Heliyon 9 (2023) e16253

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Genome sequencing of *Enterococcus faecium* NT04, an oral microbiota revealed the production of enterocin A/B active against oral pathogens

Nashwa Tarek [a,d], Ahmed F. Azmy [b], Ahmed S. Khairalla [b,c], Medhat Abdel-Fattah [a], Ohoud A. Jefri [e], Mohamed Shaban [f,g], Abdelaziz A.A. El-Sayed [h,i,*], Ahmed O. El-Gendy [b]

[a] *Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Egypt*
[b] *Microbiology and Immunology Department, Faculty of Pharmacy, Beni-Suef University, Egypt*
[c] *Department of Biology, University of Regina, 3737 Wascana Parkway, Regina, SK, S4S 0A2, Canada*
[d] *Basic Science Department, Faculty of Oral and Dental Medicine, Nahda University Beni-Suef (NUB), Beni Suef, Egypt*
[e] *Department of Biological Science, Faculty of Science, King Abdulaziz University, Jeddah, 21589, Saudi Arabia*
[f] *Department of Physics, Faculty of Science, Islamic University of Madinah, Al-Madinah Al-Munawarah, 42351, Saudi Arabia*
[g] *Nanophotonics and Applications (NPA) Lab, Physics Department, Faculty of Science, Beni-Suef University, Beni-Suef, 62514, Egypt*
[h] *Biology Department, Faculty of Science, Islamic University of Madinah, Al-Madinah Al- Munawarah, 42351, Saudi Arabia*
[i] *Zoology Department, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt*

ARTICLE INFO

Keywords:
Probiotics
Enterococcus faecium
Oral cavity
WGS

ABSTRACT

Objective: This study aimed to isolate and investigate a bacterium from an Egyptian adult's healthy oral cavity, focusing on its probiotic properties, especially its antagonistic activity against oral pathogens.
Methods: The isolated bacterium NT04 using 16S rRNA gene sequencing, was identified as *Enterococcus faecium*. In this study, the whole genome of *Enterococcus faecium* NT04 was sequenced and annotated by bioinformatics analysis tools.
Results: Numerous genes encoding the production of diverse metabolic and probiotic properties, such as bacteriocin-like inhibitory substances (Enterocin A and B), cofactors, antioxidants, and vitamins, were confirmed by genomic analysis. There were no pathogenicity islands or plasmid insertions found. This strain is virulent for host colonization rather than invasion.
Conclusion: Genomic characteristics of strain NT04 support its potentiality as an anti-oral pathogen probiotic candidate.

1. Introduction

Enterococci are a group of Gram-positive facultative anaerobic lactic acid-producing bacteria. Several strains of enterococci are used as probiotics; it is part of the microbiota of the human and animal gut [1]. Enterococci are highly tolerant to different environmental conditions, such as varying pH levels, a wide range of temperatures (from 10 °C to >45 °C), and salt concentrations [2]. The enterococci are the most argumentative opportunistic microbial family among the lactic acid bacteria (LAB) group. Although they have

* Corresponding author. Zoology Department, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt.
E-mail address: abdelaziz.a.elsayed@gmail.com (A.A.A. El-Sayed).

https://doi.org/10.1016/j.heliyon.2023.e16253
Received 28 August 2022; Received in revised form 25 April 2023; Accepted 10 May 2023
Available online 24 May 2023

been related to normal oral microbiota, some contain genes encoding virulence factors. These include aggregation substance protein, cell wall adhesins, gelatinase, cytolysin, enterococcal surface proteins, and hyaluronidase [3,4]. Due to their ability to produce bacteriocins called Enterocins, which are active against closely related pathogenic microorganisms, enterococci have garnered significant attention in recent years [5,6]. Enterocins are commonly highly effective against pathogens, such as *Staphylococcus aureus, Bacillus cereus, Listeria innocua,* and *Clostridium* spp. [7]. Their function as probiotics is still debatable due to their multiplied relation with nosocomial superinfections and the assumption that they harbor antibiotic-resistant genes and can be spread to other pathogenic microorganisms utilizing horizontal gene transfer [8]. Before deciding to use enterococci in probiotics applications or as a partner in microbiome-based treatment, it is necessary to screen enterococci extensively for the presence or absence of virulence genes and other metabolic pathways [9].

The term "probiogenomics" was developed to describe the whole genome sequencing (WGS) of probiotics as a means of gaining new insights into their functional diversity, metabolic pathways, and health-stimulating mechanisms of probiotics [10].

Genomic analysis techniques of LAB were started in 2001 [11], which can be used to track the antimicrobial susceptibility and resistance genes of LAB isolates [12].

In the current research, we tested the probiotic ability and bacteriocin production of an oral microbiota isolated from healthy Egyptian adults via a genome sequencing approach, as well as their bacteriocins' antimicrobial activities against oral pathogens. This research employed an integrated strategy of genomic analysis to characterize the metabolic characteristics of this isolate (Fig. 1).

2. Results

2.1. Antimicrobial activity of NT04 against different pathogens

Our selected NT04 isolate displayed antibacterial activity against 6 of 13 indicator bacterial samples used in this study, including Gram-positive and Gram-negative bacteria. This isolate exhibited antimicrobial activity against oral pathogens and other Gram-positive indicator bacteria, including two methicillin-resistant *S. aureus* (MRSA), *Streptococcus mutans, Listeria innocua,* and *Micrococcus luteus*. Additionally, this strain inhibits one Gram-negative indicator bacteria, *Salmonella typhi* ATCC 55669, as depicted in (Table 1).

2.2. Physiochemical properties of bacteriocin-like inhibitory substances (BLIS)

The antibacterial compound's proteinaceous nature was determined by treating the crude BLIS with proteolytic enzymes. According to our findings, the activity was completely abolished following treatment with Proteinase K enzyme, whereas after treatment with α-chymotrypsin enzyme, the activity was reduced by 6%. In contrast, BLIS activity was completely stable after treatment of CFS with both RNase and amylase enzymes. The crude BLIS was exposed to various temperatures (55 °C, 80 °C, 100 °C, and 121 °C), with the activity remaining stable at 55 °C for 30 min and decreasing by 5.5% at 80 °C. At 100 °C and 121 °C, the activity was eliminated entirely. Our investigation into the stability of BLIS at pH 3 and pH 9 revealed that the enzyme's activity was utterly lost at acidic pH, whereas it was completely stable at alkaline pH. The activity of BLIS was entirely stable after treatment with DTT, as shown in Fig. 2 and Table 2.

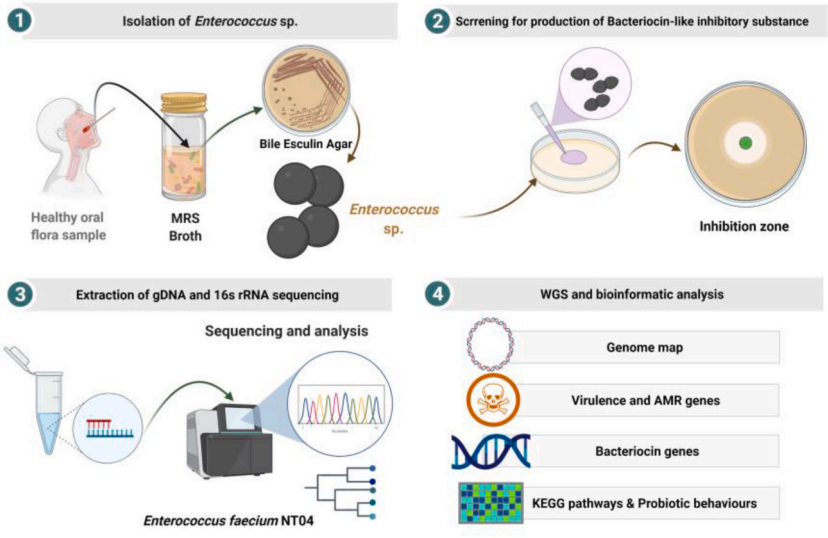

Fig. 1. Graphical abstract showing the overall aim and steps followed in this study.

Table 1
Indicator bacteria, their growth conditions, and their inhibitions by BLIS of *E. faecium* NT04.

Indicator bacteria	Strain	Growth conditions		Activity
		Medium	Temp (°C)	
Staphylococcus aureus **(MRSA)**	ATCC 43300	BHI	37	++++
Streptococcus mutans	ATCC 25175	BHI	37	++
Streptococcus pyogenes	ATCC 19615	BHI	37	–
Micrococcus luteus	ATCC 10240	BHI	30	++++
Staphylococcus aureus **(MRSA)**	LMGT 3242	BHI	37	+++
Mycobacterium phili	AOE 14	BHI	37	–
Lactobacillus plantarum	LMGT 2003	BHI	30	–
Listeria innocua	LMGT 2710	BHI	30	+
Salmonella typhi	ATCC 55669	BHI	37	+
Escherichia coli	ATCC 5087	BHI	37	–
Proteus vulgaris	AOE 21	BHI	37	–
Enterococcus faecalis	V583	BHI	37	–
Sarcina lutea	AOE 6	BHI	30	–

(+) inhibition zone 5–10 mm.
(++) inhibition zone 10–15 mm.
(+++) inhibition zone 15–20 mm.
(++++) inhibition zone of more than 20 mm.

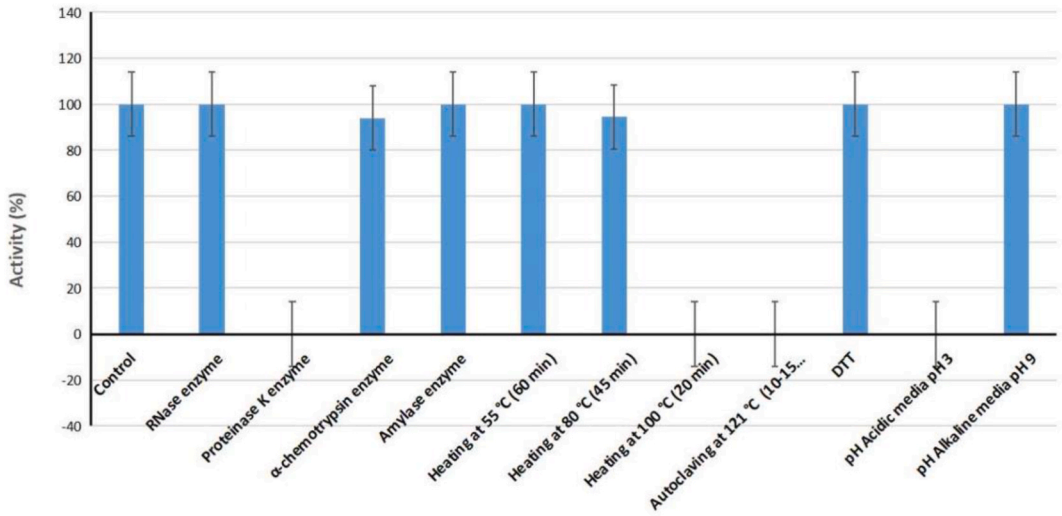

Fig. 2. Results for the experiments conducted for the characterization of physicochemical properties of *E. faecium* NT04. This study showed the stability of bacteriocin NT21 against different conditions of hydrolytic enzymes (RNAase enzyme, proteinase enzyme, α-chymotrypsin enzyme, and amylase enzyme), PH (acidic media 3 and alkaline media 9), temperature (heating to 55 °C, 80 °C, 100 °C, and 121 °C) and DTT. *Staphylococcus aureus* ATCC 25923 (MRSA) was used as a control. The percentage of activity was calculated (% activity = inhibition zone of the treated sample/ inhibition zone of the control sample × 100).

2.3. Bacterial identification and general genome features

Our tested NT04 isolate was identified as *Enterococcus faecium* based on the complete genome and 16S rRNA gene sequencing. Accordingly, this isolate was designated *E. faecium NT04*.

Whole-genome annotation of *E. faecium* NT04 using the RAST server (Fig. 3) revealed a total of 2554 genes belonging to 222 subsystems such as cofactors, vitamins, prosthetic groups, pigments, cell wall, capsule, virulence, membrane transport, amino acids, and carbohydrate metabolism. According to RAST and PATRIC databases, the complete genome of *E. faecium* NT4 comprises circular chromosomes and has no plasmids. The complete genome sequencing has been deposited at GenBank under the accession number JADDKK000000000, and assembly information is illustrated (Table 3). A phylogenetic tree was constructed and presented in (Fig. 4). The graphical circular map and genome atlas of the NT04 genomes are depicted in (Fig. 5).

2.4. Genome analysis for bacteriocin encoding gene cluster

Using both antiSMASH v.4 (https://antismash.secondarymetabolites.org) and BAGEL4 (http://bagel.molgenrug.nl), the

Table 2
Physicochemical properties of crude BLIS in response to different treatment conditions.

Physicochemical properties	BLIS activity
	Inhibition zone by diameters (mm)
Control	15.00 ± 0.00
Enzymes	
Rnase enzyme	15.37 ± 0.4
Proteinase K enzyme	0.00 ± 0.00
α-chemotrypsin enzyme	14.07 ± 0.15
Amylase enzyme	15.30 ± 0.26
Temperature, °C (min)	
Heating at 55 °C (30 min)	15.0 ± 0.10
Heating at 80 °C (30 min)	14.17 ± 0.25
Heating at 100 °C (30 min)	0.00 ± 0.00
Autoclaving at 121 °C (15 min)	0.00 ± 0.00
DTT	15.13 ± 0.06
pH reactivity	
Acidic media pH 3	0.00 ± 0.00
Alkaline media pH 9	15.17 ± 0.06

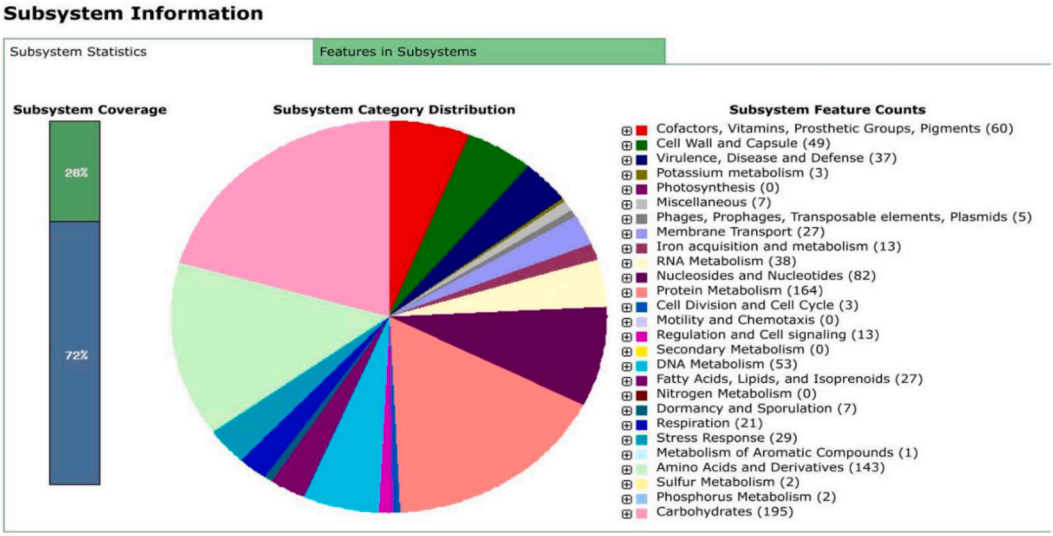

Fig. 3. Summary of annotation for *E. faecium* NT04 based on RAST subsystem.

Table 3
Genome assembly of *Enterococcus faecium* NT04.

Parameter	Data
Genome size (bp)	2,582,527
No. of contigs	34
Size of largest contig (bp)	313,135
N50 (bp)	124,292
GC content (%)	37.93
No. of rRNAs	3
No. of coding sequences	2554
No. of tRNAs	58
MLST sequence type (Clonal Complex)	22 (CC17)
BioProject accession no.	PRJNA669259
GenBank accession no.	JADDKK000000000
BioSample accession no.	SAMN16450691

identification of bacteriocinogenic gene clusters was achieved. According to antiSMASH v.4, our strain's genome has only two secondary metabolite gene clusters, which are categorized under the T3PKS (Type III PKS) and bacteriocin families, whereas bacteriocin cluster was found with a 100% similarity to enterocin A. The bacteriocin contains three areas: enterocin induction factor *entF*

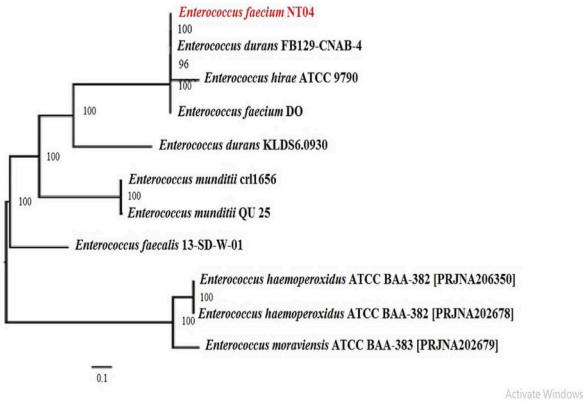

Fig. 4. Phylogenetic tree developed based on investigation of single-nucleotide polymorphisms (SNPs) of the center gene of the examined strain, with intently associated taxa primarily based on 16S rRNA gene sequences. The phylogenetic trees have constructed the usage of the neighbor-joining approach.

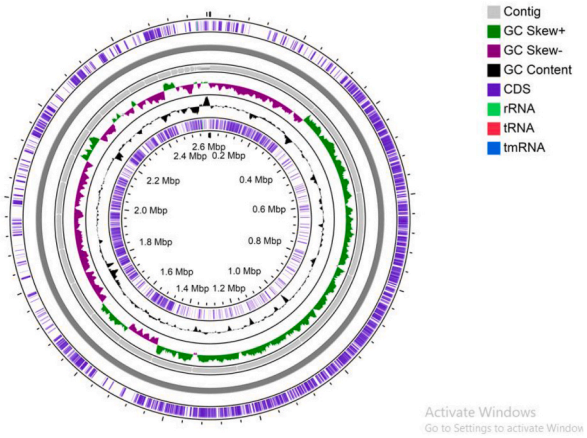

Fig. 5. Genome atlas of strain NT04. The atlas shows a circular view of the complete DNA genome sequence of Enterococcus faecium NT04. This circle was constructed by using a server CG viewer. The innermost circle 1 and circle 6 show CDS's (dark blue). Circle 2 shows GC content (black), circle 3 shows GC skew^{+} (green) and GC skew^{-}(violet), and circle 4 represents contigs.

(2,312,434–2,312,580), enterocin A immunity protein *entI* (2,312,684–2,313,022), and enterocin A *entA* (2,312,997–2,313,194). BAGLE 3 predicted the presence of two bacteriocin biosynthetic Gene clusters (enterocin A and enterocin B), Fig. 6(a and b).

2.5. Assessment of antibiotic resistance genes, virulence genes, CRISPR regions, and pathogenicity islands

The most prevalent resistance genes of our strain were identified using CARDs and the MGE finder on the CGE homepage. There were three antibiotic-resistant genes: *aac(6)-1i* (99.45% identity) for aminoglycoside resistance; *tet*M (97.65% identity) conferring tetracycline resistance; and msrC (>99% identity) for macrolide antibiotic (Table 4). Additionally, two insertion sequences (IS3 and IS982) were identified. The analysis by Virulence Finder (version 1.5) demonstrated the dominant virulence genes, as mentioned in (Table 5). In contrast, pathogenicity islands were not recorded in the PAIDB v2.0 database based on our genomic sequence results, which agree with RAST results searching for gene functional annotation. Finally, the genomic analysis using Crisper Cas Finder software revealed that this strain has only one confirmed CRISPR regions associated gene.

2.6. Genome-based metabolic pathways

The DNA genomic sequence of our strain was used to map and further define the biological function and metabolic pathways, including amino acids synthesis, glutathione metabolism, folate biosynthesis, siderophore biosynthesis, butyrate metabolism, and carbohydrate metabolism, using both the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and annotation from RAST.

E. faecium NT04's genome encodes a number of genes capable of synthesizing and metabolizing glutathione. Table S1 depicts the enzymatic pathway that explains this metabolismThe genome of *E. faecium* NT04 encodes number of genes that can synthesize and

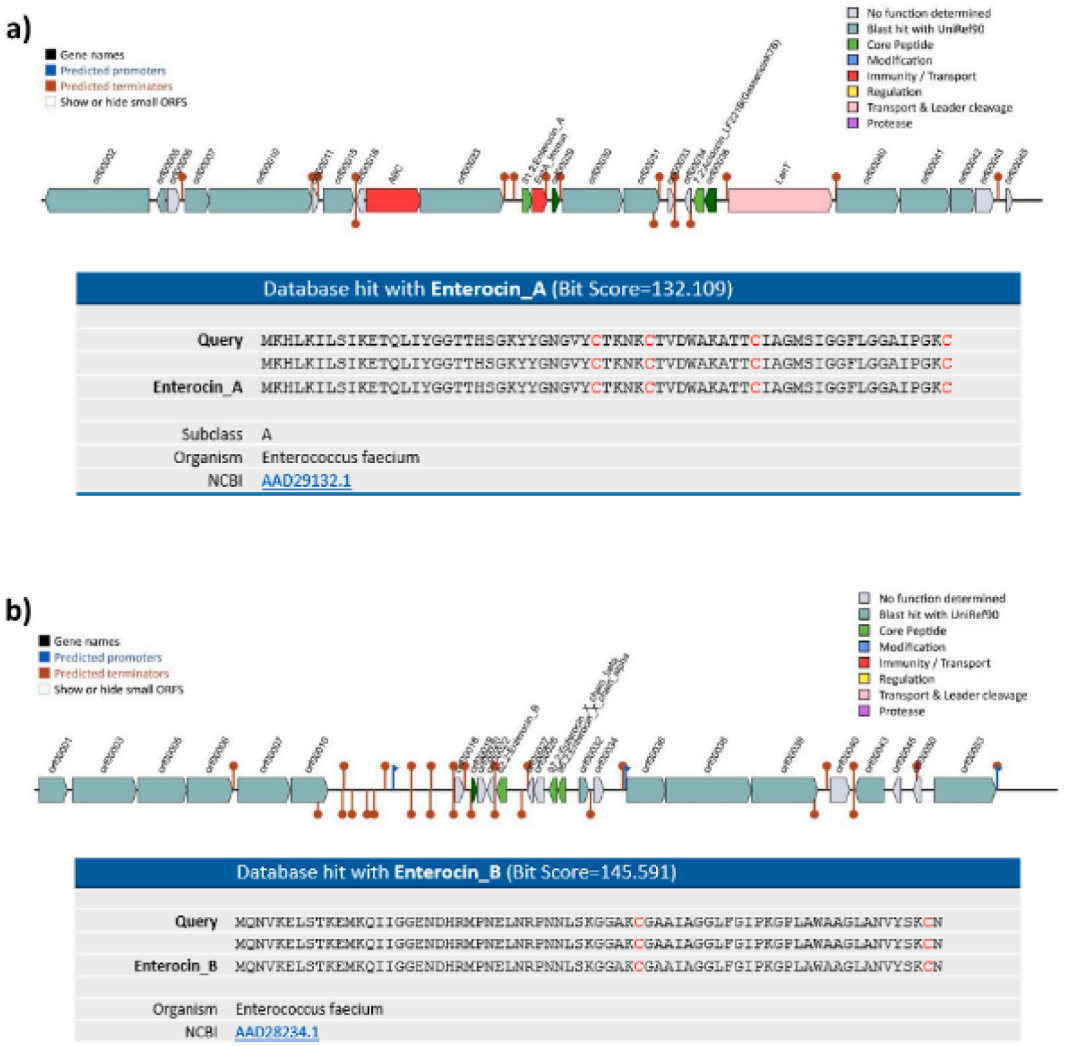

Fig. 6. a) Bacteriocin Gene clusters of enterocin A and alignment of the DNA sequences containing the genetic determinants for enterocin A; b) Bacteriocin Gene cluster of enterocin B and alignment of the DNA sequences comprising the enterocin B genetic determinants.

Table 4
Characteristics of the dominant antibiotic resistance genes.

Antibiotic gene term	Gene Family	Drug Class	Resistance Mechanism	Identity %
*tet*M	tetracycline-resistant ribosomal protection protein	tetracycline antibiotic	antibiotic target protection	97.65%
aac(6)-1i	AAC(6′)	aminoglycoside antibiotic	antibiotic inactivation	99.45%
msrC	msr-type ABC-F protein	macrolide antibiotic,	antibiotic target protection	>99%

metabolize glutathione. The enzymatic pathway that explain this metabolism is mentioned in (Table S1).

The metabolic pathway for folate synthesis was traced in the genome of our strain *E.faecium* NT04 through six enzymatic pathways from 7, 8 dihydroneopterin to folate. The six enzymatic pathway (GTPCHI, DHNA, HPPK, DHPS, DHPS/FPGS, and DHFR) explaining this metabolism are displayed in (Table S2). In addition, our strain could produce seven different types of siderophore group non-ribosomal peptides: Mycobactin, Yersiniabactin, Pyochelin, Vibriobactin, Enterochelin (Enterobactin), Bacillibactin, and Myxochelin. Each of these types is produced through an enzymatic pathway encoded by several genes, as depicted in (Table 6).

Our genomic analysis of our strain *E. faecium* NT04 found its enrollment in butyrate biosynthesis. This biosynthesis occurs within the citrate cycle through four enzymatic systems. The biosynthetic process is illustrated in (Table S3). Furthermore, the KEGG database predicted many biosynthesis pathways, such as the synthesis of some amino acids such as cysteine, serine, and threonine; carbohydrate metabolism like lactose, fructose, galactose, sucrose, glucose; secondary metabolite biosynthesis; aminoacyl-tRNA biosynthesis; peptidoglycan biosynthesis; and fatty acid biosynthesis.

Table 5
Characteristics of the dominant virulence genes.

Category	Gene	Alternative names	Designation(s)
CWAPa (surface exposed LPXTG cell wall-anchored proteins) or Fms (Efm surface proteins)	*acm*	fms8 (MSCRAMM)	adhesin of collagen from *Efm*
	scm	fms10 (MSCRAMM)	second collagen adhesion of *Efm*
PGC-3 cluster	*ebp*Afm	fms 1	endocarditis- and biofilm-associated pili A (MSCRAMM)
	*ebp*Bfm	fms5	endocarditis- and biofilm-associated pili B (MSCRAMM)
	*ebp*Cfm	fms9	endocarditis- and biofilm-associated pili C, pilB (MSCRAMM)
	*srt*C		biofilm production sortase gene (encoding sortase C; SrtC)
Endocarditis-specific antigen A	*efa*A		Manganese ABC superfamily and ATP binding cassette transporter
Type III secretion Proteins	*bop*D		Biofilm formation
Antiphagocytosis and capsular polysaccharide	*cps*A	*upp*S	Di-trans,poly-cis-decaprenylcistransferase
	*cps*B	*cds*A	Phosphatidate cytidylyltransferase

Table 6
Types of Siderophore group non-ribosomal peptides.

Types	Enzymatic pathway	The cytosolic proteins	Genes responsible for Siderophore group production
Pyochelin	salicylate biosynthesis isochorismate synthase [EC:5.4.4.2] isochorismate pyruvate lyase [EC:4.2.99.21]	PchF PchG PchD PchE	*pch*F *pch*G
Mycobactin		MbtG MbtF MbtC MbtD MbtE MbtA MbtB	*mbt*C *mbt*D *mbt*E
Yersiniabactin		Inp1 Inp2 Inp5 Inp3	*inp*1 *inp*2 *inp*3
Vibriobactin	salicylate biosynthesis isochorismate synthase [EC:5.4.4.2] bifunctional isochorismate lyase [EC:3.3.2.1] 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase [EC:1.3.1.28]	VibE VibB VibH VibF	*vib*H *vib*F
Myxochelin		MxcG MxcL MxcE MxcF	*mxc*G
Bacillibactin		DhbF DhbE DhbB	*dhb*F
Enterobactin	salicylate biosynthesis isochorismate synthase [EC:5.4.4.2] bifunctional isochorismate lyase [EC:3.3.2.1] 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase [EC:1.3.1.28] enterobactin synthetase component D [EC:6.3.2.14]	EntF EntE EntB EntH	*ent*F

3. Discussion

A deep understanding of genome sequences may contribute to gaining a precise genetic analysis of bacteria, including the genetic features associated with beneficial effects and those potentially associated with undesirable characteristics. The genus Enterococcus contains strains associated with severe infections, while other strains form part of the commensal human microbiome of the mouth, skin, and gut [13]. Through our research, we catalog and characterize the genomic features, probiotic potential, and metabolic potential obtained by the genomic analysis of *E. faecium* NT04, an oral microbiota-producing bacteriocin-like inhibitory substance active against oral pathogens. This strain lacks plasmids and has a smaller genome than other virulent or non-virulent Enterococcus sp. pathogenic strains with up to six plasmids [14], as determined by a complete genome analysis [14]. First, in our study, we test the

probiotic potential and tolerance of the bacteriocin obtained from *E. faecium* NT04 to different conditions (PH, temperatures, DTT, and some proteolytic enzymes). *E. faecium* NT04 had a broad antimicrobial spectrum and inhibited several pathogens, including methicillin-resistant bacteria. The sensitivity of the CFS to proteolytic enzymes demonstrated the proteinaceous nature of the antimicrobial compound, where bacteriocin retained its activity after treatment with RNase enzyme and amylase, as confirmed by [15]. According to [16], treatment with proteinase K resulted in a complete loss of activity. As previously reported. As previously reported [17], the crude bacteriocin exhibited a slight decrease in activity following treatment with -chymotrypsin. Consistent with our findings, Grosu and his colleagues [18] demonstrated that bacteriocin remained stable at pH 9 [19] but lost all its activity at acidic PH. The significant stability under alkaline pH conditions might be an advantage for the potential use as a bio preservative, for instance, in combination with the thermal processing of the foods [18]. Similar to our results, Kumar and his coworkers [20] showed that enterocins from *E. faecium* were stable at 55 °C for 10–20 min. Cocolin and his coworkers [21] also revealed that the crude BLIS produced by *E. faecium* M241 was stable at 85 °C for 10 min. DTT did not affect bacteriocin activity, indicating the absence of disulfide bridges [18].

Furthermore, our strain NT04 was able to produce both enterocin A and enterocin B. Enterocin A (*EntA*) is grouped as a class II bacteriocin [22]. It is synthesized by many strains of *E. faecium* [23]. Enterocin B is similar to the class IIa bacteriocins. It is synthesized by various *Enterococcus* sp. that also synthesize enterocin A, such as *Enterococcus faecium* T136 and CTC492 [24]. Several pathogens, including *C. perfringens*, *L. monocytogenes*, *S. aureus*, and *Lactobacillus* spp., are susceptible to *entB* antimicrobial activity when combined with enterocin A [25].

Additionally, the virulence factors genes were detected through *E. faecium* NT04 via using a genomic sequencing database, like genes encoded biofilm formation (*ebpAfm, ebpBfm, ebpCfm, srtC, bopD*), cell wall adhesion proteins (*acm, scm*) [26], and enzymes related to capsular polysaccharide biosynthesis and antiphagocytosis (*cps*A, *cps*B). The presence of specific virulence factors does not mean that the harboring organism may be harmful [27]. It is well known both that adhesion and capsule formation are essential even for commensal and probiotic enterococci to persist, promote colonization, and avoid elimination from the host. Moreover, genes involved in adhesion and capsule formation have also been identified among enterococci designated as starter/probiotic strains [26].

Our strain NT04 was resistant to tetracycline (tetM), aminoglycoside (aac(6′), and macrolide (msrC), but there was no genetic evidence of resistance to quinolones or vancomycin. Although antibiotic resistance is not considered a virulence trait in enterococci, it is linked to their ability to proliferate during infection [28].

Based on the genomic results, the strain NT04 does not contain any genomic islands that have a vital role in the bacterium's virulence [29]. Furthermore, our genome has two insertion sequences (IS elements) belonging to IS3 and IS982 families. The presence of IS elements in our strain indicates that they may have a role in transmitting these ARGs and other virulence factors and determinants of antibiotic resistance [30].

In our strain, one CRISPR area was recognized. It has been recommended that CRISPR-cas tend to have a stable defense mechanism within the prokaryotic chromosome toward viral contamination and mobile element integration [31]. The presence of CRISPR-cas systems and restriction enzyme-encoding genes could account for the absence of plasmids and pathogenicity islands [28].

The genomic analysis of our strain NT04 revealed the presence of enzymatic pathways responsible for folate biosynthesis (also known as Vitamin B9). Folate is used by all cells for several essential metabolic and biological functions. It is involved in essential pathways like DNA replication, cell division [32], repair and methylation, nucleotide biosynthesis, and amino acid metabolism as cofactors of metabolic enzymes [33].

In addition, our strain, NT04, can metabolize glutathione, which acts as an antioxidant agent. Glutathione is the best cellular defense tool for the organism against oxidative damage that occurs under both osmotic stress and peroxide stress [9], where it combines with toxic compounds to produce non-toxic products.

Siderophores are secondary polypeptides natural products that play a vital role in cellular growth [34], biosynthesized by non-ribosomal peptide synthetase (NRPS) members [35]. Bacteria often produce siderophores to facilitate iron uptake, which is essential in bacterial growth, replication, and metabolism [36].

According to our findings, our isolate is capable of synthesizing butyrate. Butyrate is one of the primary short-chain fatty acids (SCFAs) classified as volatile fatty acids. It is recognized for its importance as a colonic inflammatory response mediator (anti-colonic inflammation) [37], and some studies showed that it also acts as an anticancer [38].

In conclusion, the genome of our *E. faecium* NT04 presents a variety of genes that can be associated with the production of antimicrobial compounds belonging to bacteriocin (enterocin A and B) against several pathogens. In addition, *E. faecium* NT04 has multifunctional probiotic abilities to synthesize vitamins (folate), antioxidant (glutathione), antibiotic activity (bacteriocin), promotion of cellular growth (siderophores), and anti-colonic inflammation and anticancer (butyrate). The genome lacks plasmids and pathogenicity islands. Finally, the availability of our strain genome and its analysis should facilitate further investigation on the physiological functions and biotechnological applications.

4. Materials and methods

4.1. Isolation of enterococcus bacteria

A healthy oral microbiota sample was collected using a sterile swab from an adult male 23 years old. The swab was aseptically transferred to MRS broth, incubated at 37 °C for 18 h, and then plated on bile esculin agar (Oxoid, U.K). Plates were incubated under aerobic conditions for 24–48 h at 37 °C, and suspected colonies were Gram stained. The isolated bacteria were preserved as frozen stocks at − 80 °C of its pure culture in 20% (v/v) glycerol [39].

4.2. Cell-free supernatant (CFS) preparation and screening for the production of bacteriocin-like inhibitory substances (BLIS)

The NT04 isolate was inoculated (1% v/v) into MRS broth (Thermo Scientific^{tm} Oxide, U.K) and then incubated aerobically for 24 h at 37 °C. The pH of the CFS was adjusted at 6.5 using 0.1 M NaOH, and catalase (1 mg/mL) was added to avoid the inhibitory effects of organic acids and hydrogen peroxide (H_2O_2). The fermented broth was centrifuged at 5000×*g* for 10 min at 4 °C (Sigma-3-16K, UK), and the supernatant was collected. Finally, the CFS was filtered with a sterile 0.22-μm pore size filter (Millipore, Merck). The crude CFS was preserved at 4 °C [40]. The antimicrobial assay was performed by the agar well diffusion test (ADT) against different microorganisms listed in (Table 1), as described by [41]. Briefly, 50 μl aliquots of cell-free culture supernatants were placed into wells (6-mm diameter) cut in cooled agar media (0.7%, wt/vol) plates previously seeded (10^5 CFU/ml) with the indicator microorganisms. After pre-diffusion for 2 h at 4 °C, the plates were incubated at 37 °C for 24 h to allow the growth of the target microorganisms and then analyzed for the presence of inhibition zones around the wells. The indicator microorganisms used in this study and their growth conditions are listed in (Table 1).

4.3. Characterization and physicochemical properties of BLIS

The sensitivity of BLIS to hydrolytic enzymes such as proteinase K, α-chymotrypsin, RNAase enzyme, and α-amylase was detected. These enzymes were diluted (1 mg/ml), sterilized, added to CFS samples, and incubated at 37 °C for 2 h. Heat resistance of CFS was tested by heating to 55 °C, 80 °C, 100 °C (30 min) and, finally, autoclaving at 121 °C (15 min).

The activity was calculated by measuring the inhibition zone diameters of the treated samples compared to the untreated control. According to [42], CFS samples were evaluated for their ability to withstand at acidic and alkaline conditions by acidifying the CFS to (pH 3) with 0.1 M HCl and by alkalifying the CFS to (pH 9) with 0.1 M NaOH. In order to determine the effect of disulfide bonds reduction on the BLIS activity, the CFS sample was incubated at 37 °C for 1 h with dithiothreitol (DTT) at a final concentration of 1% (v/v). The inhibitory activity was measured as described by [43].

4.4. Bacterial DNA extraction, 16S rRNA gene sequencing, and whole-genome sequencing (WGS) of the NT04 isolate

Genomic DNA extraction was managed as stated in the manufacturer's protocol (QIAamp genomic DNA kit, Germany). The 16S rRNA gene was amplified by using specific primers 27F (5-AGA GTT TGATCMTGG CTC AG-3′) and 1492R (5′-ACG GCTACC TTGTTA CGA CTT-3′) [44]. Moreover, TruSeq Nano DNA Kit (Illumina Inc., USA) was used to sequence DNA of each cluster on a flow cell to generate 151 bp paired-end reads. The reads were extracted in FASTA format bearing DNA sequence with respective quality values for each base in the sequence. The genome of *E. faecium* Aus0004 (GenBank accession number: CP003351) was used as a reference.

4.5. Comparative genomic and bioinformatics analysis

The complete genome sequence was assembled by SPAdes v3.13.0 and then annotated with Rapid Annotation using Subsystem Technology (RAST) version 4.0 [45] and the Pathosystems Resource Integration Center (PATRIC) [46]. The complete genome sequence of our strain NT04 was investigated for the presence of gene clusters responsible for bacteriocins, putative virulence genes, antibiotic resistance genes, pathogenicity islands, CRISPR-Cas, and secondary metabolite biosynthetic gene clusters, and genes associated with physiological and functional properties. Virulence and antibiotic resistance genes were identified using Virulence Finder (version 1.5) [47] and the Comprehensive Antibiotic Resistance Database (CARDs) [48]. Bacteriocin genes were explored using the Bagel 4 (Bagel automated bacteriocin mining version.4) web software [49] and were completed with the antiSMASH: antibiotics and Secondary Metabolite Analysis Shell that also identified gene clusters of any bioactive compounds [50]. Additional databases were searched for genes associated with pathogenicity islands (PAIDB v2.0) [51] and CRISPR-cas region prediction (CRISPR Cas finder) [52]. Our strain genes' potential metabolic and biological pathways were carried out with the KEGG Automatic Annotation Server for orthologue assignment and pathway mapping (KAAS), using gene prediction in amino acids [53]. The production of secondary metabolites, antioxidant activity, and polypeptide biosynthesis was predicted by identifying genes corresponding to the complete metabolic pathway and searching for genes that encode enzymes reported for specific reactions. The genome map was shaped using CGview software (http://cgview.ca/).

4.6. Accession number(s)

This whole-genome shotgun project has been deposited at GenBank under the accession JADDKK000000000. The version described in this paper is version JADDKK010000000.

4.7. Ethical consideration

This study was carried out in accordance with the recommendations of the Research Ethical Committee at Faculty of Medicine, 143 Beni-Suef University (FM-BSU REC), Beni-Suef, Egypt, with approval number: (144 FMBSUREC/05072020/Mahdy) approved the protocol for this study.

4.8. Statistical analysis

The data were analyzed using Minitab 19 to find out the means and standard deviation for the different replicates of each treatment.

5. Limitations of the study

There are some limitations common in the study design and sampling method. Firstly, authors depend on genomic sequencing only to detect the metabolic activities of the strain NT04 without laboratory detection. Another limitation is that the authors did not use a combination of in-vitro and genomic investigation to test the antibiotic resistance of the strain. Furthermore, the authors did not perform bacteriocin detection to test the probiotic attributes of the strain against a wide range of microbes. Finally, the authors did not show comprehensive testing against a suitable collection of oral pathogens.

Author contribution statement

Ahmed O. El-Gendy: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ahmed F. Azmy: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data. Ahmed S. Khairalla: Conceived and designed the experiments.

Medhat Abdel-Fattah: Conceived and designed the experiments; Analyzed and interpreted the data.

Nashwa Tarek: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Ohoud A. Jefri: Contributed reagents, materials, analysis tools or data.

Mohamed Shaban: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data؛ Wrote the paper.

Abdelaziz A. A. El-Sayed: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16253.

List of abbreviation

LAB	Lactic acid bacteria
WGS	Whole genome sequencing
MRSA	Methicillin-resistant *S. aureus*
BLIS	Bacteriocin-like inhibitory substances
DTT	Dithiothreitol
KEGG	Kyoto Encyclopedia of Genes and Genomes
DHPS	Dihydropteroate synthase
FPGS	Folylpolyglutamate synthetase
DHFR	Dihydrofolate reductase
EPS	Extracellular polysaccharides
CHO	Carbohydrate
NRPS	Non-ribosomal peptide synthetase
CFS	Cell-free supernatant
ADT	Agar well diffusion test
RAST	Rapid Annotation using Subsystem Technology
PATRIC	Pathosystems Resource Integration Center
CARDs	Comprehensive Antibiotic Resistance Database

References

[1] B. Krawczyk, et al., The many faces of Enterococcus spp.—commensal, probiotic and opportunistic pathogen, Microorganisms 9 (9) (2021) 1900.
[2] R. Zaheer, et al., Surveillance of Enterococcus spp. reveals distinct species and antimicrobial resistance diversity across a one-health continuum, Sci. Rep. 10 (1) (2020) 3937.
[3] E. Aun, et al., Molecular characterization of Enterococcus isolates from different sources in Estonia reveals potential transmission of resistance genes among different reservoirs, Front. Microbiol. 12 (2021).
[4] B.B. Xavier, et al., Novel vancomycin resistance gene cluster in Enterococcus faecium ST1486, Belgium, June 2021, Euro Surveill. 26 (36) (2021), 2100767.

[5] A. Baran, et al., Proteolytic activities and safety use of Enterococcus faecalis strains isolated from Turkish white pickled cheese and milk samples, J. Hellenic Veter. Med. Soc. 72 (4) (2022) 3391–3400.
[6] H.M. Abuohashish, et al., Pharmacological Effects of marine-derived Enterococcus faecium EA9 against acute lung injury and Inflammation in cecal ligated and punctured septic rats, BioMed Res. Int. 2021 (2021), 5801700.
[7] A.C. Almeida-Santos, et al., Enterococcus spp. as a producer and target of bacteriocins: a double-edged sword in the antimicrobial resistance crisis context, Antibiotics 10 (10) (2021) 1215.
[8] S. Arredondo-Alonso, et al., Plasmids shaped the recent emergence of the major nosocomial pathogen Enterococcus faecium, mBio 11 (1) (2020), e03284-19.
[9] V.C. Ghattargi, et al., Genomic and physiological analyses of an indigenous strain, Enterococcus faecium 17OM39, Funct. Integr. Genom. 18 (4) (2018) 385–399.
[10] O.S. Stergiou, et al., Whole-genome sequencing, phylogenetic and genomic analysis of lactiplantibacillus pentosus L33, a potential probiotic strain isolated from fermented sausages, Front. Microbiol. 12 (2021), 746659.
[11] C. Wu, J. Huang, R. Zhou, Genomics of lactic acid bacteria: current status and potential applications, Crit. Rev. Microbiol. 43 (4) (2017) 393–404.
[12] Y. He, et al., Evaluation of the inhibitory effects of Lactobacillus gasseri and Lactobacillus crispatus on the adhesion of seven common lower genital tract infection-causing pathogens to vaginal epithelial cells, Front. Med. 7 (2020).
[13] T.J. Hossain, Functional genomics of the lactic acid bacterium Limosilactobacillus fermentum LAB-1: metabolic, probiotic and biotechnological perspectives, Heliyon 8 (11) (2022), e11412.
[14] J. Mbanga, et al., Genomic analysis of Enterococcus spp. isolated from a wastewater treatment plant and its associated waters in umgungundlovu district, South Africa, Front. Microbiol. 12 (2021).
[15] Q. Xi, et al., Purification and characterization of bacteriocin produced by a strain of Enterococcus faecalis TG2, Appl. Biochem. Biotechnol. 184 (4) (2018) 1106–1119.
[16] Y. Jiao, et al., Probiotic effects and metabolic products of Enterococcus faecalis LD33 with respiration capacity, Foods 11 (4) (2022) 606.
[17] D.C. Dezwaan, et al., Purification and characterization of enterocin 62-6, a two-peptide bacteriocin produced by a vaginal strain of Enterococcus faecium: potential significance in bacterial vaginosis, Microb. Ecol. Health Dis. 19 (4) (2007) 241–250.
[18] S.-S. Grosu-Tudor, et al., Characterization of some bacteriocins produced by lactic acid bacteria isolated from fermented foods, World J. Microbiol. Biotechnol. 30 (9) (2014) 2459–2469.
[19] R. Hajikhani, et al., Assessment of some metabolic activities and potential probiotic properties of eight Enterococcus bacteria isolated from white cheese microbiota, Antonie Leeuwenhoek 114 (8) (2021) 1259–1274.
[20] M. Kumar, S.K. Tiwari, S. Srivastava, Purification and characterization of enterocin LR/6, a bacteriocin from Enterococcus faecium LR/6, Appl. Biochem. Biotechnol. 160 (1) (2009) 40.
[21] L. Cocolin, et al., Description of the bacteriocins produced by two strains of Enterococcus faecium isolated from Italian goat milk, Food Microbiol. 24 (7) (2007) 752–758.
[22] E. Escamilla-Martínez, et al., Identification of structural and immunity genes of a class IIb bacteriocin encoded in the enterocin A operon of Enterococcus faecium strain MXVK29, J. Food Protect. 80 (11) (2017) 1851–1856.
[23] R. Rahmeh, et al., Characterization of semipurified enterocins produced by Enterococcus faecium strains isolated from raw camel milk, J. Dairy Sci. 101 (6) (2018) 4944–4952.
[24] G. Liu, et al., Genetic and biochemical evidence that Enterococcus faecalis Gr17 produces a novel and sec-dependent bacteriocin, enterocin Gr17, Front. Microbiol. 10 (2019).
[25] D. Ankaiah, et al., Cloning, overexpression, purification of bacteriocin enterocin-B and structural analysis, interaction determination of enterocin-A, B against pathogenic bacteria and human cancer cells, Int. J. Biol. Macromol. 116 (2018) 502–512.
[26] J. Anjum, et al., A potentially probiotic strain of Enterococcus faecalis from human milk that is avirulent, antibiotic sensitive, and nonbreaching of the gut barrier, Arch. Microbiol. 204 (2) (2022) 158.
[27] L. Avram-Hananel, et al., E durans strain M4–5 isolated from human colonic flora attenuates intestinal inflammation, Dis. Colon Rectum 53 (12) (2010) 1676–1686.
[28] M. Olvera-García, A. Sanchez-Flores, M. Quirasco Baruch, Genomic and functional characterisation of two Enterococcus strains isolated from Cotija cheese and their potential role in ripening, Appl. Microbiol. Biotechnol. 102 (5) (2018) 2251–2267.
[29] M. Desvaux, et al., Pathogenicity factors of genomic islands in intestinal and extraintestinal Escherichia coli, Front. Microbiol. (2020) 11.
[30] Z. Udaondo, et al., Insertion Sequences Associated with Antibiotic Resistance Genes in Enterococcus Isolates from an Inpatient with Prolonged Bacteremia, bioRxiv, 2021, p. 2021.07.22.453447.
[31] E.P. Rocha, D. Bikard, Microbial defenses against mobile genetic elements and viruses: who defends whom from what? PLoS Biol. 20 (1) (2022), e3001514.
[32] A. Greppi, et al., Ability of lactobacilli isolated from traditional cereal-based fermented food to produce folate in culture media under different growth conditions, LWT 86 (2017) 277–284.
[33] F. Moens, et al., A four-strain probiotic exerts positive immunomodulatory effects by enhancing colonic butyrate production in vitro, Int. J. Pharm. 555 (2019) 1–10.
[34] R.A. Ansari, et al., Siderophores: augmentation of soil health and crop productivity, in: V. Kumar, et al. (Eds.), Probiotics in Agroecosystem, Springer Singapore, Singapore, 2017, pp. 291–312.
[35] I.J. Schalk, C. Rigouin, J. Godet, An overview of siderophore biosynthesis among fluorescent Pseudomonads and new insights into their complex cellular organization, Environ. Microbiol. 22 (4) (2020) 1447–1466.
[36] M.G.P. Page, The role of iron and siderophores in infection, and the development of siderophore antibiotics, Clin. Infect. Dis. 69 (Supplement_7) (2019) S529–S537.
[37] D. Parada Venegas, et al., Short chain fatty acids (SCFAs)-Mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases, Front. Immunol. 10 (2019).
[38] J. Chen, K.-N. Zhao, L. Vitetta, Effects of intestinal microbial–elaborated butyrate on oncogenic signaling pathways, Nutrients 11 (5) (2019) 1026.
[39] W. Chingwaru, S.F. Mpuchane, B.A. Gashe, Enterococcus faecalis and Enterococcus faecium isolates from milk, beef, and chicken and their antibiotic resistance, J. Food Protect. 66 (6) (2003) 931–936.
[40] T. Choeisoongnern, et al., Potential probiotic Enterococcus faecium OV3-6 and its bioactive peptide as alternative bio-preservation, Foods 10 (10) (2021) 2264.
[41] L.M. Cintas, et al., Isolation and characterization of pediocin L50, a new bacteriocin from Pediococcus acidilactici with a broad inhibitory spectrum, Appl. Environ. Microbiol. 61 (7) (1995) 2643–2648.
[42] L.-S. Chou, B. Weimer, Isolation and characterization of acid- and bile-tolerant isolates from strains of Lactobacillus acidophilus1, J. Dairy Sci. 82 (1) (1999) 23–31.
[43] R. Kemperman, et al., Identification and characterization of two novel clostridial bacteriocins, circularin A and closticin 574, Appl. Environ. Microbiol. 69 (3) (2003) 1589–1597.
[44] V.S. Patil, et al., Enterobacillus tribolii gen. nov., sp. nov., a novel member of the family Enterobacteriaceae, isolated from the gut of a red flour beetle, Tribolium castaneum, Antonie Leeuwenhoek 107 (5) (2015) 1207–1216.
[45] R. Overbeek, et al., The SEED and the rapid annotation of microbial genomes using subsystems Technology (RAST), Nucleic Acids Res. 42 (D1) (2013) D206–D214.
[46] A.R. Wattam, et al., PATRIC, the bacterial bioinformatics database and analysis resource, Nucleic Acids Res. 42 (D1) (2013) D581–D591.
[47] K.G. Joensen, et al., Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli, J. Clin. Microbiol. 52 (5) (2014) 1501–1510.
[48] A.G. McArthur, et al., The comprehensive antibiotic resistance database, Antimicrob. Agents Chemother. 57 (7) (2013) 3348–3357.

[49] A.J. van Heel, et al., BAGEL3: automated identification of genes encoding bacteriocins and (non-)bactericidal posttranslationally modified peptides, Nucleic Acids Res. 41 (W1) (2013) W448–W453.
[50] M.H. Medema, et al., AntiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences, Nucleic Acids Res. 39 (suppl_2) (2011) W339–W346.
[51] S.H. Yoon, Y.-K. Park, J.F. Kim, PAIDB v2.0: exploration and analysis of pathogenicity and resistance islands, Nucleic Acids Res. 43 (D1) (2014) D624–D630.
[52] I. Grissa, G. Vergnaud, C. Pourcel, CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats, Nucleic Acids Res. 35 (suppl_2) (2007) W52–W57.
[53] H. Ogata, et al., KEGG: Kyoto Encyclopedia of genes and genomes, Nucleic Acids Res. 27 (1) (1999) 29–34.