clad3: exhibit closely related for Bifidobacterium adolescentis strain BBMN23 CP010437 and Bifidobacterium dentium Bd1 CP001750 in the same clad it means are related to each other but not to Bifidobacterium adolescentis ATCC 15703 AP009256 in other clad.

Clad 4: *Bifidobacterium longum* strains classify in to sub clads. *Bifidobacterium longum subsp longum* JCM 1217 DNA AP010888, *Bifidobacterium longum subsp. infantis* 157F DNA AP010890, *Bifidobacterium longum subsp. longum* KACC 91563 CP002794 and *Bifidobacterium longum subsp. longum* strain NCIMB8809 CP011964 are related for each other. Taxa1 *Bifidobacterium longum subsp. longum* GT15 CP006741, Taxa2 *Bifidobacterium longum* strain BXY01 CP008885, Taxa3 *Bifidobacterium longum subsp. infantis* ATCC 15697 CP001095, Taxa4 *Bifidobacterium longum subsp.* infants strain BT1 CP010411 and Taxa5 *Bifidobacterium longum* strain BG7CP010453, Sub clad2 *Bifidobacterium longum subsp. longum* JDM301 CP002010-Bifidobacterium longum strain BXY01, CP008885 are related for each other,Taxa6-Bifidobacterium longum NCC2705- AE014295, Taxa7-Bifidobacterium longum DJO10A CP000605, Out of group *Bifidobacterium pseudolongum* PV8-2CP007457.

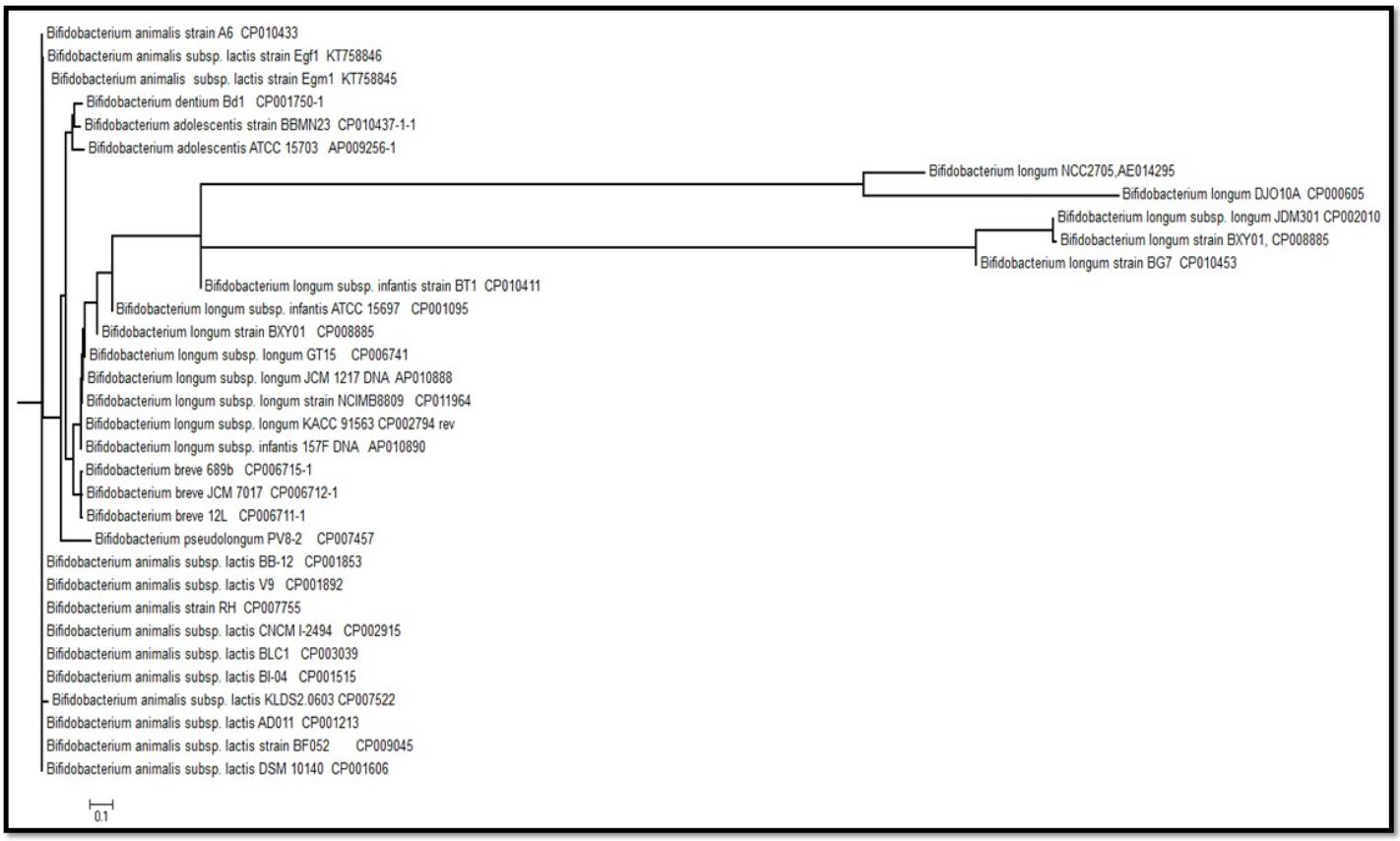

Figure 4: Phylogenetic based on 16S-23S ITS sequences, shown the inter and intraspecific relationships of members of the genus Bifidobacterium.

Our data provide the first demonstration that 16S-23S (ITS) sequences allow scientist to determine relationships among closely related species. In most cases, the (ITS) Phylogenetic (Figure 4) showed that the strains belong to the same species are more closely related than to members of another species. We indicated that levels of divergence of (ITS) sequences were able to differentiate between subspecies in the same species of *Bifidobacterium longum* and *Bifidobacterium* adolescentis, on the other hand the divergence of (ITS) sequences was not able to differentiate between *Bifidobacterium animalis subsp. Lactis* and *Bifidobacterium. breve*. The taxonomic standing of the species Bifidobacterium lactis has been long debated since its described by, and several studies have investigated its affiliation with the closely related and earlier described Bifidobacterium animalis [26,27]. Based on phenotypic characteristics, 16S rRNA sequence analysis and DNA–DNA hybridization proposed that Bifidobacterium lactis should be considered as a junior synonym Bifidobacterium animalis [28]. However, new genotypic evidence, recently reported by suggested that B. lactis and B. animalis should still be considered as two separate taxonomic entities, not at the species level but at the subspecies level [6,23]. The rRNA gene has been used widely to infer phylogenetic relationships among bacteria [29]. However, as evolutionary distances decrease, the diversity found in the 16S rDNA is often insufficient and genetic relationships of closely related species cannot be accurately defined [30]. It has been recently suggested that sequencing of the internally transcribed spacer (ITS) region could overcome this problem since ITS regions might be under less evolutionary pressure and could provide greater genetic variation. Indeed, analysis of this region has already successfully differentiated between strains and species of many bacterial groups [31]. The comparisons of 16S rRNA have been demonstrated to be highly useful for inferring phylogenetic relationships among Bifidobacteria from the level of domains to the level of species [32,33]. However, the evolutionary distances exhibited by the 16S rRNA gene sequences among closely related taxa between subspecies or at the intraspecific level are very modest, and thus, this molecular marker cannot be used in order to perform phylogenetic analysis below the species level. Our results here showed that the evolutionary rate of the ITS sequences is significantly higher than 16S rRNA gene sequences, suggesting that these two molecules provide different but complementary phylogenetic information. In fact, the analysis of 16S rRNA gene sequences represents a valid tool for inferring inter and intrageneric relationships, whereas we show here that the comparison of the ITS sequences provides data about intraspecific evolutionary development by applying a multi sequence tree approach based on the concatenation of the 16S rRNA gene and ITS sequences we were able to simultaneously analyze isolated Bifidobacteria at both interspecific and intraspecific levels [6,34].

Conclusions

This is the first report that describes the identification of probiotic *B. animalis ssp. Lactis* strains from different location in Egypt using molecular identification and bioinformatics tools for identification. *Bifidobacterium animalis subsp. lactis* is the most widely used Bifidobacterium species in probiotic applications

although the health promoting roles of Bifidobacterium are widely accepted; the diversity of Bifidobacterium among the human intestinal microbiota is still poorly understood. The PCR method allow the highly sensitive detection of specific bacteria and will have a significant effect on the analysis of gut community structure emphasis on the species specific primers for Bifidobacterium which can decrease the workload and save the identification time for the investigation of complex microbial populations, we found that the data obtained by sequencing the 16S-23S ITS region are useful for rapid identification or intraspecific phylogenetic studies of strains.

Further experiments to identify strain-specific oligonucleotides are in progress. The next significant challenges for researchers are the selection of bacterial genera and species for the preparation of further primers, and the selection of appropriate subjects for analysis [35].

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