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Unveiling the Brazilian kefir microbiome: discovery of a novel *Lactobacillus kefiranofaciens* (LkefirU) genome and in silico prospection of bioactive peptides with potential anti-Alzheimer properties

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Abstract

Background Kefir is a complex microbial community that plays a critical role in the fermentation and production of bioactive peptides, and has health-improving properties. The composition of kefir can vary by geographic localization and weather, and this paper focuses on a Brazilian sample and continues previous work that has successful anti-Alzheimer properties. In this study, we employed shotgun metagenomics and peptidomics approaches to characterize Brazilian kefir further.

Results We successfully assembled the novel genome of *Lactobacillus kefiranofaciens* (LkefirU) and conducted a comprehensive pangenome analysis to compare it with other strains. Furthermore, we performed a peptidome analysis, revealing the presence of bioactive peptides encrypted by *L. kefiranofaciens* in the Brazilian kefir sample, and utilized in silico prospecting and molecular docking techniques to identify potential anti-Alzheimer peptides, targeting β-amyloid (fibril and plaque), BACE, and acetylcholinesterase. Through this analysis, we identified two peptides that show promise as compounds with anti-Alzheimer properties.

Conclusions These findings not only provide insights into the genome of *L. kefiranofaciens* but also serve as a promising prototype for the development of novel anti-Alzheimer compounds derived from Brazilian kefir.

Keywords *Lactobacillus kefiranofaciens*, Metagenome, Kefir, Next Generation DNA Sequencing, Peptidome, Alzheimer's disease

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Introduction

Kefir is a fermented milk drink that contains a variety of beneficial microorganisms and its origins in the Caucasian, Tibet, and Mongolian mountains [1]. Studies have characterized kefir based on its microorganisms, metabolites, and peptides in various samples, providing valuable insights into the puzzle pieces of this probiotic. However, since the composition of kefir varies depending on its geographical origin, the type of milk used, and the fermentation temperature, analyzing a single sample is crucial for obtaining a comprehensive "metabolic blueprint" of these compounds [2–5].

During fermentation, kefir generates functional compounds such as peptides and metabolites that possess health-improving properties including neuroprotective effects, antioxidant activity, anti-inflammatory properties, and modulation of the gut microbiota. The gut-brain axis may also play a role, as the probiotics present in kefir are known to influence the gut microbiota, which has been linked to brain function and neurodegeneration [6–8].

Previously, our group investigated the application of brazilian kefir sample in the treatment of Alzheimer's disease, one of the most common causes of dementia. Alzheimer's disease is characterized by the cleavage of the Amyloid Precursor Protein (APP) by enzymes gamma-secretase and beta-secretase, resulting in the production of β-amyloid peptide. This process leads to the formation of β-amyloid plaques, neuronal cell death, reduced acetylcholine neurotransmission, and impaired cognitive functions [9]. We used transgenic *Drosophila melanogaster* as a model for Alzheimer's disease and demonstrated that kefir, its metabolites, and peptides can improve lifespan and mobility while reducing the accumulation of β-amyloid peptides in the brain. These studies suggest the potential therapeutic effects of kefir on Alzheimer's disease [10, 11].

Genomics is a powerful method for studying microbial communities without the need for culturing [12]. Over the past decade, it has been used to analyze the microbial communities in kefir grains from various countries in Europe, America and Asia [13–18]. In this paper, we employed shotgun metagenomics and peptidomics approaches to thoroughly characterize the bacterial diversity and species richness of Brazilian kefir grains collected in Uberlandia, Minas Gerais, which demonstrated anti-Alzheimer properties in our previous studies. Additionally, we conducted in silico screening of peptides targeting the key players involved in Alzheimer's disease. These findings can serve as a foundation for the design of future anti-Alzheimer's drugs.

Methods

Kefir sample

The kefir grains were generously donated by the community of Uberlandia, Minas Gerais, Brazil, and the fermentation process was conducted for 24 h by inoculating the grains into milk at a concentration of 4% (w/v).

DNA extraction and sequencing

Total DNA from both the kefir grains and their fermented product was sequenced. Genomic DNA purification followed the in-house protocol of BGI Americas, and the integrity of the DNA was assessed using 1% agarose gel electrophoresis. The concentration of each sample was determined using the Qubit Fluorometer (Invitrogen, Waltham, MA, USA).

For library construction, 1000 ng of DNA was used to create a whole metagenome library with fragment sizes of ≤ 800 bp, following the in-house protocol of BGI Americas. The quality of the library was assessed using the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and paired-end sequencing was performed using the DNBseq™ sequencing strategy at BGI Americas.

Genomic assembly and bioinformatic analysis

The raw data underwent pre-analysis using SOAPnuke software, developed by BGI, to filter out adapter or low-quality sequences, remove contamination, and obtain valid data. Scaffold assemblies were performed using SPADES version 3.14.0, with the default k-mer value (21, 33, and 55 bp). RAGTAG software was utilized for scaffold alignment to a reference genome. The reference genome was selected by comparing the similarity and sizes of the scaffolds with all nucleotide sequences from the offline NT database at the NCBI database. For functional annotation, we employed the genome annotation pipeline PANNOTATOR [19]. PANNOTATOR assigns color tags based on three similarity levels of alignment size and protein identity: green for certain annotations (≥ 95%), yellow for high similarity (< 95% and ≥ 70%), and red for annotations with lower confidence (< 70%). To assess pangenome conservation involving over two hundred genomes simultaneously, we used the GENPPI software [20]. Although initially developed for predicting protein interactions, GENPPI also generates core and accessory pangenomes for subsequent interaction analyses. Additionally, we investigated annotated proteins for conserved interactions within the genus. The GENPPI parameters were set based on examples from the software tutorial, employing dynamic neighborhood expansion and phylogenetic profile conservations [20].

Shotgun peptidomes analysis

The proteomic and peptidomic data from Malta et al. [11], deposited in PRIDE with the accession number PXD034148 were employed here for the prospecting of bioactive peptides. Spectrum Mill software (Agilent Technologies, Santa Clara, CA, USA) was used for data analysis, and the "*Lactobacillus kefiranofaciens*" database from Uniprot (6,162 results in April 2023) was employed.

In silico bioactive predictive analyses

To estimate toxicity, bioactivity, and passage through the blood–brain barrier, we employed the pipeline developed by Malta et al. [11]. The pipeline uses ToxinPred software for toxicity prediction, Pepdite ranker for bioactivity prediction, and BBPred for predicting passage through the blood–brain barrier [21–23]. Peptides with a score of 0.8 or higher were considered for subsequent molecular docking analysis.

Molecular docking

The three-dimensional (3D) peptide structures were generated using PEP-FOLD 3,5 [24]. The RCSB Protein Data Bank (PDB) files for BACE (3TPJ), Human acetylcholinesterase (3LII), Monomer Amyloid beta-peptide (6SZF), Beta-Amyloid Plate (2MXU), were obtained from the PDB [25]. The peptides and these enzymes were subjected to molecular docking using ClusPro [26] and PyMol. The best model was selected based on a lower weight coefficient value.

Results

Data quality

After filtering the sample data and removing adapter, the metagenomic sequencing depth was 6,208,391,400 bases, and there were 62,083,914 clean reads with a GC content of 37.29% and a Q20 value of 98.95% indicating a high level of data quality.

Scaffolding

Using the SPADES software, a total of 6,387 scaffolds were assembled, with a median size of 334 base pairs (Supplementary material—Table 1). The three largest scaffolds have sizes of 2.35×10^5, 1.87×10^5, and 1.75×10^5 base pairs, respectively. These scaffolds were then aligned to reference genomes using the RAGTAG software. While only 3.2% of our scaffolds aligned with the reference genome (GenBank number: CP045033), the sum of nucleotides from our aligned scaffolds amounted to approximately 1,635 million base pairs. A query to our standalone NCBI genome database using the code CP045033 returned the reference genome *Lactobacillus kefiranofaciens* ASM1465658v1, CP061341 in GenBank. This genome consists of 2,149,348 bases pair and includes two plasmids with sizes of 4,472 and 19,814 bases pair, respectively.

Genome assembly of *Lactobacillus kefiranofaciens ueira* (LkefirU)

Our analysis of the kefir DNA sample revealed the scaffolds aligned with the CP061341 reference genome, which consists of 2,241,619 base pairs, along with two plasmids of sizes 4,527 and 48,532 base pairs. We named the DNA strand *Lactobacillus kefiranofaciens ueira* or LkefirU for short.

The LkefirU genome has a GC content of approximately 38%, and a gene density of 1037 genes per megabase, with 88% of coding bases. Within the LkefirU genome, there are 2326 protein-coding sequences (CDS), although this number does not account for potential false genes. Among these total CDSs, 1643 (70.6%) show greater than 95% size and protein identity similarity (SPIS) to the reference genome CP061341. Another 363 (15.6%) fall within the 95% to 70% ranges, while the remaining 320 (13.7%) CDS exhibit SPIS below 70%.

The LkefirU genome consists of 396 named genes with repetition and 325 in isolation. When including the number of CDSs with SPIS < 70% (357) and CDSs annotated as hypothetical based on the reference genome (203); LkefirU presents 560 CDSs with unknown functions, accounting for 24% of its coding sequences. Fig. 1 provides an example of these sequences with unknown functions compared to the LkefirU reference genome.

Unlike the reference genome, LkefirU does not possess any plasmids, and proteins of assembled sequences from these two plasmids have no correspondence in the leading strand of LkefirU.

Additionally, compared to its reference, the LkefirU genome does not possess the expected number of ncRNA and tRNA. The CP061341 genome, contains 15 ncRNAs and 63 tRNAs, while in LkefirU, predicted to have 41 tRNAs and no ncRNAs. The lack of a minimal amount of ncRNAs indicates that LkefirU genome still in the draft stage. We used the RNAMMER program to predict ncRNAs among the 6837 scaffolds generated during the assembly with SPADES, resulting in the identification of ten ncRNAs (Table 1). However, it cannot be definitively concluded that these ncRNAs belong to LkefirU since there is a possibility of other organisms in the metagenome. Considering the significant anticipation of *L. kefiranofaciens* genomes in our assemblies, it is plausible that a significant amount of DNA is related to LkefirU. However, only the NODE_924 exhibits GC content that aligns with both LkefirU and its reference, falling within the range of 35 to 38%. To confirm this, we performed a

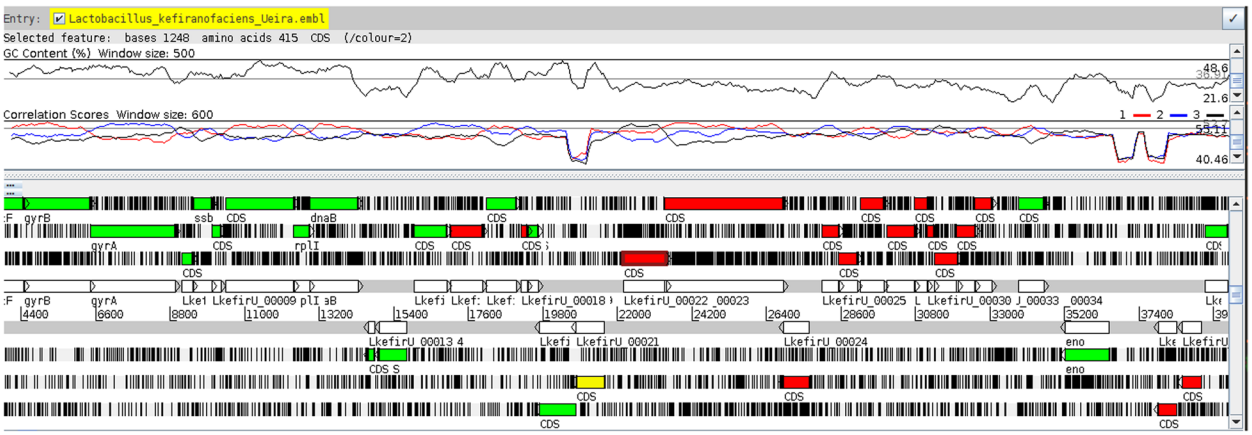

Fig. 1 The initial region of the LkefirU genome displaying regions with absent coding sequences (CDS) in the reference genome of *Lactobacillus kefiranofaciens* ASM1465658v1 (CP061341) is highlighted in red. Notice the distinctive change in GC content pattern on the flanks of the region lacking CDSs in the reference genome, indicating a potential gene transfer phenomenon in LkefirU

Table 1. Predicted ncRNAs in the scaffolds of our kefir sample

seqname	start	end	score	+/-	attribute
NODE_2071_length_560_cov_29.855446	467	558	47.7	+	5s_rRNA
NODE_266_length_10158_cov_4.677324	15	131	38.6	-	5s_rRNA
NODE_637_length_3198_cov_4421.287623	2	117	77.4	-	5s_rRNA
NODE_72_length_28997_cov_6.123005	3623	3737	86.6	+	5s_rRNA
NODE_924_length_1608_cov_2.560206	*473*	*588*	*35.8*	+	*5s_rRNA*
NODE_811_length_2056_cov_3902.386807	114	1665	1943.8	-	16s_rRNA
NODE_929_length_1578_cov_17.926461	323	1578	1336.5	+	16s_rRNA
NODE_637_length_3198_cov_4421.287623	192	3092	3323.6	-	23s_rRNA
NODE_682_length_2821_cov_29.172451	336	2821	2223.4	+	23s_rRNA
NODE_72_length_28997_cov_6.123005	771	3509	3335.2	+	23s_rRNA

Blastn to compare 16 s rRNA with *L. kefiranofaciens* and the NODE_811_length_2056_cov_3902.386807 indicates 100% of identity and 0.0 of e-value, when the NODE_929_length_1578_cov_17.926461 indicates 79.24% of identity and e-value 3e-130.

Pangenome analysis

To study the relationship between our LkefirU genome and other genomes of the *Lactobacillus* genus, we collected 204 genomes of the *Lactobacillus* genus deposited at the NCBI. Using the GENPPI tool, we examined the LkefirU pangenome and found 63% (1474 out of 2326) of the predicted proteins had one or more similar counterparts with over 90% identity in the at least one of the 204 *Lactobacillus* genomes. This includes both the core and accessory genomes of the LkefirU. However, we could not consider 37% of the LkefirU genome as unique because the GENPPI tool was set to a minimum protein identity based on conserved neighborhood relationships (CN) and phylogenetic profile (PP) with threshold above 90%. The central and accessory pangenome of LkefirU exhibited protein similarities with approximately 7000 proteins in 156 *Lactobacillus* genomes. The five genomes with the highest number of similar proteins to LkefirU, in descending order, were *Lactobacillus* sp. (401), *Lactobacillus paragasseri* (290), *Lactobacillus kefiranofaciens* (267), *Lactobacillus gallinarum* (188) and *Lactobacillus* sp. UMNPBX14 (184).

We generated a network of interactions for LkefirU, which consisted of approximately 159,000 edges, 7,645 for CN, and 151,671 for PP. This network comprised 80% of the predicted open reading frames (ORFs) in LkefirU, distributed across 21 interconnected components. In the Fig. 2, the colors white, green, red, and blue mean cytoplasmic, membrane, surface exposed, and secreted proteins, respectively. The larger nodes represent the thirty proteins with the highest Bridging Centrality scores [27].

The LKefirU genes absent in the reference genome were confirmed using its interaction network. Since the GENPPI software evidenced conserved neighborhood

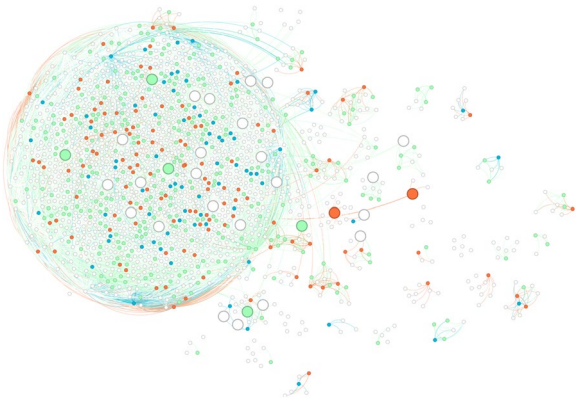

Fig. 2 The LKefirU interaction network depicting. the colors white, green, red, and blue represents cytoplasmic, membrane, surface exposed, and secreted proteins, respectively. These figure show that LkefirU have a solide network and preserve some philogenetics characteristics

or phylogenetic profile, it means the gene exists in other genomes. For this analysis, we focused on 319 genes from the LkefirU chromosome, excluding genes that were assigned hypothetical protein annotations by the reference genome. The presence of non-annotated gene interactions with the reference genome (indicated in red) suggests that these genes, which were not accounted for in the reference annotation, may exist in one or more of the 204 *Lactobacillus* genomes obtained from the NCBI. These genes likely share conserved gene neighborhoods, conserved phylogenetic profiles, or both. Out of these 319 genes examined, 141 genes (44%) interacted with other *Lactobacillus* genomes. On average, these proteins indicated in red displayed thirteen interactions each. Notably, the two proteinsLKU01457.1 and LKU00889.1 stood out with 564 and 555 interactions, respectively, representing the highest number of interactions among the non-annotated genes. Aligning these protein sequences against the NCBI pool revealed 51 alignments for LKU01457.1, and over 100 LKU00889, predominantly with *Lactobacillus* sp.. Therefore, we estimate that there are approximately 178 unique proteins in the LkefirU chromosome, which accounts for around 8% of the entire genome. This proportion of unique proteins aligns with expectations for an unpublished genome [28].

Peptidomes analysis and in silico bioactive prediction of LkefirU

A total of 91 peptides were identified in peptidomics analysis from an intact peptide fraction encrypted by LkefirU in the Kefir sample.To analyze the bioactivity of screening peptides in an effective, cheap and fast way, we use in silico analysis techniques to evaluate toxicity, the possibility of passing through the blood–brain barrier and the interaction against targets for Alzheimer's disease. Among these peptides, only one peptide was predicted to be toxic, while nine peptides passed the bioactivity filter, and nine passed on blood–brain barrier peptide (BBP) filter. Remarkably, two peptides, namely VPGYPFLPI and KSPCVFILDQKKRL, met the criteria for both bioactivity and BBP filters (Supplementary Table 2).

The peptide VPGYPFLPI is encrypted within Amino Acid Permease, a transmembrane protein, while the KSPCVFILDQKKRL peptide is encrypted within Glycolate Oxidase, a protein associated with the cytoplasmatic membrane. Molecular docking was then performed using these two peptides to identify bioactive peptides that could affect important molecules involved in the pathophysiology of Alzheimer's diseases. Both peptides demonstrated a low Weight Coefficient with all predictions target β-amyloid monomer, and β-amyloid plaque, BACE, and AChE. Specifically, VPGYPFLPI displayed the lowest Weight Coefficient against the target AChE, while the KSPCVFILDQKKRL peptide exhibited the lowest Weight Coefficient against the target β-amyloid plaque (Table 2).

Molecular docking

The KSPCVFILDQKKRL peptide exhibited interactions with 7 out of the 42 amino acids residues (aa) in the Aβ (1–42) monomer (Fig. 3a and Supplementay Table 3). Moreover, the VPGYPFLPI peptide showed interactions with 5 out of the 42 aa in the Aβ (1–42) monomer (Fig. 3b and Supplementay Table 3).

During the molecular docking analysis of the Aβ plate, the KSPCVFILDQKKRL peptide displayed interactions with only two aa. Specifically, the LYS-32 of the L chain

Table 2 In silico docking results of potential bioactive peptides

Peptide Sequence	Protein origin	Bioactivity	BBP Probability	Weight Coefficient			
				β-amyloid monomer	β-amyloid plaque	BACE	AChE
VPGYPFLPI	Amino Acid Permease	0.849297	0.831898	-652.5	-916.3	-762,9	-974.0
KSPCVFILDQKKRL	Glycolate Oxidase	0.814756	0.868010	-640.7	-833.4	-678.9	-792.2

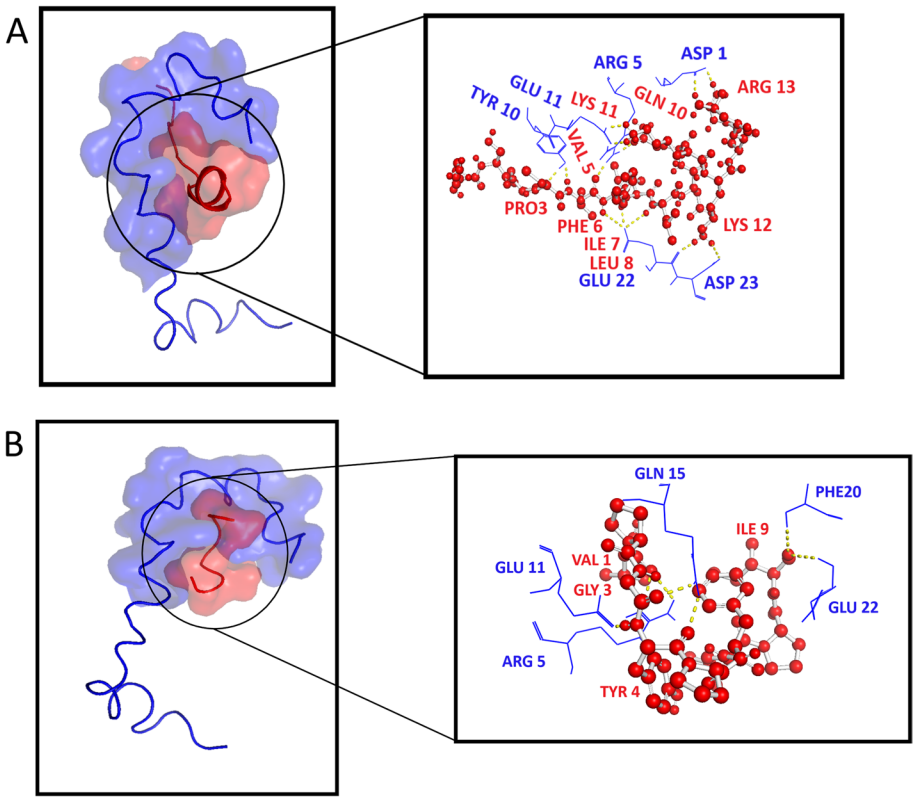

Fig. 3 Molecular docking of Aβ monomer and peptides KSPCVFILDQKKRL and VPGYPFLPI. **A** Molecular docking analysis of the KSPCVFILDQKKRL peptide (indicated in red) and their interaction with Aβ monomer (indicated in blue). Zoomed-in image of the panel of amino acids residues (aa) interactions in the left. **B** Molecular docking analysis of the VPGYPFLPI peptide (indicated in red) and their interaction with Aβ monomer (indicated in blue) and zoomed-in image of a panel of aa interactions in the left. The yellow color is interactions between the aa of both peptides

interacted with Arg13, and ILE-32 of the I chain interacted with Ser2 (Fig. 4a and Supplementay Table 3).

On the other hand, the VPGYPFLPI peptide exhibited interactions with 10 peptides of the plate, and sometimes one amino acid residue of the peptide interact with different amino acids residues of the plate, such as the amino acids residues Tyr4 interact with amino acids residues of D and E chain of plaque Aβ (1–42)—Leu34 of D chain and Leu17 and His14 of E chain- and Phe6 interact with amino acids residues of E and F chain of plaque Aβ (1–42)—Ile32 and Gly33 of E chain and Leu17 and Leu34 of F chain—(Fig. 4b and Supplementay Table 3).

The KSPCVFILDQKKRL peptide, when docked with BACE, it demonstrated interactions with six aa (Fig. 5a and Supplementay Table 3). Similarly, the VPGYPFLPI peptide, when docked with BACE, interacted with six aa, with two of these aa forming the flap region, which plays a crucial role in determining whether binding to the BACE cleavage site occurs or not (Fig. 5b and Supplementay Table 3).

The KSPCVFILDQKKRL peptide, when docking to the AChE, interacted with nine aa of the A chain. Among these interactions, one aa was located within the catalytic active site (CAS), and another aa was part of the peripheral anionic site (PAS) (Fig. 6a and Supplementay Table 3). In addition, the VPGYPFLPI peptide, when docked to AChE, interacted with five aa of the B chain, with one aa bordering the PAS binding site, located close to the CAS active site (Fig. 6b and Supplementay Table 3).

Discussion and conclusion

The microbial composition of kefir is diverse, comprising more than 50 species of bacteria and yeasts that have been described composing the microbiota of Kefir grains. Among them, the species of the genera *Lactobacillus, Lactococcus, Leuconostoc, Kluyveromyces, Pichia* and *Saccharomyces* have been identified [29–34]. Several studies have focused on characterizing the kefir microbiota through culture-dependent and sequencing techniques [32, 35–39]. The Kefir microbiota used in this work was previously characterized using the 16S technique, consisting of *Lactobacillus kefiranofaciens,*

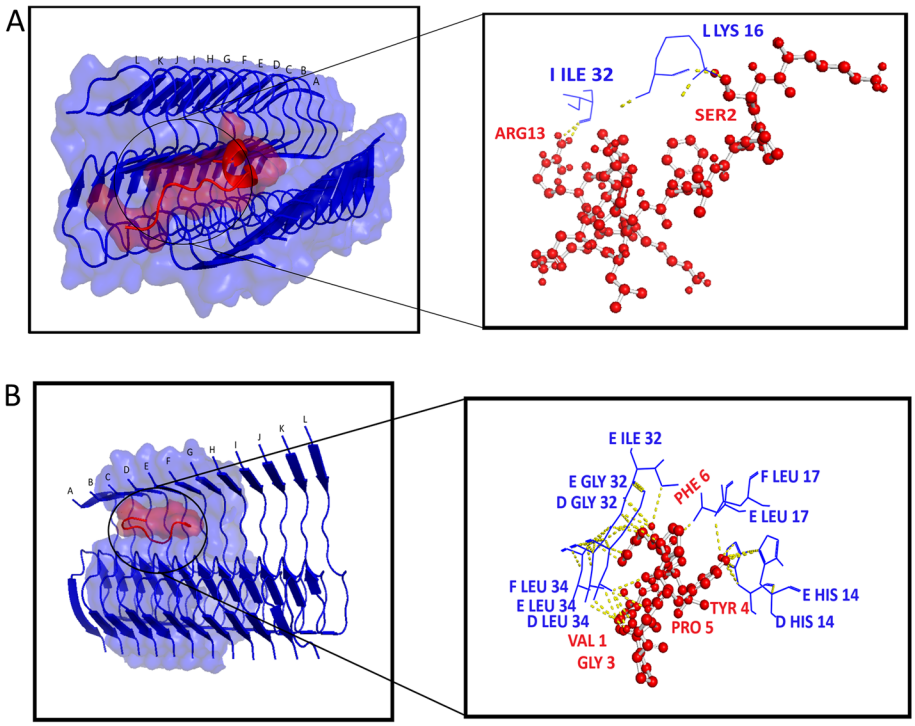

Fig. 4 Molecular docking of Aβ plate and peptides KSPCVFILDQKKRL and VPGYPFLPI. The plate has twelve chains of β-amyloid peptide named by letters A to L. (**A**) Molecular docking analysis of the KSPCVFILDQKKRL peptide (indicated in red) and their interaction with Aβ plate (indicated in blue) and zoomed-in image of the panel of aa interactions on left (**B**) Molecular docking analysis of the VPGYPFLPI peptide (indicated in red) and their interaction with Aβ plate (indicated in blue), and zoomed-in image of the panel of aa interactions on left. The yellow color is interactions between the aa of both peptides

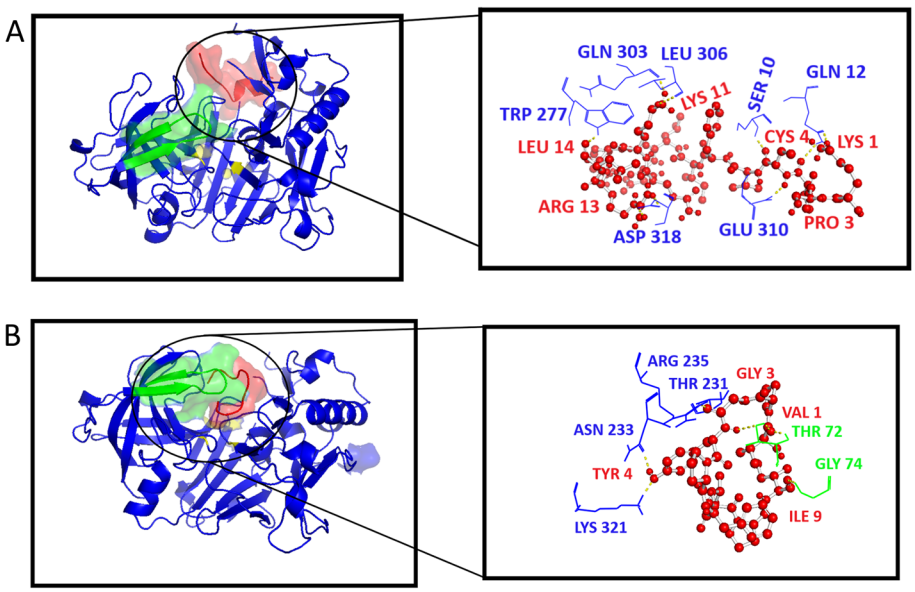

Fig. 5 Molecular docking of BACE1 and peptides KSPCVFILDQKKRL and VPGYPFLPI. The color yellow represents the active site and green represents the flap of BACE1 (A)- Molecular docking analysis of the KSPCVFILDQKKRL peptide (indicated in red) and their interaction with BACE enzyme and zoomed-in image of the panel of aa interactions on left. (B) Molecular docking analysis of the VPGYPFLPI peptide (indicated in red) with the BACE enzyme and zoomed-in image of the panel of aa interactions on left

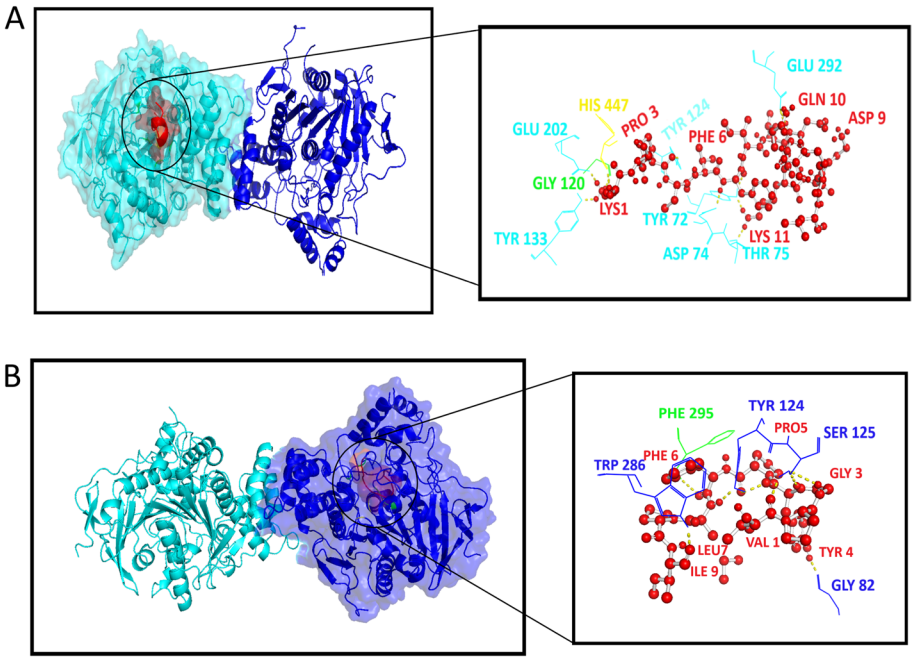

Fig. 6 Molecular docking of AChE and peptides KSPCVFILDQKKRL and VPGYPFLPI. The yellow represents catalytical active site (CAS) and green represents the peripheral anionic site (PAS) of AchE (**A**) Molecular docking analysis of the KSPCVFILDQKKRL peptide (indicated in red) and their interaction with acetylcholinesterase enzyme and zoomed in the image of the panel of aa interactions on left. **B** Molecular docking analysis of the VPGYPFLPI peptide (indicated in red) with acetylcholinesterase enzyme and zoomed-in image of the panel of aa interactions. The yellow color is interactions between the aa of both peptides on left

Lactobacillus kefir, Acetobacter fabarum, Lactococcus lactis, Rickettsiales sp [10]. Our shotgun DNA sequencing results confirm the predominance of *L. kefiranofaciens* DNA sequences, consistent with previous findings. In contrast to 16S metagenomic analysis, the shotgun sequencing approach enables the identification of extensive genomic regions or even the entire genomes of microorganisms [40].

Lactobacillus kefiranofaciens is essential for the fermentation of kefir and is responsible for producing a glucogalactan polymer that forms a matrix around the bacterial cells [35]. Our focus in the present work was to study the genome and peptidome of *L. kefiranofaciens*, and the shotgun metagenomic analysis facilitated this investigation. Previous work reported the isolation of these bacteria from kefir and named the genome *L. kefiranofaciens* ZW3. However, there are some differences compared to our results. For instance, the genome of *L. kefiranofaciens* ZW3 contains approximately 2.04 million base pairs and 2,067 protein-coding genes, but LkefirU contains 2,241,619 base pairs and 2,326 protein-coding genes [41]. The pagenome analysis showed the *L. kefiranofaciens Ueira* estimated 178 unique proteins in the LkefirU chromosome, which accounts for around 8% of the entire genome contributing to understanding the genetic profile of this specie. Therefore, sequencing the LkerfirU genome is crucial for understanding potential variations in the peptide profile present in the kefir sample studied. This knowledge can contribute to future research focused on optimizing the production of molecules with therapeutic properties.

The bacteria present in Kefir are responsible for the fermentative process that produces biomolecules with healthy benefits. Studies have shown the effects of kefir against pathogenic bacteria, oxidative stress, inflammation, obesity, diabetes, osteoporosis, cardiovascular disease, and neurodegeneration [42–47]. Synergic effect could be responsible for properties of kefir, however the molecules present in kefir have to be teste isolated to understand properties of each one, and in this paper two peptides were identified as potential responsible for effect against Alzheimer disease. Our previous works show that our sample of Brazilian kefir may contain metabolites and peptides with anti-Alzheimer's effects. Therefore, we conducted a new peptidome analysis of our database to search for other possible bioactive peptides that could be responsible for the anti-Alzheimer's effects observed in our previous work.

Bioactive peptides refer to small peptide fragments present in the primary structure of proteins but are inactive in their natural state [48]. Upon hydrolysis and release

from the parent protein, bioactive peptides can regulate the metabolism of living organisms, potentially treating chronic illnesses with targeted potency and minimal side effects, including toxicity [49].

In our research on putative bioactive peptides with anti-Alzheimer effects, we selected the main possible target: β-amyloid peptide and plate, BACE, and AChE [50]. Targeting the β-amyloid peptide can involve cleavage by other peptidases and prevent the formation of β-amyloid plate. Targeting the β-amyloid plate can destabilize and cause its degradation. Peptides that bind to β-amyloid can potentially prevent forming and accumulating beta-amyloid plaques [51]. The peptides identified in our work could be effective as drug to target β-amyloid monomer and plate, mainly the VPGYPFLPI peptide, as it exhibits different points of interaction with both targets.

The production of β-amyloid can be prevented by inhibiting the BACE enzyme. It can decrease the production of amyloid beta peptides and potentially slow or halt the progression of the disease [52]. Several BACE inhibitors have been developed and tested in preclinical and clinical studies, showing promising results in reducing β-amyloid levels in the brain. However, challenges remain in finding a BACE inhibitor that can effectively cross the blood–brain barrier and achieve therapeutic concentrations in the brain without causing adverse effects [53]. The peptides identified in our work interact with important amino acid residue for the function of BACE enzyme, such as Gly74 and Thr72 interact with the amino acid residue of VPGYPFLPI. These amino acid residues are in the FLAP region, and this interaction could reults in BACE inhibition. Moreover, the VPGYPFLPI interacts with residue Asn233, similar to a BACE1 inhibitor called hydroxyethylamine [54].

Acetylcholinesterase inhibitors are drugs commonly used to treat Alzheimer's disease by reducing the symptoms caused by the degeneration of cholinergic neurons and decrease of the neurotransmitter acetylcholine. These inhibitors work by inhibiting the activity of acetylcholinesterase, an enzyme that breaks down acetylcholine in the brain. By increasing the levels of acetylcholine, these drugs can improve cognitive function and reduce the symptoms of Alzheimer's disease [55]. Acetylcholinesterase inhibitors have been shown to slow the progression of Alzheimer's disease and improve the quality of life in patients with mild to moderate dementia. Donepezil, rivastigmine, and galantamine are commonly used acetylcholinesterase inhibitors. However, these drugs are only effective for some patients, and their efficacy can diminish over time [56]. The VPGYPFLPI and KSPCVFILDQKKRL peptides interact with the peripheral anionic site (PAS) of AChE, and that interaction could impact on the function of the enzyme.

Our results should be viewed under several limitations. First, our findings are specific to Brazilian kefir grains from Uberlandia, Minas Gerais. Confirming the applicability of our findings to kefir from other regions would require similar analyses on those specific samples. Moreover, we only performed i*n silico* analysis, and further in vitro and in vivo experiments are needed to examine the anti-Alzhimer's properties in the two peptides identified in this study. The LkefirU genome has only one chromosome, and we were unable to identify genomic regions located in plasmids, this may be due to the non-specificity of the sequences found in plasmids. To better understand the genome of this *L. Kefiranofaciens* strain, it will be necessary to perform sequencing of the isolated bacteria.

In summary, the present work contributes to understanding the composition of Brazilian kefir sample and reinforces the results of previous work of our group. We assembled a new genome of *Lactobacillus kefiranofaciens* and investigated how these bacteria could be involved in the production of bioactive peptides in Brazilian Kefir with anti-Alzheimer's effect. Moreover, the VPGYPFLPI and KSPCVFILDQKKRL peptides show interactions with different targets of Alzheimer's disease and this characteristic could be beneficial in attenuating the symptoms and stopping the progression of the disease. Our results are promising and open avenues for new research to develop treatments for Alzheimer's disease. However, more studies are needed to evaluate the effect of the peptides using in vitro and in vivo methods, as well as clinical trials to reinforce these results, and understand the effects of different dosage approaches, since in silico predictions may differ from reality.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Authors' contributions

MHS contributes with the writting the main text of this paper and the peptides prections and molecular docking. LLB contributes with the kefir cultivation and DNA extraction for sequencing, SMM and ACCS contributes with the peptides prections and molecular docking. APMS, AMB, and CUV contributes with revision of text. ARS contributes with the bioinformatic analyses, assemble genome of LkefirU and revision of text.

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Availability of data and materials
Data from sequencing available on SRA, number of access: PRJNA725245 in https://www.ncbi.nlm.nih.gov/sra/SRX23160890 [accn]. The genome avaliable on GenBank, number of acess GCA_040039785.1 in https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_040039785.1/.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interest
The authors declare no competing interests.

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