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PROBIOTIC GENOMES: SEQUENCING AND ANNOTATION IN THE PAST DECADE

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ABSTRACT: Live bacteria known as probiotics have several positive effects on human health when given in sufficient doses. These positive effects on health have sparked interest in probiotics and encouraged its usage as a supplement to enhance overall health.overall well-being and as supplementary treatments for certain illnesses. A number of areas of human biology have benefited from probiotic research, which has been driven by the current uptick in demand for these products. Thanks to recent developments in genomics, it is now possible to sequence the genomes of probiotic bacteria and analyze them genetically to determine which genes are responsible for their health benefits. This article summarizes the genomic methodologies used to probiotic bacteria and provides information on the strains of probiotic bacteria for which genome sequences are already available. It also helps with comparative genomic investigations and compiles the genomic tools used for probiotic gene sequencing, assembly, and annotation.

Keywords: Probiotics, Genome, Bacteria, Disease

INTRODUCTION:

Live, non-pathogenic microbes that, when given to the host in sufficient doses, provide health advantages to the host are known as probiotics. one, two. They are functional foods (3, 4), and their health benefits cover a wide range of human health issues, such as regulating gut microbiota to promote intestinal health. 4–6. Avoidance of infections of the urinary tract, respiratory system, and intestines 2, 4, 7, 8, immune system activation, anti-allergicity, anti-cancer, anti-microbial, and cholesterol-lowering effects thirteen to sixteen. Modern scientific technology have been used to investigate the genetics and biology of probiotic bacteria, which has been driven by the rise of the global probiotic market. 3. In Greek, "pro" means "for" and "biotikos" means "pertaining to life," which is where the name "probiotic" comes from. The history of probiotics goes all the way back to the 18th century, when the first indications of their potential health benefits were found in ancient civilizations like the Roman Empire and the Bible. The study of probiotic species and their health effects was finally made possible by the discovery and isolation of gut microflora. 2,3,17,19, and 20. Probiotic bacteria include non-pathogenic strains of the genus Lactobacillus, as well as those of the genera Bifidobacterium, Clostridium, Bacillus, Escherichia, and Enterococcus 17. On the other hand, the probiotic market has been dominated by species of Bifidobacterium and Lactobacillus for a some now.

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Mycobaterium TB, Pseudomons aeroginosa, and other bacterial diseases were able to have their complete genomes sequenced in the late 90s and early 2000s, thanks to developments in sequencing technology. and E. coli, an enteropathogen. But recently, genomic technology and sequencing methodologies have been used to get and study the whole genomes of a number of probiotic bacteria, driven by demand 25–27. Therefore, the characterisation of microbial populations, especially probiotic bacteria 28, has been made possible by breakthroughs in genomic technology and computational approaches. The next part of this study will describe the most common probiotic species whose whole or partial genomic sequences are accessible online.

Genomics-Based Approaches to Probiotic Study: Published in 2004, one of the first whole genome sequencing experiments of a probiotic species was Lactobacillus johnsonii NCC 533. This experiment used full genome shotgun sequencing technology 27, a sequencing approach. The years that followed saw the publication of additional probiotic genome sequencing efforts, with the most recent years seeing an increase in the number of these projects (25, 29, 32). In addition, technology for sequencing genomes has evolved over the years, allowing for more genomes to be read, assembled, and annotated in less time (25–32).

Genome sequencing initiatives have progressed from using the age-old Sanger sequencing techniques (25), to using Next Generation Sequencing (NGS) technology (26–35), which are much more sophisticated. Prior to 2010, the conventional Sanger sequencing method and shotgun sequencing technology were used to sequence the genomes of probiotic species. A few examples are the Lactobacillus johnsonii (NCC 533) and Bifidobacterium animalis (AD01125) subspecies lactis genomes (27). Four major next-generation sequencing (NGS) technologies—454 pyrosequencing, Illumina/Solexa paired end sequencing, Ion Torrent, and Pacific BioSciences—have been used for genome sequencing since 2010. (26, 33–35).

Genomes of most probiotics were sequenced in 2011 using Roche 454 GS FLX.

protein sequencer. Among them are the genomic sequences of many species of Lactobacillus, including 33 and 36 for Amylovorus, 37 for Ruminis, 38 for Coryniformis, 39 for Animalis, 40 for Cypricasei, 41 for Sanfranciscensis, and 31 for Kefiranofaciens. It is not uncommon for two distinct sequencing methods to be used together. As an example, a hybrid approach combining Sanger sequencing and Roche

454 GS FLX pyrosequencing was used to sequence the genome of Lactobacillus sanfranciscensis (41). Likewise, the genome of Lactobacillus kefiranofaciens was sequenced using a combination of Roche 454 GS FLX pyrosequencing and Illumina Genome Analyzer IIx Solexa high throughput sequencing technology (31 samples total).

Although gsAssembler 36, 37 or the Phred-Phrap-Consed software package 41 were used in a few of instances, the majority of genome assemblies were performed using various versions of Newbler assembler 33, 39. A variety of tools, including the Rapid Assembly utilizing Subsystems Technology (RAST) server, EDGAR, tRNAscan-SE, RNAmmer, PEDANT, GeneMark, and the NCBI Prokaryotic Genome Automated Annotation Pipeline (PGAAP), were used for genome annotation.

Roche 454 GS FLX Titanium pyrosequencing technology was used to sequence the genome of Lactobacillus rossiae 34 that year, while the Illumina HiSeq 2000 platform was used to sequence the genomes of Lactobacillus rhamnosus 42, Lactobacillus vini 42, Lactobacillus curvatus 32, Lactobacillus fructivorans 43, and Lactobacillus helveticus 44. The majority of genome assemblies in this study were performed using Newbler Assembler. However, for Lactobacillus helveticus, Lactobacillus vini, and Lactobacillus rhamnosus, respectively, GS Reference Mapper, genome sequence assembler (gsAssembler), and whole genome sequence assembler (wgs Assembler) were employed 26, 42, 44. The aforementioned software was used for genome annotation, with RAST and PGAAP being the main tools for annotation.

With the introduction of new platforms such as the Ion Torrent Personal Genome Machine 46, Roche 454 GS FLX 47, Illumina Genome Analyzer Iix 48, and Illumina HiSeq 2000 49, probiotic genome sequencing in 2013 became more diverse in terms of the platforms used. During this time, the genomes of several species were sequenced: 48 Lactobacillus pentosus, 45 Lactobacillus helveticus, 49 Lactobacillus shenzhenensis, 50 Lactobacillus ginsenosidimutans, 51 Lactobacillus florum, 52 Lactobacillus pobuzihii, 46 Lactobacillus jensenii, 47 Lactobacillus gasseri. Another consequence of sequencing technology' many use is the proliferation of software for assembly and annotation. Software such as SOAP deNovo 49 and Velvet 48 were used to assemble sequences from Illumina platforms, whereas sequences from Ion Torrent PGM were assembled using Ion Torrent Assembler 46 or CLC de Novo Genomics Workbench. The majority of sequences from the Roche 454 GS FLX platform were assembled using various versions of Newbler 47. While RAST and PGAAP analysis accounted for the majority of

annotations, ERGO, GTPS, RDP, Silva, and ERGO were all recently included to the group (45, 47).

The Illumina and Ion Torrent technologies saw a surge in probiotic genome sequencing activity in 2014. Sequencing of the genomes of the following bacteria was performed by Illumina platforms: Lactobacillus equi 58, Lactobacillus animalis 59, Lactobacillus oryzae, Lactobacillus fabifermentans 60, and Lactobacillus salivarius 61. The Ion Torrent Personal Genome Machine was used to sequence the genomes of the following bacteria: Lactobacillus mucosae 53, Lactobacillus sakei 54, Bifidobacterium moukalabense 55, Lactobacillus sucicola 56, Lactobacillus farraginis 57, and Lactobacillus composti 57. The 62 genomes of Lactobacillus gasseri and Lactobacillus namurensis were sequenced using a Roche 454 GS FLX pyrosequencer.

When assembling genomes, a variety of assembly tools was utilized, each tailored to meet the specific needs of a particular genome. Genomes sequenced on Ion Torrent systems were assembled using Newbler57, NGen, and Roche 454 GS FLX genomes were assembled using Newbler assembler 62.

The following platforms are used to read data from Illumina platforms: (DNAStar) 53, CLC Genomics Workbench 54, Abyss61, 63, Velvet59, 63, Platanus60, AMOS59, Hawkeye 59, and so on. Newer tools such as GAMOLA59, MetaGene Annotator 60, MiGAP 60, SignalP 61, InterPro 61, TMHMM 61, and Artemis were used for annotation and curation, while RAST server and PGAAP remained the main platforms.

Several species of probiotics were sequenced within the subsequent two years. All kinds of sequencing technologies were in use in 2015, and several of them were used in combinatorial ways. Roche 454 pyrosequencers in conjunction with Illumina platforms (64 total) or Sanger sequencing (65 total) were the combinations used. This year, 66 also saw the usage of the single molecule real time (SMRT) Pacific Biosciences RSII sequencer. The species that were sequenced during this year include Lactobacillus delbrueckii 67, Bifidobacterium catenulatum 68, Bifidobacterium pseudolongum66, 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. In keeping with prior years, the following assemblers were utilized: Newbler 72, Velvet 29, gs Assembler 71, CLC Genomics Workbench 85, SOAP deNovo74, SPAdes 86, Ngen 67, and Phred-Phrap-Consed 68. Annotation was primarily performed using the RAST server and PGAAP pipeline 85, supplemented with Glimmer, tRNAscan-SE, Prodigal, GenePRIMP 65, 72, and PGAAP pipeline 85. In this year, MIRA 64 was one of the new assemblers utilized.

Illumina platforms accounted for the most majority of probiotic genome sequencing in 2016, while Ion Torrent, Roche 454, and Pacific BioSciences all played a small but significant role. Probiotics sequenced so far this year

The following species of bacteria are included: Lactobacillus casei (30), Lactobacillus sakei (89), Lactobacillus plantarum (88, 90, 91), Lactobacillus equigenerosi (92), Lactobacillus crispatus (93), Lactobacillus kunkeei (35), Bifidobacterium longum (94), Lactobacillus farciminis (95), Lactobacillus johnsonii (96), Lactobacillus brevis (97), and Lactobacillus collinoides (98). The following programs were used for the majority of the genome assemblies: Newbler 92, Ngen 91, SOAP deNovo 96, SPAdes 88, Abyss 94, Ray Assembler 90, and CLC Genomics Workbench 87. While Glimmer, tRNAscan-SE, and RNAmmer 91 were also used, RAST server and PGAAP pipeline 91 were the primary tools for annotation.

Efforts to analyze the massive amounts of genetic data produced in the last year have also been moving at a snail's pace. Several comparative genomic investigations of strains belonging to the aforementioned genera of probiotics 99–101 have been conducted in the last two years.

As an added bonus, research into how these creatures use carbohydrates has recently attracted a lot of attention 102. There has also been a push to characterize genomic features like motility77 and find new genes that aid in diagnosis 103.

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,
2005	*Lactobacillus paraplantarum* C7 *Lactobacillus hilgardii* 0006	PLASMID 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

Year	Organism	Type	Method
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; RAST
	Lactobacillus curvatus	Genome	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.

REFERENCES:

1. Food Working Group (FWG): On creating standards for the assessment of probiotics in food. Standards for the assessment of probiotics in food 2002; 1-11.
2. Bioengineered probiotics, a method for preventing gastrointestinal illnesses (Amalaradjou MAR and Bhunia AK). The article was published in Bioengineered in 2013 and can be found on pages 379-387.
3. In the Pakistan Journal of Pharmaceutical Sciences (2015), pages 2199–2026, Di Cerbo and Palmieri discuss the probiotics business.

Nagpal et al. (2012) published an article in FEMS Microbiology Letters, volume 334, pages 1–15.

5. Probiotic lactic acid bacteria in the gastrointestinal tract: Health benefits, safety, and mechanism of action (Dicks LMT and Botes M 2009). "Beneficial Microbes" 2010; 1: 11-29.
In their symposium, Salminen, Gueimonde, and Isolauri (2016) Ancestral Immunity and Disease-Preventing Human Milk Probiotics 135: 1294–1298 Journal of Nutrition, 2005.
Thomas LV and Ockhuizen T: A symposium report on new insights into the influence of the gut microbiota on health and illness. Part of the 2012 issue of the British Journal of Nutrition, volume 107, pages S1–S13.
8. Dasari S, Shouri RND, Wudayagiri R, and Valluru L: How Lactobacillus fights the microbes that cause infections in the

vagina. Journal of Tropical Diseases in Asia and the Pacific 2014; 4: 18-24.

9. Probiotic microbes stimulate the immune system (Ashraf R and Shah NP). Journal of the Science and Nutrition of Food, 2014, 54: 938-956.

The probiotic potential of lactobacillus strains with antimicrobial activity against some human pathogenic strains was investigated by Shokryazdan et al. (2010). Article published in 2014 by BioMed Research International, pages 1-16.

11. A Study on the Antimicrobial Activity of Honey-Isolated Strains of Lactobacillus acidophilus by Ween MM, Hassan Z, Muhialdin BJ, Noor HM, and Eljamel YA. The American Journal of Applied Science published an article in 2012 with the DOI number 9: 807-817.

12. Karska-Wysocki B, Bazo M, and Smoragiewicz W: Methicillin-resistant Staphylococcus aureus (MRSA) and the antibacterial activity of Lactobacillus acidophilus and Lactobacillus casei. Published in 2010 by Microbiological Research, volume 165, pages 674–686.

13. The study by Nguyen TDT, Kang JH, and Lee MS aimed to characterize Lactobacillus plantarum PH04, a probiotic bacteria that may have impacts on cholesterol levels. Paper published in 2007 in the 113th issue of the International Journal of Food Microbiology, pages 358–361.

In individuals with hypercholesterolemia, a microencapsulated yoghurt formulation of Lactobacillus reuteri NCIMB 30242 was shown to effectively decrease cholesterol levels (Jones ML, Martoni CJ, Parent M, and Prakash S.). Published in 2012 in volume 107, pages 1505–1513 of the British Journal of Nutrition.

In their 2012 study published in Experimental Diabetes Research, Kumar et al. (15) explored the use of cholesterol-lowering probiotics as a biotherapeutic for metabolic illnesses. The researchers involved were Nagpal, Kumar, Hemalatha, Verma, Kumar A, Chakraborty, Singh, Marotta, Jain, and Yadav.

16.This study was published in the European Journal of Clinical Nutrition in 2012 and was titled "Cholesterol lowering and inhibition of sterol absorption by Lactobacillus reuteri NCIMB 30242: a randomized controlled trial." The authors of the study were Jones ML, Martoni CJ, and Prakash S.

Malalaradjou MAR and Bhunia AK are the authors of the 17th. The American Journal of Clinical Nutrition, 2001, 73: 361S-364S.

18. Schrezenmeir J and deVrese M. Probiotics, prebiotics, and synbiotics—approaching a definition. Advances in Food and Nutrition Research, 2012, 67: 185–239.

Kim SY, Lee WK, and Lim HJ were the authors of chapter 19. Isolation of lactic acid bacteria from human intestines for the purpose of probiotics; these bacteria decrease cholesterol. Pages 391–395 of the Journal of Veterinary Science, volume 5, 2004.

Probiotics and prebiotics in the treatment and prevention of illnesses in babies and children; 20. Vandenplas Y, Veereman-Wauters G, deGreef E, Peeters S, Casteels A, Mahler T, Devreker T, Hauser B. No. 87, pages 292–300, Jornal de Pediatria, 2011.