The complete genome sequences of the two novel probiotics were isolated from the human gut microbiota: *Pediococcus acidilactici* WNYM01 and *Pediococcus acidilactici* WNYM02, vitamin B9, and B2-producers

Wagiha S. Elkalla[a], Yasser M. Ragab[b], Mohamed A. Ramadan[b], Nahla M. Mansour[c]

[a]Department of Microbiology and Immunology, Faculty of Pharmacy, Badr University in Cairo (BUC), Badr city, Cairo 11829, Egypt, [b]Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Giza, Egypt, [c]Department of Chemistry of Natural and Microbial Products, Gut Microbiome and Immunology Group, Institute of Pharmaceutical Research Industries, National Research Centre, Dokki, Egypt

Correspondence to Nahla M Mansour, PhD, Leader of Gut Microbiome and Immunology Group, Department of Chemistry of Natural and Microbial Products, Institute of Pharmaceutical Industries Research, National Research Centre, 33 El Buhouth Street, Dokki, Cairo 12622, Egypt. Tel: +2 01222174789; e-mail: nm.hassanein@nrc.sci.eg, nahla_mansour@hotmail.com

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Background
We previously isolated the two strains *Pediococcus acidilactici* (*P. acidilactici*) WNYM01 and *P. acidilactici* WNYM02 from human gut microbiota as producers of vitamin B2 and B9 and they were identified by a molecular method based on sequencing of 16S rRNA gene. Their probiotic properties were confirmed *in vitro* and *in vivo* in rat colitis model.
Objective
This study aimed to sequence the complete genome of these two valuable probiotic strains. It will provide comprehensive data about them for further applications in research and health applications.
Materials and methods
The genomic DNA from *P. acidilactici* WNYM01 and *P. acidilactici* WNYM02 were extracted using AxyPrep bacterial genomic DNA miniprep kit. The Illumina NexteraTM tagmentation protocol was used to prepare libraries and then they were sequenced using the MiSeq with an Illumina v3 cassette (Illumina, USA). The genome sequences were constructed from 3.2 million paired-end reads ranging in size from 80 to 250 base pairs. The DNA sequence contigs were aligned using whole-genome alignment within CLC Genomics and annotation was done by RAST tool.
Results and conclusion
The genome sequence of *P. acidilactici* WNYM01 includes 2,002,062 bases while *P. acidilactici* WNYM02 includes 1,999,478 bases and they submitted to the NCBI database. The folate and riboflavin genes were detected within the two genomes and the differentiation between them has been recorded. Whole-genome alignments were used to detect functional differences related to their potential use as probiotics. The genomic sequences of these two strains will give extensive data on these interesting strains for future research uses.

Keywords:
genome sequence, human microbiome, next generation probiotics, *Pediococcus acidilactici*, probiotic

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Introduction

Pediococcus acidilactici (*P. acidilactici*) WNYM01 and *P. acidilactici* WNYM02 strains are cocci in shape, gram-positive belong to *Lactobacillaceae* family. This family is a member of the lactic acid bacteria (LAB) group. The two strains WNYM01 and WNYM02 *P. acidilactici* are isolated from human gut microbiota as vitamin producers and identified on molecular basis as *P. acidilactici* [1]. The two strains demonstrated high resistance to an inclusive array of pH, and temperature up to 70°C, and showed probiotic properties *in vivo* and *in vitro* experiments. In general, *P. acidilactici* bacterium is a homofermentative, facultative anaerobe with antagonism towards other microbes and enteric pathogens. It secrets bacteriocins named as pediocins in addition to lactic acid [2–4]. *P. acidilactici* has arisen as a potential probiotic for general and mental health according to the promising outcomes in animal and human research [5–7]. *P. acidilactici* strains were isolated from different sources by other researchers who determined also their complete genome sequence for example, strain IRZ12B was isolated from fermented food [8], strain CACC 537 was isolated from canine [9], strain CLP03 was isolated from Felis catus [10], strains Alc3 and Alc5 from human feces [11].

The term probiotics was first described by (FAO/WHO 2001) [12] as 'live microorganisms which, when administered in acceptable quantities, confer a health advantage on the host'. Even while commensals in the gut are frequently the source of probiotic strains, they cannot be referred to as 'probiotics' unless they are isolated, defined, and their health benefits are reliably evaluated [13].

Although the most common probiotic bacteria are belong to *Lactobacillus* and *Bifdobacterium*, some of them are sensitive to room temperature and have difficulty surviving in low pH environments, which causes a significant barrier to storage and processing to keep the bacteria alive. *Pediococcus* have tolerating to high temperatures that may reach 65°C in addition to highly acidic and alkaline media [14].

Hence the beneficial effect of probiotics is strain-specific thus there is an urgent need to search for and obtain new and unique strains for health and medical treatments [15]. Whole genome sequencing is a reliable method that determines the order of bases in the genome of a bacterium in one process to ensure the safety evaluation and accurate identification [16,17].

The *in vitro* and *in vivo* experiments that done on the two strains *P. acidilactici* WNYM01, WNYM02 suggesting them as promising novel probiotic candidates for modulation the gut microbiome and pharmaceutical applications [1]. Here we announce the complete genome sequence of our valuable strains *P. acidilactici* WNYM01 and *P. acidilactici* WNYM02. The availability of a complete genome sequence provides a comprehensive view of probiotic strains, enabling safety assessment, functional characterization, and identification of strain-specific properties.

Patients and methods

Microorganisms and growth conditions

P. acidilactici strains were cultured at 37°C on de Man, Rogosa, and Sharpe (MRS) media (Laboratories Conda S.A., Madrid, Spain).

Extraction of genomic DNA

Following the manufacturer's instructions, the bacterial genomic DNA AxyGen kit (Biosciences, Union City, CA, USA) was used to extract genomic DNA from overnight bacterial cultures. The results were verified by 1% agarose gel electrophoresis.

Sequencing of the whole genome

To carry on the sequencing of the whole genome of the bacterial strains, first libraries were produced with an Illumina v3 cassette (Illumina, USA) then carried on the MiSeq via the Illumina NexteraTM tagmentation technique. The whole genome sequences for *P. acidilactici* WNYM01 and WNYM02 were constructed from scratch using roughly 3.2 million 80–250 bp paired-end reads each. To facilitate genome sequence assembly, DNA sequence contigs were aligned using whole-genome alignment in the CLC Genomics Workbench.

Genome annotation

The RAST server (https://rast.nmpdr.org) was used to annotate the whole genomes of *P. acidilactici* WNYM01 and WNYM02, and then their whole genome sequences were submitted to the GenBank database.

Phylogenetic analysis

The closest reference and representative genomes to the genomes of the two strains were recognized via Mash/MinHash [18]. PATRIC global protein families (PGFams) [19] were chosen from these genomes to define their phylogenetic position then their protein coding sequences (CSD) were aligned through MUSCLE [20], also their nucleotides were plotted to the protein configuration. The joint set of protein and DNA alignments was successively entered into a statistics matrix, and the tree support values were generated using RaxML [21] and fast bootstrapping [22].

Characterization of folate and riboflavin biosynthesis genes

Characterization of the folate and riboflavin subsystem was done in the SEED database https://rast.nmpdr.org/seedviewer.cgi. Phylogenetic pattern searches were done on the NMPDR SEED server, Blast search [23], and platform STRING [24].

Nucleotide sequence accession number

The complete genome sequences are submitted to GenBank and allocated the accession numbers; CP107051 and JAOSZF000000000 for WNYM01 and WNYM02 respectively.

Results and discussion

General genome features

The raw data of the two complete genome sequences was obtained as fastaq files then were cleaned and trimed at the Galaxy platform [25] followed by

bioinformatics analysis. The bioinformatics analysis of the two genome sequences revealed few differences between them in regard to the genome size where WNYM01 presented 2,002,062 bases while *P. acidilactici* WNYM02 presented 1,999,478 bases in addition to the absence of some genes. However, their genome size falls within the range documented by Li and his colleagues [26] in a comparative study of 41 *P. acidilactici* strains from different ecological samples and the genome size of all the tested strains was 1.88–2.17 Mb with average genome size 1.97 Mb. The genome sequence of our two strains WNYM01 and WNYM02 exhibit 95% identity to other *P. acidilactici* strains especially strain DSM 20284 which isolated from human feces as well.

The general genome features of the two strains are presented in Table 1, which display that the genomes of *P. acidilactici* WNYM01 holds 2,002,062 bases while *P. acidilactici* WNYM02 holds 1,999,478 bases calculating 2721 and 2515 CDSs respectively, both of them showed an average of 42% GC contents.

Phylogenetic analysis

The phylogenetic trees were constructed by MEGA software as shown at Fig. 1a,b and it is clear that *P. acidilactis* DSM 20284 is the closest evolutionary relative of *P. acidilactis* WNYM01 and WNYM02.

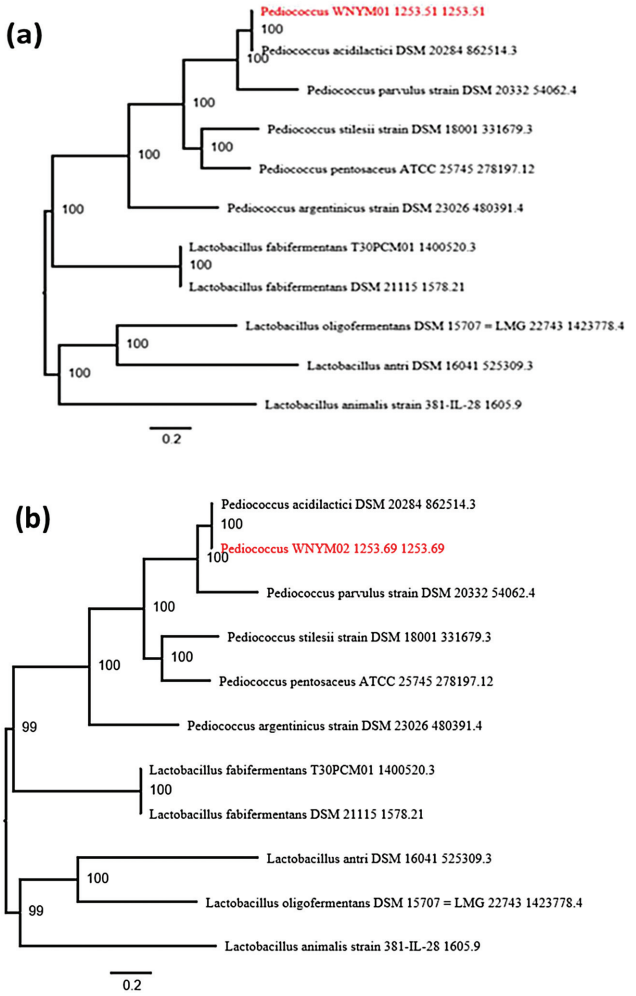
The phylogenetic analysis for the two genome sequences showed they are close to neighborhood strains *Pediococcus parvulus* strain DSM 20332, *Pediococcus stilesii* strain DSM 18001, *Pediococcus pentosaceus* ATCC 25745 (plant isolate), *Pediococcus argentinicus* strain DSM 23026 (isolated from fermented wheat flour), *Lactobacillus fabifermentans* T30PCM01 (Isolated from Fermenting Grape Marc), *Lactobacillus fabifermentans* DSM 21115 (isolated from Ghanaian cocoa fermentation),

Table 1 General genome features of *P. acidilactici* strains: WNYM01 and WNYM02

Genome	P. acidilactici WNYM01	P. acidilactici WNYM02
Size	2,002,062	1,999,478
GC Content	42.0	42.0
N50	3041	4015
L50	182	150
Number of Contigs (with PEGs*)	950	696
Number of Subsystems	201	203
Number of Coding Sequences	2721	2515
Number of RNAs	46	57

*PEGs, protein encoding genes.

Figure 1



Phylogenetic tree of the genomes: *Pediococcus acidilactici* WNYM01 (a) and *Pediococcus acidilactici* WNYM02 (b).

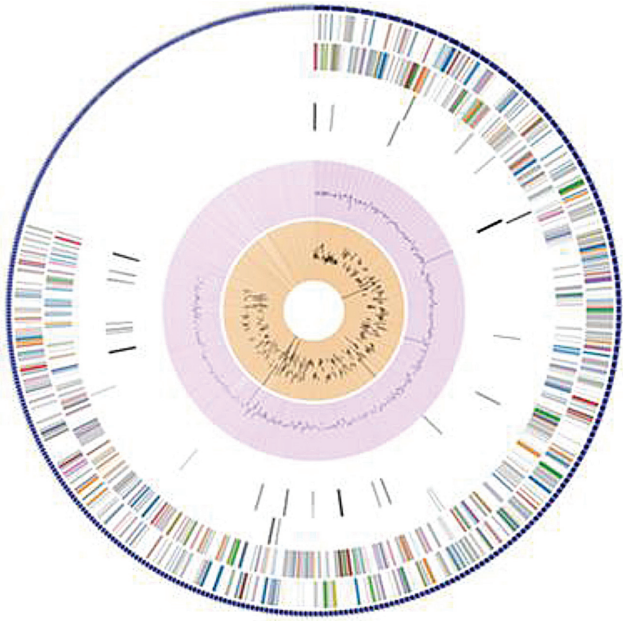
Lactobacillus oligofermentans DSM 15707 (isolated from broiler leg), *Lactobacillus antri* DSM 16041 isolated from human stomach tissue, *Lactobacillus animalis* strain 381-IL-28 isolated from kimchi.

Genome annotation

The WNYM01 and WNYM02 genomes were annotated using RAST server [22] and assigned a unique genome identifier of 1253.51. This genome is in the super kingdom Bacteria and was annotated using genetic code 11. The classification of the two genomes is: cellular organisms > Bacteria > Terr bacteria group > Firmicutes > Bacilli > Lactobacillales > Lactobacillaceae > *P. acidilactici*

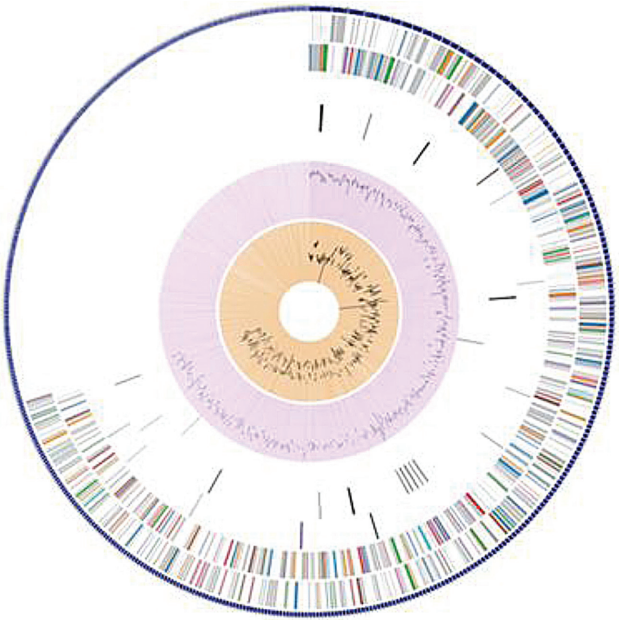
The WNYM01 genome has 2,721 CDS, 46 RNA genes, and 201 subsystems. While the WNYM02 genome has 2,515 CDS, 57 RNA genes, and 203 subsystems. The annotated features are summarized in Table 1. A circular genome maps display of the distribution of the genome annotations is provided for

Figure 2



A spherical graphical exhibition of the scattering of the WNYM01 genome annotations.

Figure 3



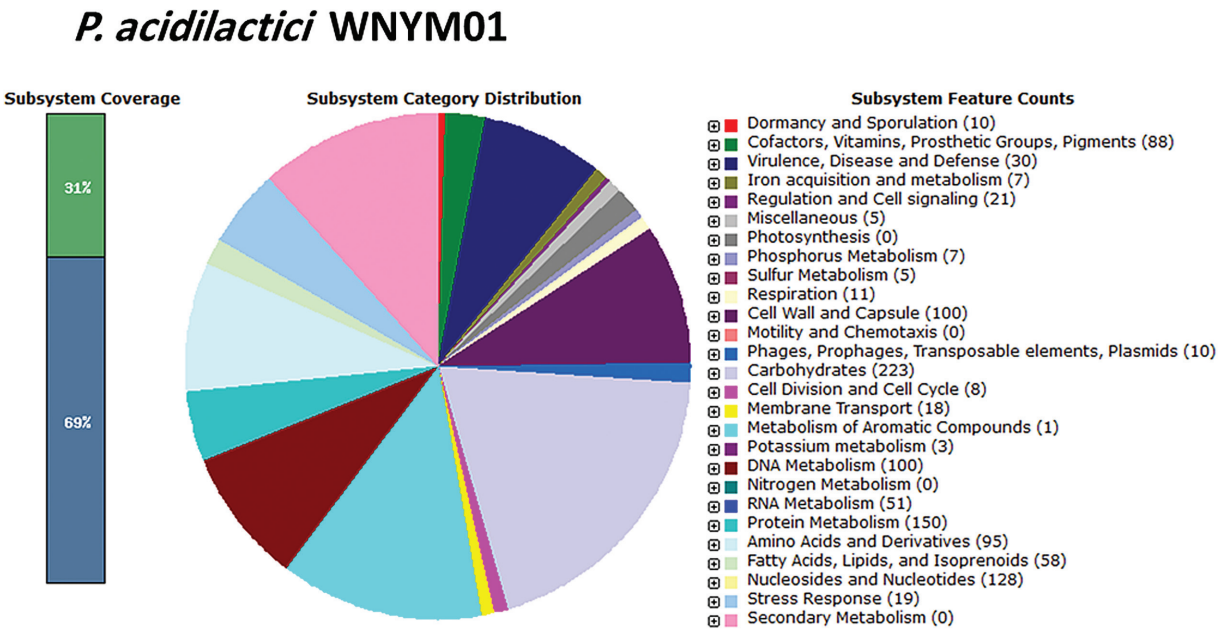
A spherical graphical exhibition of the scattering of the WNYM02 genome annotations.

P. acidilactici WNYM01 (Fig. 2) and *P. acidilactici* WNYM02 (Fig. 3). This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to identified antimicrobial resistance genes, CDS with homology to identified virulence features, GC content and GC skew. The colors of the CDS on both strands indicate the subsystems for the identified genes. Figures 4 and 5 show the subsystems category distribution for *P. acidilactici* strains WNYM01 and WNYM02, respectively.

Folate and riboflavin biosynthesis genes within the *P. acidilactici* WNYM01 and *P. acidilactici* WNYM02 genomes

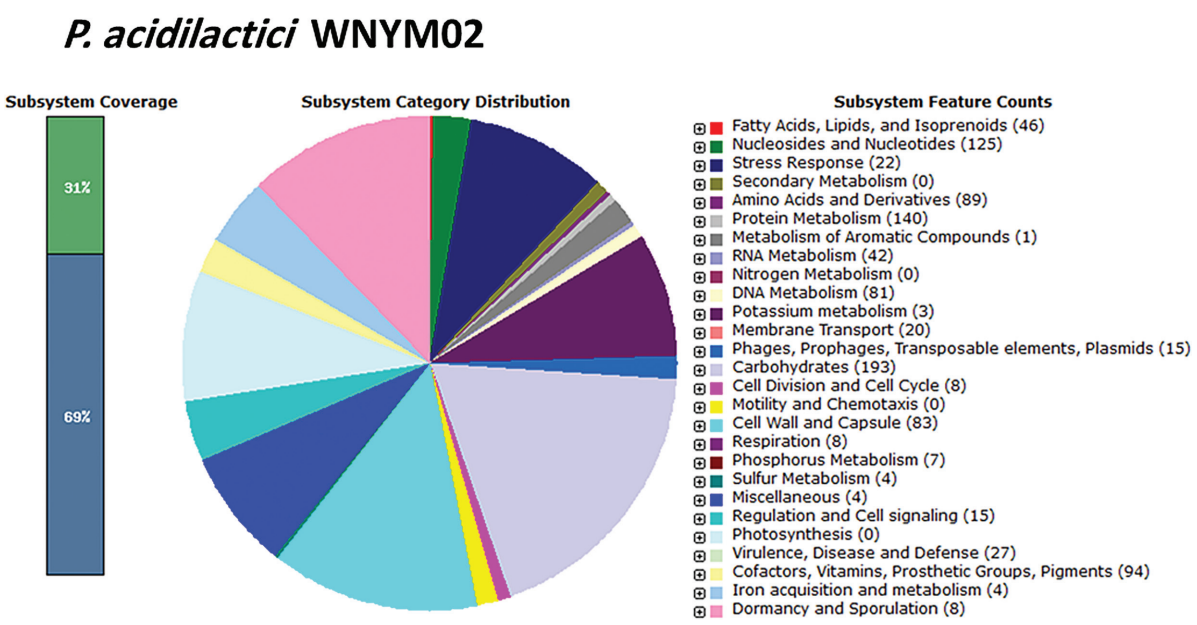
Using the folate (vitamin B9) and riboflavin (vitamin B2)-producing bacteria as probiotics in supplements

Figure 4



Subsystem category distributions of WNYM01 based on genome annotations via RAST server (https://rast.nmmpdr.org).

Figure 5



Subsystem category distributions of WNYM02 based on genome annotations via RAST server (https://rast.nmmpdr.org).

and food functional requires having all the information about the genes involved in their biosynthesis. The increasing rate of availability of different genome sequencing data provides the opportunity for the future discovery of possible novel genes related to these vitamins pathways and other pathways as well. Thus, the complete genome sequence of each of the two strains was searched for the sequences of the folate and riboflavin biosynthesis genes and related genes through seed viewer. This search allowed us to identify the genes involved and their location at the chromosomal DNA.

In respect to folate biosynthesis, the complete genome sequence of the two strains *P. acidilactici* WNYM01 and *P. acidilactici* WNYM02 revealed the presence of seven genes in different clusters. The genes are in charge of de novo biosynthesis of folate (Tables 2 and 3) are encoded as: *fol*D, *fol*B, *fol*k, *fol*T, *fol*C, *fol*E1, *fol*P. These genes are responsible for the biosynthesis of the folate precursors DHPPP (6-hydroxymethyl-7,8-dihydropterin pyrophosphate) which associated to the pterin branch. *fol*C and *fol*E1 are found in more than one copy. The 5fcl gene, the cofactor in folate metabolism was detected in addition to other co-related genes; *thy*A encoding thymidylate synthase, *HPRT* encoding hypoxanthine guanine phosphoribosyl transferase and *Til*S encoding tRNA(Ile)-lysidine synthetase. No genes found for the PABA branch.

Table 2 List and region of the folate biosynthesis genes (in bold) and related genes within the genome sequence of *P. acidilactaci* WNYM01.

P. acidilactaci WNYM01						
Start	End	Length	Strand	AA Length	Product	Gene
1	123	123	+	40	GTP cyclohydrolase I (EC 3.5.4.16) type 1	***fol*E1**
116	1270	1155	+	384	Dihydrofolate synthase (EC 6.3.2.12) @ Folylpolyglutamate synthase (EC 6.3.2.17)	***fol*C**
688	1962	1275	+	424	Dihydrofolate synthase (EC 6.3.2.12) @ Folylpolyglutamate synthase (EC 6.3.2.17)	***fol*C**
346	711	366	+	121	Dihydroneopterin aldolase (EC 4.1.2.25)	***fol*B**
704	1213	510	+	169	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase (EC 2.7.6.3)	***fol*K**
148	1278	1131	–	376	Dihydropteroate synthase (EC 2.5.1.15)	***fol*P**
133	711	579	+	192	5-formyltetrahydrofolate cyclo-ligase	*5FCL*
1259	2209	951	–	316	Thymidylate synthase (EC 2.1.1.45)	*thy*A
5172	5708	537	+	178	Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8)	*HPRT*
3808	4788	981	+	326	tRNA(Ile)-lysidine synthetase (EC 6.3.4.19)	*TilS*
4805	5182	378	+	125	tRNA(Ile)-lysidine synthetase (EC 6.3.4.19)	*TilS*

Table 3 List and region of the folate biosynthesis genes (in bold) and related genes within the genome sequence of *P. acidilactaci* WNYM02

P. acidilactaci WNYM02						
Start	End	Length	Strand	AA Length	Product	Gene
4520	5794	1275	–	424	Dihydrofolate synthase (EC 6.3.2.12) @ Folylpolyglutamate synthase (EC 6.3.2.17)	**folC**
22	531	510	–	169	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase (EC 2.7.6.3)	**folK**
524	889	366	–	121	Dihydroneopterin aldolase (EC 4.1.2.25)	**folB**
268	750	483	+	160	Dihydrofolate reductase (EC 1.5.1.3)	**folA**
1	534	534	–	178	Dihydrofolate synthase (EC 6.3.2.12) @ Folylpolyglutamate synthase (EC 6.3.2.17)	**folC**
527	847	321	–	106	Dihydrofolate synthase (EC 6.3.2.12) @ Folylpolyglutamate synthase (EC 6.3.2.17)	**folC**
850	972	123	–	40	Dihydrofolate synthase (EC 6.3.2.12) @ Folylpolyglutamate synthase (EC 6.3.2.17)	**folC**
229	603	375	–	124	GTP cyclohydrolase I (EC 3.5.4.16) type 1	**folE1**
3	236	234	–	78	Dihydrofolate synthase (EC 6.3.2.12) @ Folylpolyglutamate synthase (EC 6.3.2.17)	**folC**
2486	3616	1131	–	376	Dihydropteroate synthase (EC 2.5.1.15)	**folP**
2	187	186	–	62	GTP cyclohydrolase I (EC 3.5.4.16) type 1	**folE**
250	828	579	+	192	5-formyltetrahydrofolate cyclo-ligase	5FCL
3	122	120	–	40	Thymidylate synthase (EC 2.1.1.45)	thyA
2	250	249	+	82	Thymidylate synthase (EC 2.1.1.45)	thyA
2	595	594	–	198	Thymidylate synthase (EC 2.1.1.45)	thyA
2504	3040	537	+	178	Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8)	HPRT
1140	2120	981	+	326	tRNA(Ile)-lysidine synthetase (EC 6.3.4.19)	TilS
2137	2514	378	+	125	tRNA(Ile)-lysidine synthetase (EC 6.3.4.19)	TilS

Our results are in agreement with others where several species of lactic acid bacteria showed the occurrence and deficiency of enzymes involved in the metabolism of folate one-carbon and pABA. The pathway of folate and its connected reactions have been confirmed by some microorganisms and plants [27–29]. The genes involved in folate biosynthesis are well-defined and known as; *fol*E, *fol*B, *fol*K, *fol*Q, *fol*P, *fol*C, and *fol*A and their nucleotide sequences are available in the database. Though, it is documented that several genomes lose some of these genes, while others contain several copies of certain genes [30]. In addition to these genes, there is the 5*FCL* gene encoded by 5-formyltetrahydrofolate cyclo-ligase enzyme, 5*FCL* is a member of the folate cofactor family which works as a regulator of folate metabolism and other folate-dependent enzymes. It was documented that only *Lactococcus lactis* poses whole pathways for biosynthesis both folate and pABA in addition to one-carbon metabolism [31]. Furthermore, *Lactobacillus* genus showed different patterns of folate biosynthesis among its members [32] thus confirming the folate production as strain specific trait. The sequenced genome from the *lactobacilli* members showed the absence of the pABA genes.

The genome sequences of *B. subtilis*, *E. coli*, and *S. thermophilus*, confirmed the presence of genes responsible for folate biosynthesis at different locations on the chromosome. While the members of *Lactobacillus* and *Lactococcus* genus showed the presence of folate biosynthesis genes, excluding *fol*A, in one cluster.

In regard to the riboflavin, the four genes required for the riboflavin biosynthesis were found within the genome sequence of *P. acidilactici* WNYM01 and *P. acidilactici* WNYM02 (Tables 4 and 5). The *rib*A encoding 3,4-dihydroxy-2-butanone-4-phosphatesynthase, *rib*G encoding Di amino hydroxyl phosphor ribosyl amino-pyrimidine deaminase, *rib*B encoding riboflavin synthase subunit alpha, and *rib*H encoding 6,7-dimethyl-8-ribityllumazine (lumazine) synthase. Other genes involved in the metabolism or transport of riboflavin within the genome of the two strains are indicated in Tables 4 and 5.

The analysis of the genome sequence of the two strains revealed the presence of the complete riboflavin operon in the common order as indicated for G+ bacteria. It is well observed that bacteria are greatly varied in the occurrence, genetic organization and regulation of the genes involved in the riboflavin pathway and its related reactions not only among the species but also among the strains. Riboflavin biosynthesis genes and their associated are regulated at the transcription level in G+ bacteria, while in G- bacteria these genes are controlled on level of translation initiation. The gene order for riboflavin varies from the order of enzymatic reactions [33,34]. Transcription of the four riboflavin genes is mainly regulated by the *rib*P1 and *rib*P2 promoters in addition to the regulatory region located at the 5′ ends of the operon [35]. The annotation sequences of *P. acidilactici* WNYM01 and *P. acidilactici* WNYM02 appear in the name of

Table 4 Riboflavin biosynthesis genes (in bold) and related genes within the *P. acidilactici* WNYM01 genome

P. acidilactaci WNYM01						
Start	End	Length	Strand	AA Length	Product	Gene
856	1536	681	+	227	Diaminohydroxyphosphoribosylaminopyrimidine deaminase (EC 3.5.4.26) / 5-amino-6-(5-phosphoribosylamino)uracil reductase (EC 1.1.1.193)	**ribD/G**
138	737	600	+	199	Riboflavin synthase eubacterial/eukaryotic (EC 2.5.1.9)	**ribB**
3	137	135	+	44	Diaminohydroxyphosphoribosylaminopyrimidine deaminase (EC 3.5.4.26) / 5-amino-6-(5-phosphoribosylamino)uracil reductase (EC 1.1.1.193)	**ribD/G**
730	1020	291	+	97	3,4-dihydroxy-2-butanone 4-phosphate synthase (EC 4.1.99.12) / GTP cyclohydrolase II (EC 3.5.4.25)	**ribB/ribA**
1910	2686	777	–	258	FMN adenylyltransferase (EC 2.7.7.2) / Riboflavin kinase (EC 2.7.1.26)	**ribF**
3	707	705	+	234	3,4-dihydroxy-2-butanone 4-phosphate synthase (EC 4.1.99.12) / GTP cyclohydrolase II (EC 3.5.4.25)	**ribB/ribA**
712	1185	474	+	157	6,7-dimethyl-8-ribityllumazine synthase (EC 2.5.1.78)	**ribH**
3247	3549	303	+	100	Transcription termination protein	NusB
1	390	390	+	130	Ribulose-phosphate 3-epimerase	rpe
262	2	261	–	87	Ribulose-phosphate 3-epimerase	rpe
708	782	75	–	24	Ribulose-phosphate 3-epimerase	rpe
1	222	222	+	73	Orotidine 5'-phosphate decarboxylase	URA3
365	871	507	–	168	Orotidine 5'-phosphate decarboxylase	URA3
703	1086	384	+	127	Diacylglycerol kinase	dgkA

*rib*D gene instead of *rib*G where the two names have been proposed for the deaminase and reductase domains, respectively [36]. Furthermore, the two strains in this study showed *rib*BA gene instead of separate genes *rib*A and *rib*B as the *rib*BA gene differs from them in the sequence and function. As *rib*BA is able to do bifunctional by producing riboflavin precursors directed for secretion also producing riboflavin precursors to achieve the bacteria needs [37].

Other related genes found in the genome sequence of the two strains species as *rib*Z1 and *rib*Z2 are the riboflavin transport which is responsible for the uptake. It is documented that some bacteria depend on the uptake external riboflavin via flavin transport systems and they could have different families of bacterial riboflavin transport system [33]. Many species keep both functions the uptake and biosynthesis of riboflavin and thus confirm with the two strains WNYM01 and WNYM02.

Nucleotide sequence accession number

The whole complete genome sequences for WNYM01 and WNYM02 are available in GenBank with accession numbers CP107051 and JAOSZF0000 00000, respectively.

Table 5 Riboflavin biosynthesis genes (in bold) and related genes within the *P. acidilactici* WNYM02 genome.

P. acidilactaci WNYM02						
Start	End	Length	Strand	AA Length	Product	
1273	2220	948	+	315	FMN adenylyltransferase (EC 2.7.7.2) / Riboflavin kinase (EC 2.7.1.26)	**ribF**
3	380	378	–	126	3,4-dihydroxy-2-butanone 4-phosphate synthase (EC 4.1.99.12) /GTP cyclohydrolase II (EC 3.5.4.25)	**ribB/ribA**
3073	3522	450	–	149	6,7-dimethyl-8-ribityllumazine synthase (EC 2.5.1.78)	**ribH**
3	338	336	+	112	3,4-dihydroxy-2-butanone 4-phosphate synthase (EC 4.1.99.12) / GTP cyclohydrolase II (EC 3.5.4.25)	**ribB/ribA**
2	553	552	–	183	3,4-dihydroxy-2-butanone 4-phosphate synthase (EC 4.1.99.12) / GTP cyclohydrolase II (EC 3.5.4.25)	**ribB/ribA**
546	725	180	–	59	Riboflavin synthase eubacterial/eukaryotic (EC 2.5.1.9)	**ribB**
1	492	492	–	164	Riboflavin synthase eubacterial/eukaryotic (EC 2.5.1.9)	**ribB**
493	1566	1074	–	357	Diaminohydroxy phosphoribosylaminopyrimidine deaminase (EC 3.5.4.26) / 5-amino-6-(5-phosphoribosylamino)uracil reductase (EC 1.1.1.193)	**ribD/G**
3247	3549	303	+	100	Transcription termination protein	NusB
472	1119	648	+	215	Ribulose-phosphate 3-epimerase	rpe
508	1221	714	+	237	Orotidine 5'-phosphate decarboxylase	URA3
1991	2374	384	–	127	Diacylglycerol kinase	dgkA

Conclusion

In conclusion, strains of *P. acidilactici* are suggested as probiotics but few of them have been characterized in the molecular and genomic base. Thus, the complete genome sequencing in such important candidates is important to reveal their molecular characteristic and genetic function in addition enables genetic engineering possibility. Here, the complete genome sequences of the probiotic *P. acidilactici* WNYM01 and *P. acidilactici* WNYM02 isolated from human gut microbiome are described. We suggested them as valuable probiotics for pharmaceutical preparation and medical purposes.

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Nil.

Conflicts of interest

The authors declare there are no conflicts of interest.

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