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Complete Genome Sequence and Comparative Analysis of *Bifidobacterium bifidum* Strains Reveal Insight into Probiotic Properties

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ABSTRACT

Despite the widespread use of probiotic supplements for their beneficial health effects, there have recently been some concerns expressed over the absorption and potential colonization of the intestinal tract by the bacteria that these products contain. One of the bacterial species of the genus Bifidobacterium is the strain *Bifidobacterium bifidum*. One of the most prevalent probiotic bacteria in the bodies of mammals, including humans, is called *Bifidobacterium bifidum*. Recently, probiotics like *Bifidobacterium bifidum* have become more and more well-liked. However, it has been the focus of a few studies that demonstrate its efficacy in treating a number of ailments, including dyspepsia and malnutrition. The *Bifidobacterium bifidum* strain has genes that let the organism tolerate and be resistant to hazardous toxins and some medications, which helps with their capacity to cling to the host. Specialty genes, such as those encoding for vitamins, DNA repair, stress response, amino acids, bacteriocins, phages, and antibiotic tolerance and resistance, are responsible for certain bodily processes in hosts. In our study, eight sequencing datasets produced from *Bifidobacterium bifidum* sample were subjected to bioinformatic analysis. A particular number of ARGs were connected to integrated mobile genomic elements or prophages that assisted the HGT. In this study, we identified the genes that code for fundamental and significant properties of probiotics in the host, such as the production of bacteriocin and vitamins.

Background

The bacterium *Bifidobacterium bifidum* is used to make bacteriocins, fermented foods, and dairy products. The strains' genomes include several genes that may have been acquired through horizontal gene transfer. However, other genes may contain and disseminate virulence and antibiotic resistance determinants. Many of these genes are crucial for the regulation, metabolism, and transport of different sugars. Monitoring these genomes is crucial to the production of food in this way. By using various methods, we hope to present an overview of the genetic characteristics of *Bifidobacterium bifidum* in this work (Aslam *et al.*, 2009).

These studies all agree that the bacteria found in probiotics must stay in the intestinal tract for either a shorter or longer amount of time for the anticipated beneficial benefits to manifest over time. Nevertheless, colonization's success relies on a number of circumstances, thus each person's assurance that it will happen varies individual (Zmora *et al.*, 2018).

However, the likelihood of some adverse or possibly even dangerous effects from using probiotics has also been raised recently (Gopalakrishnan et al., 2018) Some bacterial strains used in probiotic supplements, powders, and capsules may include genes linked to antibiotic resistance, claim multiple research (ARGs).

Our recent research shows that 1,972 open-reading frames (ORF), most of which are connected to the metabolic process, are found in the *Bifidobacterium bifidum* genome. The functional genome typically has 1,305 genes that encode proteins involved in processes such as the synthesis of vitamins, cadmium and mupirocin resistance, and the generation of bacteriocins. Conclusions remarks: Based on our findings, there is a significant amount of genetic material being transferred between the strains. The strains may be valuable for biotechnology, bioremediation, and the generation of bacteriocins because we were unable to detect any virulence factors. However, only certain strains are covered by the potential applications. Because bifidobacteria are notoriously resistant to genetic manipulation, it is impossible to investigate their genomic functions, especially those that have positive effects on (human) health.

1. Introduction

Regardless of cultural background, geographic region, or societal standards, probiotics and probiotic products have established a global reputation and popularity in our daily lives. (Sanders *et al.*, 2018). Beneficial health effects assigned to probiotics have been reported in several studies. Probiotics are good bacteria of comparative genomics or yeasts that naturally

live in the body (Aslam *et al.,* 2009). The body constantly has good and bad bacteria and when there's an infection, that is, there are more bacteria knocking the body out of balance. Good bacteria help eliminate bad bacteria and probiotics are good bacteria that make the body healthy.

They are widely used for treatment of autoimmune conditions such as allergic reaction, (Vigil-stenmen *et al.,* 2017) they provide the effect of anti-inflammation and immune modulating capacity thereby providing adherence to intestinal epithelium, probiotics bacteria are most defined organisms when ingested in right proportion confer one or more benefits to the host immune system. There has been a rising attention in the benefits of probiotics on the host in recent years. As a result, different probiotic products have been developed to promote health of human organisms. Among them, B. *bifidum* is a gram-positive rod-shaped anaerobic bacterium that is neither motile nor spore forming majorly found in the colon, lower small intestine, breast milk and vagina. A small number of bifidobacterial (Sub)species such as Bifidobacterium *pseudologum,* Bifidobacterium *adolescentis,* Bifidobacterium *pseudocatenulatum,* and Bifidobacterium *bifidum,* have been isolated from various animal/mammalian hosts and for this reason are acknowledged as cosmopolitan bifidobacterial taxa (Milani *et al.,* 2017, Idrees *et al*)

Over the years, it has been proven that probiotic bacteria are of great benefit to the host, especially *Bifidobacterium bifidum* ICIS-629. This study aimed to characterize a complete genome sequence of *Bifidobacterium bifidum* ICIS-629 and determine specific probiotic properties in comparison with other strains.

2. Organism information

2.1 Classification and features

The size of the *Bifidobacterium bifidum* strain ICIS-629 genome is 2,254,433 bp, it possesses a circular chromosome consisting of a G + C content of 62.47%. Contained in the genomes, is a total of 1,972 open reading frames (ORFs) in addition with 3 genes encoding rRNAs and 52 genes encoding tRNAs. Among the ORFs present, 1,305 (66.2%) genes encode functional proteins while 667 (33.8%) encoding hypothetical (unknown proteins). The summary of the genome is presented in Table 1 below. Based on our bioinformatics analysis, there was no plasmid detected.

3. METHODS AND MATERIALS

In this study, probiotics is defined as good bacteria or live microorganisms that confer a health benefit on the host when taken in adequate or moderate quantity which is according to FAO/WHO. (FAO/WHO 2006)

3.1 DATA

We chose freely obtainable samples for the investigation from bacterial strains isolated from probiotic goods for human use or from the sequencing of such products. Strain ICIS-629 was initially identified as *Bifidobacterium bifidum* using the biochemical species identification kit ANAERO test 24 (Lachema, Czech Republic). The taxonomic identity of this strain was verified on a cell protein profile using a matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry Biotyper system (Bruker Daltonik, Bremen, Germany) and using 16S rRNA gene sequencing.

3.2 Sequencing Procedure

A single colony of ICIS-629 agar culture was inoculated and grown in 4ml of sterile Schaedler media for DNA isolation. Media Ingredients (gm/liter) -Tryptone: 5.67, Proteose peptone: 5.0, Soya peptone: 1.0, Yeast extract: 5.0, Dextrose (Glucose): 5.83, Sodium chloride: 1.67, Dipotassium hydrogen phosphate: 0.83, Tris (hydroxymethyl) aminomethane: 3.0, L-Cystine: 0.4, Hemin: 0.01, Agar: 15. After incubation, the culture was centrifuged at 4,000g for 6 minutes.nutrient with the composition of Yeast extract 5.0 Agar 13.5, Sodium Chloride 1.7, Sheep blood, defbrinated 5% Dipotassium Phosphate 0.8, pH of 7.6±0.2 The pelleted cells were resuspended in 50 l of Tris-buffered saline containing 2 g of hen egg white lysozyme (HEWL) and incubated for 60 minutes at 37°C. Using silica beads, the suspension was mechanically homogenized. Using proteinase K, DNAases were inactivated. The extracted DNA solution was purified with a standard phenol-chloroform extraction method and precipitated with ethanol (Godson et al., 1973). DNA was dissolved in deionized Milli-Q water. The genomic DNA of *Bifidobacterium bifidum* ICIS-629 was used to prepare a DNA library with the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA). The library was sequenced in a 2*300-nucleotide run using the MiSeq reagent kit v 3 and the MiSeq desktop sequencer (Illumina). A total of 251,752 sequence reads were generated. The reads were quality trimmed using the sliding window mode of the Trimmomatic v 0.40 program using default parameters (Bolger *et al.*, 2014). De novo genome assembly was performed using

the SPAdes genome assembler (St. Petersburg Genome Assembler) version 3.10.1 with default parameters (Bankewich *et al.*, 2012). The assembly yielded 24 contigs covering a total of 2,254,433bp with an N50 value of 251,752, a GC content of 62.47%, and an average coverage of 56.8.

The genome sequence was annotated using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok), which revealed 1972 gene sequences, including 1,306 proteins, 666 hypothetical proteins, 3 rRNA genes, 52 tRNA genes, and 0 noncoding RNA (ncRNA) genes. The revealed properties of *Bifidobacterium bifidum* ICIS-629 (e.g., a high level of acetate production) may be useful for probiotic development. This strain can serve as one of the models for host-microbiota interaction studies.

The table below shows the Bio-project, the Isolation source, region, and the G + C content of the samples used. The table below shows the details of the analyzed samples. One probiotic capsule was shotgun sequenced (PRJNA774131) by the authors. All further short read datasets were obtained from NCBI SRA repository.

Table 1: General Feature Table

STRAIN NAME	ACCESSION NUMBER	BIO-PROJECT	ISOLATION SOURCE	REGION	G + C CONTENT (%)
ICIS-629	JAJFBS000000000	PRJNA774131	Faeces	Russia	62.47
TMC 3115	NZ_AP018132	PRJNA224116	Faeces	Japan	62.79
S6	NZ_CP022723	PRJNA224116	Faeces	South- Korea	62.72
PRI 1	NZ_CP018757	PRJNA224116	-----	South-Korea	62.74
CNCM I-4319	NZ_CP058603	PRJNA224116	Faeces	France	62.74
YIT-10347	NZ_AP024712	PRJNA224116	_	_	62.76
HN002	CP069279	PRJNA698755	Faeces	South Korea	62.65
BF3	NZ_CP010412	PRJNA224116	Faeces	Korea	62.64

Table 1: This shows the list of meta-analyzed samples obtained from NCBI SRA, showing the strain name, accession number, bio-project, isolation, the region found, and the G + C content.

3.3 Complete Genomes

We retrieved the raw data from the query sequence that was made public on NCBI's ICIS-629 (National Center for Biotechnology Information) raw reads sequence and processed it using PATRIC (Pathosystems Resource Integration Center). The whole genome of the

Bifidobacterium bifidum substrains was downloaded as FASTA files for genome annotation. The bulk of samples were derived from feces in the People's Republic of Korea, Japan, Russia, and France, as shown in Table 1, which implies that various *Bifidobacterium bifidum* strains interact with one another in the gastrointestinal tract. An earlier study found that the *Bifidobacterium bifidum* micro flora lives in the human digestive tract (Palmer et al, 2007). This demonstrates the probiotic qualities of our *Bifidobacterium bifidum* strains, which were separated from their native ***microbiota.***

3.4 Genome Annotation

Annotation of the sub-strains of *Bifidobacterium bifidum* ICIS-629 were performed using the RAST tool kit (RASTtk) on PATRIC v3.28.9 for a comprehensive genome analysis of the sequences. The results were shown in Table 2. Featuring the genome length, G+C content, OPR, Annotated genes, Hypothetical proteins, RNA genes (tRNA+mRNA), CRISPR repeats, Pfam genes.

3.5 Specialty Genes

Bifidobacterium bifidum strain ICIS-629 was uploaded on RAST(Rast.nmpdr.org) for the annotation of the sequence. RAST(Rapid Annotation using Subsystem Technology) is a seed viewer that provides genome annotation, mapping genes of subsystems and metabolic reconstruction. And the result was shown in Table 3 , and represented in Fig. 1 and Fig. 2 as Pie chart and Bar chart respectively

3.6 Multiple Genomes Alignment

The rRNA sequence of *Bifidobacterium bifidum* ICIS-629 and the sub strains were downloaded from PATRIC. Using the Geneious software v9.0.5, multiple alignment of the rRNA sequence was done to create a phylogenetic tree.

3.7 Pan-genome

The reference sequence (*Bifidobacterium bifidum* strain ICIS-629), and the query sequence (including *Bifidobacterium bifidum* strain ICIS-629) were uploaded using the BRIG (Blast Ring Image Generator V0.95) software, and NCBI data set for mapping gene functions.

3.8 Prophage and Bacteriocin

The reference sequence (*Bifidobacterium bifidum* strain ICIS-629), and other substrains were uploaded on PHASTER (PHAge Search Tool Enhanced Release) pipeline tool for the identification of prophage sequences within the genome. ICIS-629 was uploaded on BAGEL4 (www.bagel.molgenrug.nl) for the identification of BACTERIOCIN- ribosomally synthesized peptides or proteins that are produced by bacteria. BAGEL4 could not identify bacterocins for substrain

4. RESULTS AND DISCUSSIONS

Genome sequencing and assembly

On the NCBI database, the whole genome of other strains as well as the whole genome sequence of *Bifidobacterium bifidum* strain ICIS-629, were accessed (*Bifidobacterium bifidum* CNCM I-4319, YIT-10347, BF3, PRI 1, HN002, S6, TMC-3115). The PATRIC data base has received the READ file with the accession number *Bifidobacterium bifidum* strain ICIS-629. A complete genomic study of the sub-strains' genome sequences was retrieved and submitted to the PATRIC database.

4.1 Pan-genome

The diagram as shown below illustrates comparison of the genome sequence of B. *bifidum* ICIS-629, TMC-3115, S6, PRI-1, CNCM I-4319, YIT-10347, HN002, and BF3 strains. The diagram shows a circular view of the chromosome of *Bifidobacterium bifidum* ICIS-629 strain which contains the leading (high G and low C region), and lagging (low G and high C region) strands of the genome sequence. The local GC skew deviations may indicate inversion, translocations, and newly incorporated DNA (9)

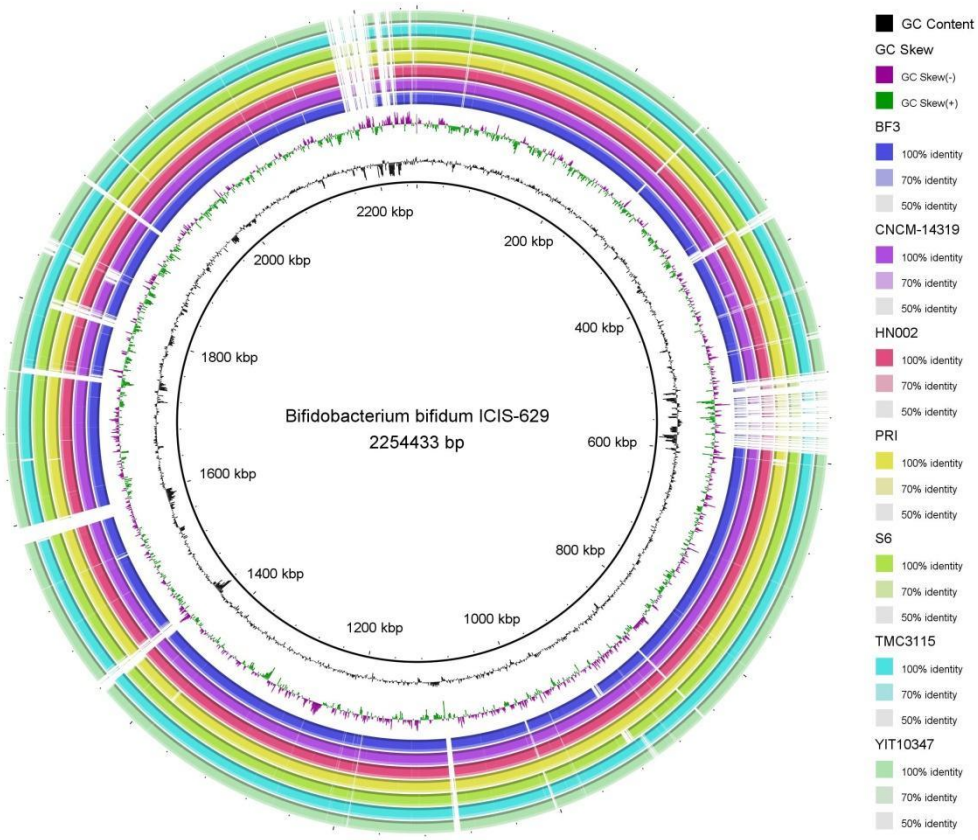

Fig.1

Bifidobacterium bifidum ICIS-629 *genome representation showing GC skew. Leading and lagging strands are shown in green and purple. BlastN comparison of the genome of Bifidobacterium bifidum ICIS-629 against those of YIT-10347, TMC-3115, BF3, PRI, CNCM-4319, HN002, and S6, are indicated by the color-coded key. The intensity of each color indicates nucleotide percentage identity. The diagram was generated using BRIGS software using an upper identity threshold of 70% and a lower identity threshold of 50%.*

4.2 Genome Properties

The size of *Bifidobacterium bifidum* train ICIS-629 genome is 2,254,433bp, with sub-strains ranging from 2,149,912bp to 2,311,342bp which possess a circular chromosome consisting of a G + C content of 62.47%. Contained in the genomes, is a total of 1,972 open reading frames (ORFs) in addition with as 3 genes encoding rRNAs and 52 genes encoding

tRNAs. Among the ORFs, 1,305 (66.2%) genes encode functional proteins while 667 (33.8%) encodes hypothetical and unknown proteins

Table 2: Comparison of Chromosomal Features among different strains

Strain	**ICIS-629**	**TMC 3115**	**S6**	**PRI 1**	**CNCM I-4319**	**YIT-10347**	**HN002**	**BF3**
Genome Length (Bp)	2,254,433	2,178,894	2,311,342	2,243,572	2,193,720	2,149,91	2,223,317	2,210,370
GC content (%)	62.47	62.79	62.72	62.74	62.74	62.76	62.65	62.64
Open reading frames	1972	1,854	1,997	1,917	1,873	1,811	1,895	1,881
Annotated genes	1305	1,283	1,295	1,286	1,273	1,260	1,265	1,271
Hypothetical and unknown proteins	667	571	702	631	600	551	630	609
RNA genes	55	61	62	59	62	61	61	61
CRISPR repeats			71		0	55	0	
Genes with Pfam domains	1,916	1,809	1,916	1,874	1,813	1,759	1,841	1,838

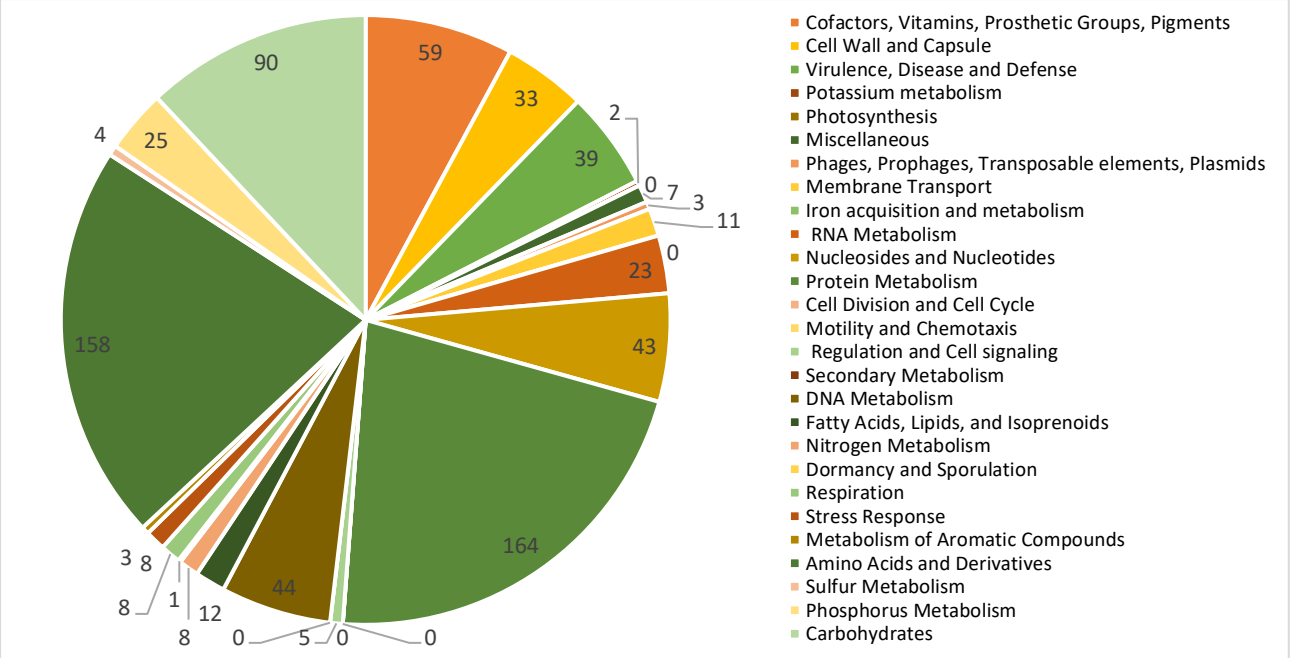

Fig 2: Pie chart representation of the subsystem genes presents in Bifidobacterium *bifidum* ICIS-62

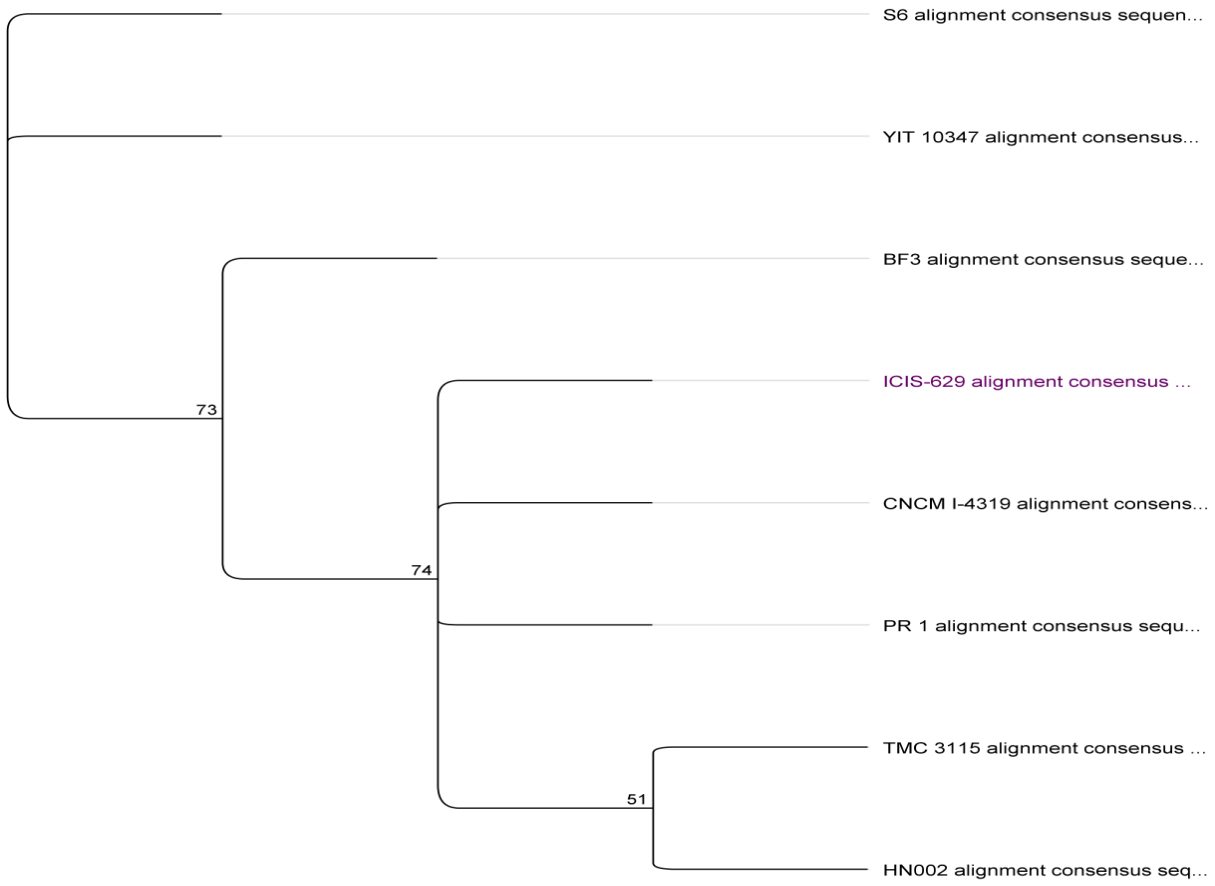

Fig 3: Phylogenetic tree analysis of B. *bifidum* ICIS-629 with seven available complete genomes sequences.

4.3Functional Capabilities

Following the subsystem result of our annotated genes on PATRIC, certain genes were found to play important roles in the gene of Bifidobacterium *bifidum* strain ICIS-629, including Vitamins, Amino acids, Stress response, DNA repair. Vitamin genes encode essential vitamins for the natural host. These genes encode vitamin B1, B2, B6 and B7 which serves a crucial role in assisting the bacteria to inhibit pathogens and harmful bacteria that colonize and infect the gut mucosa of the host. 3 genes encoded Vitamin B1, 4 genes coding for vit. B2, 8 genes coding for vit. B6 while 15 genes code for vit. B7. These genes were not just found in ICIS-629 but substrains (CNCM-4319, YIT 10347, BF3).

119 genes were seen to code for amino acids with 4 genes undefined and 6 genes coding for Alanine, serine and glycine. Other genes were seen to code for amino acids with the following number of genes. These were found in all sub-strains as the reference sequence, *Bifidobacterium bifidum* ICIS-629.

Table 4: showing the amino acid constituent and the number of genes that encodes them.

Amino acid	Number of genes
Arginine, urea cycle, creatine, polyamines	8
Aromatic amino acids and derivatives	22
Branched-chain amino acids	12
Glutamine, glutamate, aspartate, asparagine, ammonia assimilation	7
Histidine metabolism	10
Lysine, threonine, methionine, and cysteine	33
Proline and 4-hydroxyl proline	17

According to a recent study, intestinal bacteria produce bile salt hydrolase, which catalyzes the deconjugation of glyco- and tauro-conjugated bile acids by hydrolyzing the amide link.

The host's gastrointestinal tract contains the gene ICIS-629, which encodes bile hydrolysis, which aids in fat metabolism. Some genes are found to encode resistance to cadmium, a metallic compound that leads to cancer and organ-system toxicity including kidney and respiratory failure even anosmia, enabling the survival of the bacteria in the gastrointestinal tract against such toxic compounds. Other genes encode resistance to Capreomycin and

Viomycin, antibiotics against multidrug-resistant tuberculosis, ensuring the intake of that drug do not reduce the effect of this probiotic bacteria in the host. Comparing *Bifidobacterium bifidum* strain ICIS-629 to other strains, 8 genes code for Capreomycin and Viomycin resistance in sub-strains while 4 genes code for antibiotic resistance in *Bifidobacterium bifidum* ICIS-629. A gene coding for resistance of Mupirocin, an antibiotic useful against superficial skin infections could also be seen in *Bifidobacterium bifidum* ICIS-629 and sub-strains of *Bifidobacterium bifidum*, enabling the survival of this bacteria in the host with the use of this antibiotic. B.bifidum genes also take a major role in encoding for copper homeostasis which regulates the intake of copper in the bacteria and its host. Copper, an essential trace mineral, is vital for the survival of living things. In humans, copper is important to the proper functioning of organs and metabolic processes (68). Excessive copper can cause copper toxicity in the body which can result in liver damage and gastrointestinal symptoms (69). While its deficiency can lead to anemia, osteoporosis, etc(68).

Among other genes present in the genomic constituent of Bifidobacterium *bifidum* ICIS-629 are those which shows to play a role in stress response, particularly heat and cold shock, these serve as a means for the survival of bacteria in the host organism, 15 genes encode heat shock proteins such as GrpE, a nucleotide exchange factor that is important for regulation of protein folding machinery with the release of heat-inducible transmission repressor HrcA. 4 genes encode for DNA repair, a mechanism by which a cell maintains the integrity of its genetic code, in ICIS-629 (9). This ensures the survival of the probiotic bacteria from parent to offspring, ensuring the continuous availability of these bacteria in the host's gastrointestinal tract. DNA polymerase was seen as a product of DNA repair, with the role of replicating the genome in order to ensure the maintenance of genetic information. Other putative functions were shown in the table below.

Table 5: showing the putative functions of vitamin genes in B. bifidum sample strains in comparism with reference strain

Vitamin genes	**Putative functions**	**B.*bifidum* strains**			
		ICIS-629	**CNCM I-4319**	**YIT-10347**	**BF3**
Thiamin	Thiamin pyrophosphokinase	+	+	+	+
	Hydroxylethylthiazole kinase	+	+	+	+
	Cytoplasmic thiamin-binding component of thiamin ABC transporter	+	+	+	+

Riboflavin	Substrate-specific component RibU of riboflavin ECF transporter	+	+	+	+
	tRNA pseudouridine synthase	+	+	+	+
	FMN adenyltransferase	+	+	+	+
Biotin	Substrate-specific component BioY of biotin ECF transporter	+	+	+	+
	Long-chain-fatty-acid-CoA ligase	+	+	+	+
	Biotin—protein ligase	+	+	+	+
	Phosphoenolpyruvate carboxylase	+	+	+	+
	Biotin carboxylase of acetyl-CoA carboxylase	+	+	+	+
Pyridoxine	Pyridoxine Biosynthesis	+	+	+	+

Stress response genes	Putative functions	B.*bifidum* strains			
		ICIS-629	CNCM I-4319	YIT-10347	BF3
Heat/Cold Shock	Cold Shock Protein	+	+	+	+
	Translation elongation	+	+	+	+
	Factor LepA	+	+	+	+
	Nucleoside	+	+	+	+
	triphosphatase RdgB	+	+	+	+
	Ribonuclease PH	+	+	+	+
	16S rRNA	+	+	+	+

Antibiotics and toxic compounds	Choloylglycine hydrolase	+			
	Alanine racemase	+			
	LSU rRNA	+			
	Putative pre-16S				
	rRNA nuclease				
	Cadmium-transporting ATPase	+			
	Cytoplasmic copper homeostatis Protein	+			
	Magnesium and Cobalt efflux Protein	+			
	SSU ribosomal protein	+			
	Isoleucyl-tRNA synthetase	+			
DNA Repair	Exodeoxyribonuclease	+	+	+	+
	DNA polymerase	+	+	+	+
	Phosphoglycolate phosphstase	+	+	+	+

Table 6: Comparison of stress response genes of *Bifidobacterium bifidum* strains CNCM I-4319, YIT-10347 and BF3 with reference strains (ICIS-629).

Amino acids genes	Putative function	B.bifidum strains			
		ICIS-629	CNCM I-4319	YIT 10347	BF3
Alanine, serine, and glycine	Phosphoserine phosphatase	+	+	+	+
	D-3-phosphoglycerate dehydrogenase	+	+	+	+
	Phosphoserine aminotransferase	+	+	+	+
	L-serine dehydratase	+	+	+	+
Arginine; urea cycle, creatine, polyamines	Argininosuccinate lyase	+	+	+	+
	N-acetylornithine aminotransferase	+	+	+	+
	N-acetyl-gamma-glutamyl-phosphate reductase	+	+	+	+
	Ornithine carbamoyltransferase	+	+	+	+

	N-acetylglutamate kinase	+		+	+
	Glutamate N-acetyltransferase		+		
	Argininosuccinate synthase	+	+	+	+
		+		+	
Aromatic amino acids and derivatives	Chorismate mutase I	+	+	+	+
	3-dehydroquinate dehydratase II	+	+	+	+
	3-phosphoshikimate 1-carboxyvinyltransferase	+	+	+	+
	Anthranilate phosphoribosyltransferase	+	+	+	+
	Anthranilate synthase	+	+	+	+
	Arogenate dehydrogenase	+	+	+	+
	Biosynthetic Aromatic amino acid aminotransferase alpha	+	+	+	+
	Chorismate synthase				
	Indole-3-glycerol phosphate synthase	+	+	+	+
		+	+	+	+
	Phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	+	+	+	+
	Prephenate dehydratase				
	Shikimate 5-dehydrogenase	+	+	+	+
	Tryptophan synthase alpha chain	+	+	+	+
		+	+	+	+
Branched-chain amino acids	2-isopropylmalate synthase	+	+	+	+
	3-isopropylmalate dehydratase	+	+	+	+
	3-isopropylmalate dehydrogenase	+	+	+	+
	Acetolactate synthase	+	+	+	+
	Branched-chain amino acid aminotransferase	+	+	+	+
	Dihydroxy-acid dehydratase	+	+	+	+
	Ketol-acid reductoisomerase				

	Threonine dehydratase	+	+	+	+
	Valine--pyruvate aminotransferase	+	+	+	+
		+	+	+	+
Histidine Metabolism	ATP phosphoribosyltransferase	+	+	+	+
	Adenylosuccinate synthetase	+	+	+	+
	Histidinol dehydrogenase	+	+	+	+
	Histidinol-phosphate aminotransferase	+	+	+	+
	Imidazole glycerol phosphate synthase	+	+	+	+
	Imidazoleglycerol-phosphate dehydratase	+	+	+	+
	Phosphoribosyl-AMP cyclohydrolase	+	+	+	+
	Phosphoribosyl-ATP pyrophosphatase	+	+	+	+
	Phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	+	+	+	+
Lysine, threonine, methionine, and cysteine	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	-	+	+	+
	4-hydroxy-tetrahydrodipicolinate synthase	+	+	+	+
	4-hydroxy-tetrahydrodipicolinate reductase	+	+	+	+
	Aspartate-semialdehyde dehydrogenase	+	+	+	+
	Aspartokinase	+	+	+	+
	Diaminopimelate decarboxylase	+	+	+	+
	Diaminopimelate epimerase	+	+	+	+
	Homoserine dehydrogenase	+	+	+	+
	Homoserine kinase	+	+	+	+
	N-succinyl-L				

	Polyribonucleotide nucleotidyltransferase	+	+	+	+
	SSU ribosomal protein S15p	+	+	+	+
	Threonine synthase	+	+	+	+
		+	+	+	+
		+	+	+	+
Glutamine, aspartate, asparagine, ammonia	Glutamine synthetase	+	+	+	+
	Aspartokinase	+	+	+	+
	Homoserine dehydrogenase	+	+	+	+
	NADP-specific glutamate dehydrogenase	+	+	+	+
Proline and 4-hydroxyproline	Argininosuccinate lyase	+	+	+	+
	Argininosuccinate synthase	+	+	+	+
	Gamma-glutamyl phosphate reductase	+	+	+	+
	Glutamate 5-kinase	+	+	+	+
	Glutamate N-acetyltransferase	+	+	+	+
	N-acetyl-gamma-glutamyl-phosphate reductase	+	+	+	+
	N-acetylglutamate kinase	+	+	+	+
	N-acetylornithine aminotransferase	+	+	+	+
	NADP-specific glutamate dehydrogenase	+	+	+	+
	Pyridoxal phosphate-containing protein YggS	+	+	+	+
	Pyrroline-5-carboxylate reductase	+	+	+	+

4.4 Prophages

PHASTER software identified one region containing a phage attachment site in *Bifidobacterium bifidum* ICIS-629. A 24.5kb region of 1,185bp – 25, 740 bp includes a number of genes encoding for lysin. Another 15.6kb (PRI- 1 strain) region of 1,214,988bp – 1,230,606bp with no attachment includes a number of genes that encode transposase and lysin with a total protein of 17ORFs and another 21.2kb was identified with 2 region (1,283,980bp-1,305,203bp) encodes terminase with a protein of 26ORF in total. A 27.4kb region of CNCM I-4319(683326bp-710740bp) Includes a total number of 26ORFs protein encoding terminase. The strain of 6.9kb region (1,887,347bp-1,894,296bp) identified two regions encoding for transposase and tail protein. The BF3 strain of 20.7kb region (205,256bp-226,045bp) encodes transposase and integrase with a total protein of 11 ORFs. Another 6.9kb region (1,079,022bp-1,085,971bp) of strain TMC 3115 encodes transposase and tail protein. Two prophage region were identified in strain HN002 with 13.3kb region length encoding for terminase, portal and head protein with total protein of 16ORFs. Four prophage regions were identified in the S6 strain. The 15.6 kb region (453620bp- 469305bp) encodes head, portal and terminase protein. The 13.8kb region(1097009bp-1110895bp) has no prophages. Another 10.6kb region (2254593bp-2265204 bp) encodes the tail protein.

Region between 1,079,022bp and 1,400,834bp were seen to encode majorly for transposase. Transposase is an enzyme that binds to the end of a transposon and catalyzes its movement to another part of the genome by replicative transposition mechanism. Transposases are mobile genetic elements suggested to have an important role in bacterial genome plasticity and host adaptation but their transcriptional activity in natural bacterial communities is largely unexplored. (12) From the previous studies, this mobile genetic element is suggested to have an important role in bacterial genome plasticity and host adaptation. (13) Lysine was the only prophage found in ICIS-629 and was also found in sub-strain PRI-1. Lysines are hydrolytic enzymes produced by bacteriophages in order to cleave the host's cell wall. (14) This explains the adherence ability of ICIS-629 to its host. Terminase, Tail, head, and portal proteins were found in sub-strains of ICIS-629.

4.5. Bacteriocin

Revealed in *Bifidobacterium bifidum* ICIS-629 is a unique region encoding bacteriocin, a ribosomally synthesized peptides produced by bacteria (4). Previous studies of bacteriocins

proof to have shown antibacterial activity against other bacteria. Using BAGEL 4 to predict bacteriocins from Bifidobacterium *bifidum* ICIS-629, geobacillin I was discovered alongside other genes encoding promoters, terminators, transporters and regulators as shown in figure 2.0. Geobaccilin I belongs to class I bacteriocins with the antimicrobial spectrum shown to be generally similar to nisin A (8) which was desmonstrated to have increase in activity against streptococcus dysgalactiae. This was investigated with a range of Gram-positive bacteria.

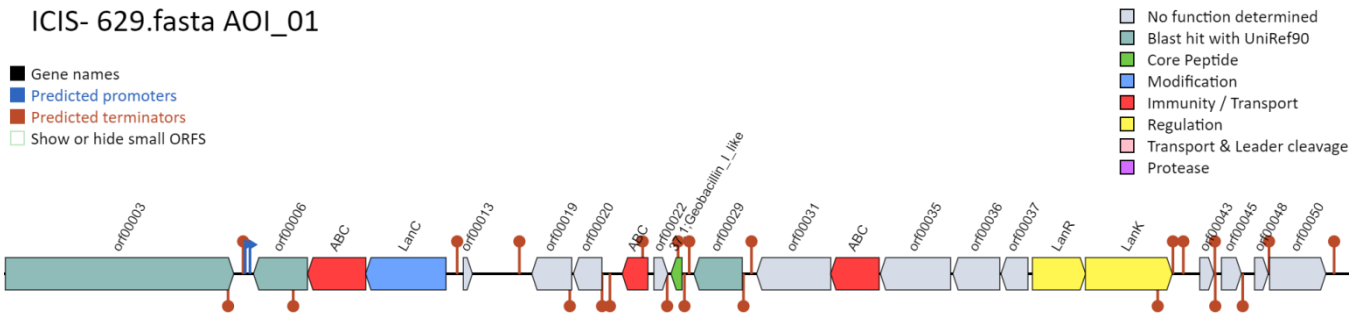

Fig 2.0 Diagram of Bacteriocin showing the core peptide Geobacilius_I_like, present in *Bifidobacterium bifidum* strain ICIS-629

5 CONCLUSION

There are numerous *Bifidobacterium bifidum* strains that have been shown to have positive benefits, including antibacterial properties against infections. Probiotic strains of *Bifidobacterium bifidum* must be able to colonize, compete with, and endure in the human intestine in order to live in this environment. After *Bifidobacterium bifidum* ICIS-629's genome sequencing was complete, the probiotic bacteria's diverse abilities could be identified. Some genes that encode for vitamins, such as thiamine, which the body cannot generate and aids in the breakdown of food in the host to maintain a healthy neural system, were found in the sequence that contributes to the probiotic qualities of the bacteria. Other vitamins include Biotin and Riboflavin, which are also important for the metabolism of protein and fat and for maintaining the health of the host's body. Other genes aid in the attachment of the bacteria to the host so they can survive in the hostile environment of the host, particularly in the GIT.

Our research indicates that *Bifidobacterium bifidum* ICIS-629 and its substrains possess genes encoding proteins that facilitate adherence to the host's acidic and harsh environment. Lysine is unique to strain ICIS-629 and helps the bacteria connect to the host cell wall, while the majority of the genes in the sub-strains code for transposases. It was also found that some genes code for heat/cold shock, a trait that helps bacteria endure extremes of temperature while still

inside their host. This aids the host's homeostasis as a result. Tolerance and resistance to harmful and antibiotic chemicals are not the sole purview of the genes formerly thought to be responsible for these phenomena. Mupirocin is a topical antibiotic cream that prevents the host from suppressing the probiotic effect by encoding resistance in the bacterium. There are other genes that protect the host against the toxic effects of cadmium by preventing the system from absorbing the metal. The host's copper level is regulated by a set of genes in ICIS-629 responsible for copper tolerance and copper homeostasis. Copper and iron are examples of trace elements that aid in hemoglobin production. Thus, this probiotic can control copper levels when they are too high or too low. Proline, a crucial amino acid, was found to have genes that translate for it in probiotic bacteria. It's believed that this amino acid helps the skin maintain moisture and works to prevent the production of fine lines and wrinkles. The genes in bacteria responsible for DNA repair are also identified in this study, ensuring the long-term viability of probiotic bacteria within the host. The bacteriocin gene was one of the unique features of ICIS-629 that couldn't be found in any other strain sequencing. The bacteria produce a bacteriocin with unique fermentative and preservation properties, making the dairy product last longer. The bacteriocin's potent antimicrobial activity, especially against heat bacteria, has implications for the search for new antibiotics and the promotion of host-infection prevention. More studies are being conducted to learn more about the bacteriocin that was engineered.

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