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PROBIOTIC GENOMES: SEQUENCING AND ANNOTATION IN THE PAST DECADE

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ABSTRACT: Probiotics are live microorganisms that confer many health benefits to the host when administered in adequate quantities. These health benefits have garnered much attention towards Probiotics and have given an impetus to their use as dietary supplements for the improvement of general health and as adjuvant therapies for certain diseases. The increased demand for probiotic products in the recent times has provided the thrust for probiotic research applied to several areas of human biology. The advances in genomic technologies have further facilitated the sequencing of the genomes of such probiotic bacteria and their genomic analyses to identify the genes that endow the beneficial effects they are known to exert. This work reviews the application of genomic strategies on probiotic bacteria, while providing the details about the probiotic strains whose genome sequences are available. It also consolidates the Genomic tools used for the sequencing, assembly and annotation of the probiotic genes and how it has helped in comparative genomic analyses.

INTRODUCTION: Probiotics can be defined as live non-pathogenic microorganisms that present health benefits to the host when administered in adequate quantities [1, 2]. They fall under the class of functional foods [3, 4], and their health benefits encompass multiple facets of human health including improvement of intestinal health through the regulation of gut microflora [4 - 6], prevention of enteric, respiratory tract, and urogenital infections [2, 4, 7, 8], stimulation of immune system [9], anti-allergic activity [2], anti-cancer effects [4, 7], anti-microbial effects [10 - 12], and cholesterol-lowering effects [13 - 16]. The growth of the global probiotic market has in turn served as an impetus to probiotic research driving the adoption of modern scientific technologies in studying the genetics and biology of probiotic microorganisms [3].

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The term *Probiotic* finds its origin from the Greek words *pro* meaning for and biotikos meaning pertaining to life [17]. With the earliest clues about the involvement of probiotics in health benefits dating back to the biblical times and the times of the ancient civilizations like the Roman empire, the history of probiotics go way back in time [18]. The identification and isolation of gut microflora eventually paved way to the isolation of probiotic species and the study of their health benefits [4, 5, 17, 19, 20]. Among the bacterial species that fall under the spectrum of probiotics are the non-pathogenic species within the genera of *Lactobacillus, Bifidobacterium, Clostridium, Bacillus, Escherichia*, and *Enterococcus* [17]. However, since several years, species within the genera of *Lactobacillus* and *Bifidobacterium* have dominated probiotic market[21].

In the late 1990s and the early 2000, advances in sequencing technologies facilitated whole genome sequencing of several bacterial pathogens including *Mycobaterium tuberculosis, Pseudomons aeroginosa*, and enterohaemorrhagic *Escherichia coli* [22 - 24].

In the recent past, however, the demand for probiotics has served as an impetus for the application of sequencing strategies and genomic technologies to obtain and analyze the whole genome sequences of several probiotic bacteria [25-27]. Thus, advances in genomic technologies and computational strategies have facilitated the characterization of microbial population, particularly probiotic bacteria [28]. The forthcoming section of this review articulates predominant probiotic species whose whole genome or draft genome sequences have been made available in public databases.

Genomic Technologies in Probiotic Research: One of the earliest whole genome sequencing projects of a probiotic species (*Lactobacillus johnsonii* NCC 533) was published as early as 2004 [27]. The sequencing strategy that was used for this project was the whole genome shotgun sequencing technology [27]. There on, several sequencing projects of probiotic genomes were published in the years that followed, with a rise in the number of such projects in the very recent years [25, 29 - 32]. Furthermore, there has been a gradual change in the sequencing technologies adopted overtime for such projects thus facilitating more genomes to be sequenced, assembled and annotated in shorter durations of time [25, 29 - 32].

While initial genome sequencing strategies embraced the traditional Sanger sequencing methods [25], more advanced sequencing technologies that are collectively referred to as the Next Generation Sequencing (NGS) technologies have been eventually adopted [26, 33- 35]. The genome sequencing of probiotic species until or before 2010 was accomplished by the traditional Sanger sequencing method and shotgun sequencing technique. These include the genome sequencing of *Lactobacillus johnsonii* NCC 533 and *Bifidobacterium animalis* subsp. *lactis* AD011[25, 27]. Post 2010, NGS technologies have been adopted for genome sequencing with the prominent ones being 454 pyrosequencing technology, Illumina/ Solexa paired end sequencing technology, Ion Torrent sequencing technology, and Pacific BioSciences sequencing technology [26, 33-35].

In 2011, most of the probiotic genomes were sequenced using Roche 454 GS FLX pyrosequencer. These include the genome sequences of *Lactobacillus amylovorus* [33, 36], *Lactobacillus ruminis*[37], *Lactobacillus coryniformis* [38], *Lactobacillus animalis* [39], *Lactobacillus cypricasei* [40], *Lactobacillus sanfranciscensis* [41], and *Lactobacillus kefiranofaciens* [31] among others. In some cases, a combination of two different sequencing technologies has been adopted. For instance, in case of *Lactobacillus sanfranciscensis* genome sequencing, a combination of Roche 454 GS FLX pyrosequencing technology and Sanger sequencing was adopted [41]. Similarly, in case of *Lactobacillus kefiranofaciens*, Roche 454 GS FLX pyrosequencing technology was combined with Illumina Genome Analyzer IIx Solexa high throughput sequencing technology to sequence the genome [31].

In most cases, genome assembly was done using different versions of Newbler assembler [33, 39] except in a few cases where gsAssembler [36, 37] or CLC Genomics Workbench [37] Phred-Phrap-Consed software package [41]. Genome annotation was done using the Rapid Assembly using Subsystems Technology (RAST) server [38 - 40], often combined with Glimmer [38], tRNAscan-SE [38], RNAmmer[38], EDGAR[36], PEDANT [41], GeneMark [41], and NCBI Prokaryotic Genome Automated Annotation Pipeline (PGAAP) analysis [33, 36].

The following year also had several probiotic genomes including those of *Lactobacillus rhamnosus* [26], *Lactobacillus vini* [42], *Lactobacillus curvatus* [32], *Lactobacillus fructivorans* [43], and *Lactobacillus helveticus* [44] were sequenced using the Roche 454 GS FLX Titanium pyrosequencing technology, while the genome of *Lactobacillus rossiae* [34] was sequenced using the Illumina HiSeq 2000 platform. Even here, genome assembly was predominantly carried done using Newbler Assembler with the exception of the use of whole genome sequence assembler (wgs Assembler), genome sequence assembler (gsAssembler) and GS Reference Mapper for the assembly of *Lactobacillus helveticus*, *Lactobacillus vini*, and *Lactobacillus rhamnosus* genomes respectively [26, 42, 44]. Genome annotation was done by similar software that was mentioned before with RAST and PGAAP being the predominant tools for annotation.

In 2013, probiotic genome sequencing witnessed a more heterogeneous usage of sequencing platforms with the Applied Biosystems ABI377 and 3700 automated sequencers [45], and the Ion Torrent Personal Genome Machine [46] entering the arena where Roche 454 GS FLX [47], Illumina Genome Analyzer Iix [48], and Illumina HiSeq 2000 [49] existed. The species whose genomes were sequenced in this period include *Lactobacillus pentosus* [48], *Lactobacillus helveticus* [45], *Lactobacillus shenzhenensis* [49], *Lactobacillus ginsenosidimutans* [50], *Lactobacillus florum* [51], *Lactobacillus pobuzihii* [52], *Lactobacillus jensenii*, *Lactobacillus gasseri* [46], and *Lactobacillus otakiensis* [47]. Additionally, with heterogeneous usage of sequencing technologies came the usage of multiple assembly and annotation software. While most sequences that came out of Roche 454 GS FLX platform were assembled by different versions of Newbler [47], sequence from Ion Torrent PGM were assembled using Ion Torrent Assembler [46] or CLC *de Novo* Genomics Workbench, while the output from Illumina platforms were assembled using SOAP deNovo [49] or Velvet [48] software. Annotation was predominantly done by RAST and PGAAP analysis, but ERGO, GTPS, RDP, Silva, and ERGO were the new additions to the group [45, 47].

The year 2014 witnessed an increased use of Illumina and Ion Torrent platforms for sequencing probiotic genomes. While genomes of *Lactobacillus mucosae* [53], *Lactobacillus sakei* [54], *Bifidobacterium moukalabense* [55], *Lactobacillus sucicola* [56], *Lactobacillus farraginis* [57], and *Lactobacillus composti* [57] were sequenced using Ion Torrent Personal Genome Machine, the genomes of *Lactobacillus equi* [58], Lactobacillus animalis [59], Lactobacillus oryzae, Lactobacillus fabifermentans [60], and Lactobacillus salivarius [61] were sequenced by Illumina platforms. Roche 454 GS FLX pyrosequencer was used to sequence *Lactobacillus gasseri* and *Lactobacillus namurensis* [62] genomes.

In case of genome assembly, there was a diverse use of assembly software that was perhaps used to match the requirements of a particular genome. While genomes sequenced using Roche 454 GS FLX continued to be assembled using Newbler assembler [62], genomes sequenced using Ion Torrent systems were assembled using Newbler [57], NGen (DNAStar) [53], or CLC Genomics Workbench [54], and reads from Illumina platforms used Abyss[61, 63], Velvet [59, 63], Platanus [60], AMOS [59], Hawkeye [59] either in isolation or in concert. RAST server and PGAAP continued to be the predominant annotation platform, newer tools like GAMOLA[59], MetaGene Annotator [60], MiGAP [60], SignalP [61], InterPro [61], TMHMM [61], and Artemis being used for annotation and curation.

In the next two years, a number of probiotic species were sequenced. The year 2015 not only witnessed the use of all types of sequencing technologies, but also witnessed the combinatorial use of many of them. The combinations were either a combination of Roche 454 pyrosequencers with Illumina platforms [64] or with Sanger sequencing methods [65]. Single molecule real time (SMRT) Pacific Biosciences RSII sequencer was another technology that was used this year [66]. The species that were sequenced during this year include *Lactobacillus delbrueckii* [67], *Bifidobacterium catenulatum* [68], *Bifidobacterium pseudolongum*[66], *Lactobacillus johnsonii* [29], *Lactobacillus rhamnosus* [69], *Lactobacillus reuteri* [70], *Bifidobacterium angulatum* [71], *Bifidobacterium adolescentis* [71], *Lactobacillus kunkeei* [72], *Lactobacillus mucosae* [64], *Bifidobacterium scardovii* [65], *Bifidobacterium aesculapii* [73], *Lactobacillus curieae* [74], *Lactobacillus acidophilus* [75], *Bifidobacterium actinocoloniiforme* [76], *Lactobacillus curvatus* [77], *Lactobacillus rhamnosus* [69], *Lactobacillus fermentum* [78, 79], *Bifidobacterium kashiwanohense* [80, 81], *Lactobacillus paracasei* [82], *Lactobacillus hokkaidonensis* [83], and *Lactobacillus farciminis* [84]. The assemblers used included Newbler [72], Velvet [29], gs Assembler [71], CLC Genomics Workbench [85], SOAP deNovo[74], SPAdes [86], Ngen [67], and Phred-Phrap-Consed [68] as seen in the previous years and annotation was done mostly using RAST server and PGAAP pipeline [85], complemented with Glimmer, tRNAscan-SE, Prodigal, GenePRIMP [65, 72]. One of the new assemblers used in this year was MIRA [64].

In 2016 also, several probiotic genomes have been sequenced mainly using Illumina platforms [87] with isolated use of Ion Torrent [88], Pacific BioSciences [35] and Roche 454 [30] platforms as well. The probiotics that have been sequenced this year

include *Lactobacillus casei* [30, 87], *Lactobacillus sakei* [89], *Lactobacillus plantarum* [88, 90, 91], *Lactobacillus equigenerosi* [92], *Lactobacillus crispatus* [93], *Lactobacillus kunkeei* [35], *Bifido bacterium longum* [94], *Lactobacillus farciminis* [95], *Lactobacillus johnsonii* [96], *Lactobacillus brevis* [97], and *Lactobacillus collinoides* [98]. Genome assemblies were mostly done with the help of software like Newbler [92], Ngen [91], SOAP deNovo [96], SPAdes [88], Abyss [94], Ray Assembler [90], and CLC Genomics Workbench [87]. Annotation was predominantly done using RAST server and PGAAP pipeline [91] with the additional use of Glimmer, tRNAscan-SE, and RNAmmer [91].

With the explosive amount of genomic data generated in the recent year, efforts towards their analyses have also been slowly progressing. The last two years have seen several comparative genomic analyses of the strains belonging to the aforementioned genera of probiotics [99 - 101].

Furthermore, in the recent years, a special interest is also observed in studies pertinent to carbohydrate utilization in these organisms [102]. Also, there has been an impetus for the identification of novel genes helpful in diagnostics [103], and genomic characterization of important traits like motility[77].

TABLE 1: SPECIES, TYPE OF GENOME SEQUENCE AND TECHNOLOGY USED

Year	Species	Type of Genome sequence	Technology used
2004	*Lactobacillus johnsonii* NCC 533	Whole genome	Whole genome shotgun; Assembler: PHRED; Annotation: tRNSscan-SE, COG, ORF,
	Lactobacillus paraplantarum C7	PLASMID	
2005	*Lactobacillus hilgardii* 0006	Gene sequence	
2009	*Bifidobacterium animalis* subsp. lactis AD011		Traditional Sanger paired end sequencing of plasmid and fosmid libraries; Assembly: PHRED, PHRAP, CONSED; Annotation: Glimmer, CRITICA; AUTOFACT; Artemis for annotation verification
2011	*Lactobacillus amylovorus* GR1112	Genome	454 GS FLX pyrosequencer; Assembler: gsAssembler; Annotation: PGAP, EDGAR
	Lactobacillus amylovorus GR1118	Genome	454 GS FLX pyrosequencer; Assembler: Newbler; Annotation: PGAP
	L. crypricaesei *Lactobacillus ruminis* SPM0211	Genome	454 GS FLX pyrosequencer; paired end; correction by Illumina IIx genome analyzer; Assembler: GS deNovo Assembler 2.5 and CLC Genomics Workbench 4.5.1
	Lactobacillus iners AB-1 *Lactobacillus coryniformis*	Whole genome	shotgun 454 GS FLX; paired reads; Assembler: Newbler 2.3; Annotation; RAST, Glimmer 3.02, tRNAscan-SE, RNAmmer
	Lactobacillus aviaries *Lactobacillus cypricasei* KCTC 13900	Genome	454 Titanium pyrosequencing (Roche); Assembler: Newbler2.3; Annotation: Glimmer3.02, RNAmmer1.2, RAST
	Lactobacillus coryniformis KCTC 3167	Genome	454 GS FLX pyrosequencer;whole genome shotgun; Assembler: Newbler2.3; Annotation: RAST, Glimmer3.02, tRNAscan-SE 1.21, RNAmmer 1.2
	Lactobacillus animalis KCTC 3501	Genome	454 GS FLX pyrosequencer;whole genome shotgun; Assembler: Newbler2.3; Annnotation: RAST, Glimmer3.02, tRNAscan-SE 1.21, RNAmmer 1.2
	Lactobacillus sanfranciscensis	Genome	Combined Sanger/454 pyrosequencing; Annnotation: PEDANT, GenMark2.8
	Lactobacillus kefiranofaciens ZW3	Whole Genome	combo of 454 sequencing and GA IIx Solexa HTS; Assembler: Newbler; Annotation: PHRED, PHRAP, CONSED, Glimmer, GenMark; Verification by Artemis

2012	*Bifidobacterium asteroids* PRL 2011 *Lactobacillus rhamnosus* MTCC5462	Complete Genome	Shotgun; Roche GS 454; Assembler: GS Reference Mapper v 2.3;
	Lactobacillus vini LMG 23202T, JP7.8.9	Genome	Roche 454 GS FLX Titanium; Assembler: gsAssembler2.3; Annotation: RAST
	Lactobacillus curvatus CRL705	Draft	454 GS Titanium pyrosequencer; Assembler: Newbler 2.5.3; Annotation: RAST
	Lactobacillus rossiae DSM 15814T	Genome	Shotgun Illumina sequencing HiSeq 2000; paired end; Annotation: RAST
	Lactobacillus fructivorans KCTC 3543	Genome	454 GS FLX Titanium pyrosequencer; Assembler: Newbler 2.3; Annnotation: RAST, Glimmer3.02, tRNAscan-SE 1.21, RNAmmer 1.2
	Lactobacillus helveticus R0052	Complete Genome	454 GS FLX Titanium; Assembler: wgsAssembler v6.0; Annotation: PGAAP
2013	*Lactobacillus pentosus* KCA1	Genome	Paired end Next Gen Illumina GAII sequencing; Assembly: VELVET assembler; Mauve and Artemis comparison tool
	Lactobacillus helveticus CNRZ 32	Genome	Shotgun sequencing; Applied Biosystems ABI377 and 3700 automated sequencers; PE 377 automated DNA sequencers; Annotation: ERGO
	Lactobacillus shenzhenensis strain LY-73	Whole Genome	Illumina HiSeq 2000; paired end; Assembler: SOAP deNovo 1.05; Annotation: Glimmer 3.0, RAST
	Lactobacillus ginsenosidimutans sp *Lactobacillus florum*	Draft	Paired end Illumina HiSeq 2000; Assembler: Velvet 1.2.07; Annotation: RAST
	Lactobacillus pobuzihii E100301T	Draft	Illumina GAIIx; Assembler: Velvet; Annotation: RAST
	Lactobacillus jensenii MD IIE-70	Draft	Ion Torrent PGM; Assembler: Ion Torrent Assembler and CLC Genomics Workbench deNovo assembler; Annotation: PGAP and RAST
	Lactobacillus gasseri Strain 2016	Draft	Ion Torrent PGM; Assembler: Ion Torrent Assembler and CLC Genomics Workbench deNovo assembler
	Lactobacillus otakiensis JCM 15040 T	Whole Genome	454 GS FLX pyrosequencer; whole genome shotgun; Assembler: Newbler 2.7; Annotation: Glimmer3.02, GTPS, RDP, Silva, tRNAscan-SE
2014	*Lactobacillus gasseri* K7	Improved Draft	454 GS FLX+; Assembler: Newbler 2.6; Annotation: PGAAP, IMG-ER; Artemis and IMG-ER for curation
	Lactobacillus mucosae CRL573	Draft	Whole genome shotgun Ion Torrent Personal Genome Machine (PGM); Assmbler: NGen (DNAStar); Annotation: PGAAP, tRNAscan-SE
	Lactobacillus sakei wikim 22	Draft	Ion Torrent and a 318 chip; Assmbler: CLC Genomics Workbench v7.0.4; Validation of assembly by OSlay; Annotation: GenemarkS, RNAmmer, tRNAscan, RAST
	Bifidobacterium moukalabense DSM 27321	Genome	GenProBio srl using Ion Torrent PGM
	Lactobacillus salivarius	Draft	Illumina HiSeq2000; Assembler: Abyss; Annotation: Glimmer3, GeneMark, Artemis, InterPro, SignaalP, TMHM
	Lactobacillus sucicola JCM 15457 T	Draft	Ion Torrent PGM system; Assembler: Newbler v2.8; Annotation: RAST, Glimmer3

	Lactobacillus fabifermentans T30PCM01	Genome	Illumina MiSeq; Assembler: Abyss 1.3.6 and Velvet 1.2.10; Assemblies aligned using Mauve; Annotation: RAST, GeneMark.hmm 2.8,
	Lactobacillus oryzae Strain SG293 T	Draft	Illumina MiSeq; Assembler: Platanus v1.2.1; Annotation: MiGAP, MetaGene Annotator 1.0, tRNAscan-SE 1.23, RNAmmer 1.2
	Lactobacillus animalis 381-IL-28	Draft	Illumina GAIIx and IonTorrent PGM; Assembly: Velvet; manually validated with AMOS and Hawkeye; Annotation: GAMOLA v2
	Lactobacillus namurensis Chizuka 01	Draft	Roche 454 GS FLX, Assembler: Newbler 2.7; Annotation: MiGAP
	Lactobacillus equi	Genome	Illumina HiSeq2000; Annotation: Metagene
	Lactobacillus gorilla sp. Nov. *L. farraginis* JCM 14108 T	Draft	Ion Torrent PGM; Assembler: Newbler v 2.8; Annotation: RAST
	L. composti JCM 14202 T	Draft	Ion Torrent PGM; Assembler: Newbler v 2.8; Annotation: RAST
2015	*Lactobacillus delbrueckii* subsp. bulgaricus CRL871	Draft	Whole genome shotgun Ion Torrent (life technologies); Assembler: Ngen (DNASTAR); Annotation: RAST
	Bifidobacterium catenulatum JCM 1194T	Complete genome	Whole genome shotgun with sanger sequencing; Assembly: Phred-Phrap-Consed; Annotation: Glimmer 3.0, tRNAscan-SE
	Bifidobacterium pseudolongum PV8-2	Genome	Single molecule real time (SMRT) PacBio RSII; Assembly: Heirarchical genome assembly process; Annotation: PGAP, RAST
	Lactobacillus johnsonii strain 16	Draft	Illumina Genome analyzer IIx; paired ends; Assembler was Velvet0.7.54; Mapping MAQ0.7.1 and BWA 0.5.8c
	Lactobacillus rhamnosus CNCM I -3698	Draft	Illumina GAIIx; paired end; Assembler: deNovo CLC Genomics Workbench 5.0; Annotation: RAST and PGAP
	Lactobacillus reuteri *Bifidobacterium angulatum* GT102	Draft	Whole genome shotgun Roche 454; Assembler: gsAssembler v3.0
	Bifidobacterium adolescentis 150	Draft	Whole genome shotgun Roche 454; ; Assembler: gsAssembler v3.0
	Lactobacillus kunkeei	Genome	454 GS FLX pyrosequencer Titanium; Assembler: Newbler; Verified by BWA, Artemis, Artemis COMparison tool, Mauve; Annotation: DIYA, Prodigal, tRNAscan, RNAmmer, genePRIMP
	Lactobacillus mucosae DPC 6426	Draft/Genome	454 GS FLX and Illumina MiSeq; Assembly: MIRA; Artemis Comparison Tool; Annotation: RAST, Prodigal, Glimmer 3.02
	Bifidobacterium scardovii JCM 12489T	Complete Genome	Sanger and 454 GS FLX; Assembly: Phred-Phrap-Consed, Newbler; Annotation: Glimmer 3.0, tRNAscan-SE
	Bifidobacterium aesculapii DSM 26737 T	Draft	Illumina MiSeq; Assembler: Newbler v 2.8;
	Lactobacillus kunkeei EFB6	HQ Draft	Genome Analyzer II (Illumina); paired end; Assembler: SPAdes 2.5; Annotation: Glimmer3, YACOP, IMG-ER
	Lactobacillus curieae CCTCC M 2011381 T	Draft	Illumina Solexa HiSeq2000; Assembler: SOAP deNovo; Annotation: Glimmer 3, PGAP
	Lactobacillus acidophilus ATCC 4356	Draft	454 GS Titanium; Assembly: Newbler v 2.6; Annotation: RAST, PGAP
	Bifidobacterium actinocoloniiforme DSM 22766 T	Complete Genome	MiSeq and HiSeq 2000; paired end Draft genome assembler: SPAdes v3.50 and A5 miseq;

	Lactobacillus curvatus	Genome	RAST HiSeq 2000: Assembly: Velvet 1.2.07; Annotation: Glimmer
	Lactobacillus acidophilus FSI4	Complete Genome	Illumina GIIx; paired ends; Assembler: Velvet; Error correction by Illumine HiSeq 2000
	Lactobacillus sp. strain TCF032-E4	Draft	Illumina HiSeq 2500; Contigs ordered by Mauve 2.3.1; Assembler: Velvet 1.2.10; Annotation: RAST
	Lactobacillus rhamnosus CLS17	Draft	Roche 454 GS FLX Titanium; Assembler: Newbler v 2.3;
	Lactobacillus rhamnosus	Draft	Roche 454 GS FLX Titanium; Assembler: Newbler 2.6; Annotation: RAST
	Lactobacillus fermentum 3872	Genome	Ion Torrent PGM 314 v2 chip; Assembler: Torrent Assembler and CLC Genomics Workbench combined using CISA contig integrator; Annotation: RAST, PGAP
	Bifidobacterium kashiwanohense JCM 15439^{T}	Complete Genome	WGS Sanger and 454 GS FLX pyrosequencing; Assembler: Newbler, Phred-Phrap-Consed; Annotation: Glimmer 3, tRNAscan-SE
	Lactobacillus paracasei	Genome	Illumina Genome Analyzer II; Assembler: Velvet deNovo; Annotation: MiGAP, tRNAscan-SE
	Lactobacillus fermentum LfQi6	Draft	Illumina MiSeq; Assembler: Velvet and SPAdes;
	Lactobacillus hokkaidonensis LOOC260(T)	Complete Genome	PacBio SMRT RSII sequencer; Also, independent Illumina MiSeq; Assembly: deNovo by HGAP method, Platanus; Annotation: APBRO
	Lactobacillus farciminis CNCM-I-3699	Genome	Illumina GAIIx; 454 GSFLX; Assembly: CLC Genomics Workbench 5.0; Newbler 2.6; Annotation: RAST, GO and Pfmagainst UFO web browser
	Bifidobacterium scardovii Strain JCM 12489T	Genome	Sanger and 454 GSFLX; Assembler: Phred-Phrap-Consed; Annotation: Glimmer 3.0,
	Lactobacillus gorillae KZ01 T	Draft	Illumina MiSeq; Assembler: CLC Genomics Workbench 8.0.1; Annotation: PGAP, ARDB
	Bifidobacterium kashiwanohense PV20-2	Complete Genome	SMRT PacBio RSII; Assembly: Heirarchical genome assembly; Annotation: PGAP, RAST
	Lactobacillus curieae CCTCC M 2011381 T	Draft	Illumina SOlexa HiSeq 2000; Assembler: SOAP deNovo; Annotation: Glimmer 3.0, NCBI PGAP
	Lactobacillus plantarum P-8	Complete genome	454 GS FLX and Illumina Solexa GAIIx paired end combined; Assembler: Newbler
	Lactobacillus panis DSM 6035 T	Draft	Illumina MiSeq; Assembly: Velvet; Annotation: RAST
2016	*Lactobacillus casei* N87	Draft	Illumina HiSeq 1000; Assembler: CLC Genomics Workbench v 8.0.3; Annotation: PGAP
	Lactobacillus sakei FBL1	Draft	Ion Torrent PGM; Assembler: Ref based SPAdes v 3.1.0; Annotation: RAST
	Lactobacillus plantarum 2025	Draft	Ion Torrent PGM; Assembler: SPAdes and GWB, consensus combined by CISA; Annotation: RAST
	Lactobacillus plantarum SF2A35B	Draft	WGS Illumina HiSeq 2000; Assembly: deNovo by Ray Assembler; Annotation: RAST server
	Lactobacillus plantarum CRL1506	Draft	WGS Illumina MiSeq;

		Assembler: Ngen (DNAStar); Annotation; RAST, PGAP, tRNAscan-SE; RNAmmer
Lactobacillus equigenerosi NRIC 0697 T	Draft	Illumina MiSeq; Assembler: Newbler 2.8
Lactobacillus crispatus JCM5810	Draft	Illumina MiSeq; Assembler: CLC Genomics Workbench 8.5.1; scaffolds by Sanger seequencing
Lactobacillus casei DPC6800	Draft	Roche 454 FLX; Assembler: Ngen (DNAStar); Annotation: Glimmer 3.0.2, RAS; verified by BLASTp and Artemis
Lactobacillus kunkeei MP2	Genome	using one SMRT cell (P6-C4 Chemistry) on a PacBio RSII sequencer (Pacific Biosciences)
Bifidobacterium longum infantis TPY12-1		Illumina HiSeq2500; paired ends; Annotation Abyss v.1.9.0
Bifidobacterium longum suis BSM11-5		Illumina MiSeq; paired ends; annotation by RAST, Annotation Abyss v.1.9.0
Lactobacillus farciminis NBRC 111452	Draft	Ion Torrent PGM system; Assembler: Newbler v2.8; Annotation: RAST server using Glimmer3
Lactobacillus johnsonii strain W1	Genome	Illumina MiSeq; paired ends; Assembler: SOAP denovo 2.04.r240; Annotation: PGAP analysis
Lacobacillus brevis strain D6	Whole genome	Roche 454 GS FLX; Assembler: Newbler; Annotation: PGAAP analysis
Lactobacillus collinoides CUPV237	Draft	Illumina GAIIx; Assembler: Genomics Workbench v 7.0; Annotation: PGAP

CONCLUSION: In conclusion, the application of genomic technologies in probiotic research has facilitated better understanding of probiotic bacteria and the genes and the molecular mechanisms that endow them with characteristic traits. The advances in sequencing technologies through the years, represented by the four generations of high throughput sequencing technologies, have eventually enabled easier and faster acquisition of genome data as seen by the reports of the genome sequences published over the years. A parallel advance has also been witnessed in the development of genome assembly and annotation software and tools to facilitate the analysis of the genome data. Furthermore, studies pertinent to the biomolecule utilization and comparative genomics studies of probiotic genomes have been gaining momentum in the recent years.

Future Work: As understanding complete genome maps of probiotic bacteria give us insights into the characteristic traits of particular species, it is important to analyze and understand the genomes of these probiotics. It is also crucial that we look deeper into the genome to see what they actually possess. Comparative Genomics studies have to be carried out as they could reveal genes that are critical in rendering the probiotics non-pathogenic, distinguishing them from the other bacteria. This will also help us connect the similar traits present in different probiotic species, helping us understand the evolutionary relationship among the bacterial communities that form the intestinal microbiota. It is therefore, the need of the hour to develop databases and tools that aid in the analysis of probiotic genomes through comparative genomics studies.

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