Journal of Applied Biology & Biotechnology Vol. 11(1), pp. 36-44, Jan-Feb, 2023
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2023.110105-1

Enterococcus species and their probiotic potential: Current status and future prospects

Kondapalli Vamsi Krishna, Koushik Koujalagi, Rutiwick U. Surya, M. P. Namratha, Alok Malaviya*

Applied and Industrial Biotechnology Laboratory (AIBL), Department of Life Sciences, CHRIST (Deemed to be University), Hosur Road, Bengaluru, Karnataka, India

ARTICLE INFO

Article history:
Received on: June 14, 2022
Accepted on: September 17, 2022
Available online: November 22, 2022

Key words:
Anti-oxidant properties,
Enterocins,
Enterococcus spp.,
Pathogenecity,
Probiotics.

ABSTRACT

Probiotics are described as live microbes that, once consumed in sufficient quantities, provide a health advantage to the host. A rising number of research works have verified the health benefits of probiotics. *Enterococci* are common bacteria that may be found almost anywhere. For their opportunistic pathogenicity, *Enterococci* have been associated with numerous nosocomial infections resulting from resistance to antibiotics and the existence of other virulence factors, notably the development of vancomycin-resistant *Enterococci*. However, some Enterococcal strains such as *E. faecium* and *E. faecalis* strains are being utilized as probiotics and are widely marketed, usually in the form of pharmaceutical solutions. *Enterococcus* spp. based probiotics are used to treat irritable bowel syndrome, infectious diarrhea, and antibiotic-associated diarrhea, along with decreasing cholesterol levels and enhancing host immunity. To be used as probiotics in the future, Enterococcal strains must be properly defined and thoroughly evaluated in terms of safety and can be beneficial. Here, in this work, we have reviewed various aspects of *Enterococcus* spp. pertaining to its possibility of being utilized as a probiotic strain.

1. INTRODUCTION

Probiotics could be used as part of a strategy to accomplish and treat infections in an era when new approaches are being looked-for. The idea of probiotics emphasizes the usage of competitive elimination for enhancing a particular ecosystem. Probiotic treatment or prophylaxis purposefully introduces advantageous bacteria to fight off harmful microorganisms. In animals and people, probiotics have been utilized. Some of the recognized probiotics have been mentioned in Table 1. Lactic acid bacteria (LAB) are the most frequent type of microbes that have the capability to digest lactose, turning it into lactic acid and consequently decreasing the gastrointestinal pH. *Bifidobacteria*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, and *Streptococcus* are all members of the LAB group. *Bacillus* and the yeast *Saccharomyces* are examples of non-LAB. Each possesses distinct modes of action, metabolism, and antibiotic sensitivity. *Lactobacillus* and *Bifidobacterium* group of strains are the most common genera of probiotic organisms [1]. Milk from various sources and milk products is proved to be good probiotic sources. Probiotics have been isolated from different sources such as raw milk [2], cheese [3], fermented milk [4,5], Koumiss [6], and yogurt [7]. Different research studies have indicated that probiotic bacteria may lighten lactose intolerance [8,9], have a helpful impact on the gut flora of the host [10], stimulate/control mucosal immunity [11], lessen inflammatory or allergic reactions [12], lessen blood cholesterol [13,14], possess anti-colon cancer effects [15,16], reduce the clinical manifestations of atopic dermatitis [17,18], Crohn's disease [19-22], candidiasis [23], and urinary tract infections [24].

Until recently, *Bifidobacterium*, *Lactobacillus,* and *Lactococcus* species were the most commonly accepted probiotic strains. Other bacteria with probiotic potential should be investigated for probiotic admissibility to find new applications. *Enterococcus* spp. is a major contender among them [25]. *Enterococci* are a significant bacterial group, and their association with humans is well documented. They are Gram-positive cocci, which proliferate in short chains or pairs and are facultative anaerobes that can withstand high temperatures. They can grow in high NaCl concentrations and at pH = 9.6 and temperatures range from 10°C to 45°C, with optimal growth at 35°C. With 37 species defined by phylogenetic evaluation utilizing DNA-DNA hybridization methods and 16S rRNA sequencing, this is the third biggest genus in the group of LAB, following *Lactobacillus* and *Streptococcus* [26-28] and of all *E. faecalis* and *E. faecium* are the most widely dispersed species in nature.

The current article aims to summarize the recent updates on various aspects of *Enterococcus* spp. including their potential to be used as a probiotic candidate.

2. HABITAT AND OCCURRENCE OF *ENTEROCOCCUS*

Enterococcus species have been discovered in food, water, soil, plants, animals (insects, birds and mammals), and people. These organisms appear to have their primary natural home in the gut of humans and

*Corresponding Author:
Alok Malaviya,
Applied and Industrial Biotechnology Laboratory (AIBL), Department of Life Sciences, CHRIST (Deemed to be University), Hosur Road, Bengaluru, Karnataka, India.
E-mail: alokkumar.malaviya @ christuniveristy.in

other animals, where they structure a large percentage of the typical gut microbiota. A few species are host-specific, whereas others are widespread. *Enterococcus columbae*, which is unique for pigeons, and *Enterococcus asini*, which has up to now only been identified in donkeys, are two examples of host-specific *Enterococci*. The most common *Enterococcal* species seen in farm animal intestines are *E. durans*, *E. faecium*, *E. faecalis*, *E. hirae*, and. In chickens, *E. faecalis* is present early in life and is eventually replaced by *E. faecium*, which is ultimately replaced by *E. cecorum*, *E. casseliflavus*, *E. mundtii*, and *E. gallinarum* [29]. In addition to that, *Enterococci* occur in different types of sources such as dairy, meat, and seafood. A list of various Enterococcal strains isolated from different sources is summarized in Table 2. *Enterococci* are one of the first LAB to inhabit the neonatal gut [30], and they may be linked to infant health and formation of the human microbiome [31]. *Enterococci* represent approximately 1% of human gut flora and are the most prevalent gram-positive organisms in feces. *E. faecalis* may be detected in 90–100% of animals and human feces, whereas *E. faecium* is prevalent in 25%. In the oropharyngeal secretions, urogenital tract, and on the skin, specifically in the perineum, small quantities of *Enterococci* may also be detected [32].

3. OPPORTUNISTIC PATHOGENICITY AND VIRULENCE OF *ENTEROCOCCUS* SPECIES

Concerns about the safety of *Enterococcus* spp. have not been incorporated in the Qualified Presumption Safety (QPS) list [33] nor have generally been regarded as safe (GRAS) status [34]. Through the past two decades, *Enterococcus* has become a more common source of nosocomial infections [35]. According to recent studies, the growth of antibiotic-resistant *E. faecium* isolates has amplified the incidence of *E. faecium* infections [36-39]. Other Enterococcal species, for instance *E. durans*, *E. casseliflavus*, *E. gallinarum*, *E.lactis,* and *E. raffinosus*, are less commonly connected with Enterococcal infections. *Enterococci* are often seen in polymicrobial intra-abdominal infections, finding it challenging to define their role in the illness [40,41]. Enterococcal infections are frequently linked to preliminary colonization of the patient's gastrointestinal tract and translocation of *Enterococci* across the intestinal epithelial barrier is leading to bacteremia [42,43]. The influence of vancomycin resistance and high-level gentamicin resistance on mortality has been the subject of some debate [44-47]. Enterococcal bacteremia can progress to endocarditis, the most serious infection caused by *Enterococci* to treat in the bloodstream and has been linked to a higher mortality rate. After *Streptococci* and *S. aureus*, the third most common cause of endocarditis is known to be caused by *Enterococci*, predominantly *E. faecalis* which are responsible for 5–20% of endocarditis [48,49]. Antibiotic resistance in *E. faecalis* and *E. faecium* has been thoroughly investigated. *Enterococci* have an exceptionally exuberant rate of inherent tolerance to low doses of various antibiotic classes, including aminoglycosides, beta-lactams (third-generation cephalosporins), and quinolones, due to their hardness. Clinical isolates of *E. faecium* are particularly resistant to high amounts of penicillin [50]. *Enterococci* have acquired genetic determinants that confer resistance to several antibiotics, including glycopeptides (Vancomycin and Teicoplanin), as well as the synergistic action of -lactams and aminoglycosides [51]. The *Enterococcus* genus's high genetic diversity suggests that specific mutations have adapted to various environments. Thus, prolonged antibiotic exposure may induce a mutation that imparts resistance to a specific antimicrobial agent, allowing bacteria to survive [52,53]. In *Enterococcus* spp., six phenotypes of glycopeptide resistance have been identified (*van*A, *van*B, *van*C, *van*D, *van*E, and *van*G). The *van*A operon in strains presents a high-level resistance to vancomycin and teicoplanin. With minimum inhibitory concentrations between 4 and 1000 mg/mL, the *van*B operon incites different levels of resistance to vancomycin. Only *van*A and *van*B have the ability to transfer vertically and horizontally as well as impart high resistance levels [53]. *van*C strains have a phenotype that demonstrates minimal vancomycin resistance and intrinsic sensitivity to teicoplanin [69]. Vancomycin and teicoplanin resistance is generated by the operon

Table 1: Various microorganisms used as Probiotics. This table summarizes the probiotic strains approved by Food Safety and Standards Authority of India as well as probiotics used in other countries [41,46].

***Lactobacillus* strains**	***Bifidobacterium* strains**	**Others**
L. acidophilus	*B. bifidum*	*E. faecalis*
L. amylovorus	*B. infantis*	*E. faecium*
L. brevis	*B. lactis*	*E. durans*
L. bulgaricans	*B. breve*	*Streptococcus boulardii*
L. casei	*B. longum*	*S. thermophilus*
L. caucasicus	*B. animalis*	*Bacillus cereus*
L. debrueckii		*B. subtilis*
L. fermentis		*Lactococcus lactis*
L. gallinarum		*Propionibacteria*
L. helveticus		*Escherichia coli*
L. infantis		*Saccharomyces boulardii*
L. johnsonii		*S. cerevisiae*
L. lactis		
L. plantarum		
L. reuteri		
L. rhamnosus		
L. salivarius		
L. sporogenes		

Table 2: Different Enterococcus strains isolated from various sources.

Strains	**Source**	**References**
	Dairy Products	
E. faecalis, E. casseliflavus	Raw-Milk Cheese	[54]
E. faecalis	Tunisian cheese	[55]
E. lactis sp. nov.	Italian raw milk cheeses	[56]
E. faecium	Raw bovine milk	[57]
*E. bulliens*sp. nov	Camelus dromedaries (Camel) milk	[58]
E. faecium		[59]
Enterococcus lactis PMD74	Ezine Cheese	[60]
	Meat and meat products	
E. faecalis	Refrigerated poultry meat	[61]
E. faecalis, E. faecium, E. casseliflavu	Fermented meat products	[62]
	Sea water food	
E. faecium	Cyprinus carpio Macrobrachium rosenbergii	[63]
E. faecium E. mundtii	Psetta maxima	[64]
E. feacium	Tunisian Fish Viscera	[65]
E. faecalis E. faecium	Retail shrimps	[66]
E. faecium	Tunisian freshwater fishes	[67]
E. lactis	Penaeus vannamei Palaemon serratus	[68]

Table 3: Different classes of enterocins produced by various Enterococcus species.

Enterocins	Class	Organism	References
Enterocin AS-48	Class IId	*E. faecalis*	[70]
Enterolisin	Class III	*E. faecalis LMG 2333*	[71]
Enterocin CRL35	Class IIa	*E. mundtii*	[72]
Enterocin 96	Class II	*E. faecalis* WHE96	[73]
ST4SA	Class IIa	*E. mundtii*	[74]
E 50-52	Class IIa	*E. faecium*	[75]
Hiracin JM79	Class II	*E. hirae* DCH5	[76]
E- 760	IIb	*Enterococcus* spp. NRRL B-30745	[77]
Avicin A	IIa	*E. avium*	[78]
ESL 5	Unknown	*E. faecalis*	[79]
Enterocin C	Class IIb	*E. faecalis* C901	[80]
Enterocin M	Unknown	*E faecium* AL41	[81]
Enterocin R5	Class II	*E. lactis* RS 5	[82]
Enterocins A, B and P		*E. lactis* 4CP3	[83]

*van*D, which is present in the chromosome and varies from other resistance genes. Because of its stability in its genome, this trait does not appear to be transferrable [84]. Vancomycin resistance is encoded by the *van*E and *van*G operons, which are assumed to be acquired and inducible [85,86]. *van*G discovered in an *E. faecalis* showed moderate resistance to teicoplanin [53].

Insulin, casein, hemoglobin, fibrinogen, collagen, and gelatin are all hydrolyzed by gelatinase, a metalloendopeptidase [83]. Cytolysin is a bifunctional bacteriocin/hemolysin protein. Hyaluronidase induces the lysis of hyaluronic acid, which is the major component of the extracellular matrix's connective tissue [35]. These virulence factors (gelatinase, cytolysine, and hyaluronidase) are found in almost all species. β-hemolytic strains of *Enterococcus* increased the risk of death fivefold when compared to those with bacteremia caused by non-hemolytic strains [44]. The ability to produce this protease has been proven by the presence of *gel*E gene which might not be expressed in *E. faecium* and *E. faecalis* strains [87]. However, Enterococcal virulence factors' expression may differ depending on the ecological environment, resulting in greater pathogenicity potential, particularly in vulnerable populations like the elderly and immunocompromised individuals.

4. ENTEROCINS: CLASSIFICATION AND SIGNIFICANCE

Enterococci have been thoroughly studied as a potential probiotic candidate. Some desirable qualities in the selection of a probiotic strain include molecular identification, safety, potential to survive the intestinal passage, targeted application, and bacteriocin production [88]. Strains produce a broad range of bacteriocins, which are referred to as enterocins and have been studied widely due to their antimicrobial activity against Gram-positive food-borne pathogens such as *L. monocytogenes* [89]. *E. faecium and E. faecalis* are the main producers of enterocins and to a lesser extent *E. mundtii*, *E. avium*, *E. hirae*, *and E. durans* [90]. The bacteriocins were divided into four groups by Franz *et al.* (2011): Class I lantibiotic enterocins, which are found only rarely in *Enterococci* and are represented only by cytolysin [91] and enterocin W [92] both from *E. faecalis* isolates. The enterocin of this class is two-component bacteriocin made up of two linear peptides that differ structurally from other linear lantibiotics such as nisin A and Z, as well as smaller globular peptides lantibiotics. It contains lanthionine residues, suggesting that these constitute two-component lantibiotics.

Class II, enterocins are of the pediocin family. The Class II.1 of pediocin-like bacteriocins is divided into two subgroups according to sequence similarities, which has two sub groups enterocin A [93], mundticin [94], and enterocin CRL5 [95] and Subgroup 2 includes enterocin P [96] and enterocin M [97] which is a variant of enterocin P. Enterocins lacking a leader peptide, such as two peptide bacteriocin L50 (A, B), are classified as Class II.2, enterocin Q [96], and enterocin C [80]. Different classifications of enterocins produced by Enterococcus species are mentioned in Table 3. Enterocin B falls under Class II three liner-non-pediocin-type enterocins [97]. Enterocin AS-48, produced by *E. faecalis* S-48, is classified as a cyclic antibacterial peptide regroup under class III [98]. The Class IV enterocins include enterolysin A majorly produced by *E. faecalis* [71].

CBT SL-5 bacteriocin lotion generated by *E. faecalis* SL-5 decreased inflammatory lesions due to *Propionibacterium acnes* substantially and suggested a possible function as an alternative to antibiotics during acne therapy [79]. In recent times, the *E. durans* LAB18s strain has been validated to be used as a dietary selenium source [99]. Thirty-eight different *spp*. strains were isolated from the feces of 34 healthy babies and analyzed for virulence genotype and phenotype, biofilm formation, and antibiotic resistance. Ten of the strains were determined to be harmless due to the lack of virulence factors and their susceptibility to conventional antimicrobials. These strains demonstrated good resistance to bile salt and the digestion of the gastrointestinal tract. These bacteria can thus be seen as possible candidates for probiotics [100]. Enterococcal strains, such *E. faecium* SF-68 and *E. faecium* M74, were used in various probiotic products that proved to be efficacious and safe, such as FortiFlora® and Cernivet® (which has *E. faecium* SF68) and Symbioflor® (has *E. faecalis*) and are included as dietary supplements [27]. In a study by Bhardwaj *et al.* (2010), *E. faecium* KH 24 strain was evaluated for the presence of virulence determinants and bacteriocin production, the strain was found to be non-virulent and a bacteriocin producing strain, which was confirmed by a study conducted on mice group which were fed with *E. faecium* KH 24, the mice showed prominent weight gain and nearly 1 log cfu/g decrease in *Salmonella enteritidis* count in the intestines. Decreased coliform counts and an increase in *lactobacilli* growth were observed in the test group [101]. *Enterococcus* strains can survive, compete with, and adhere to host cells in the GIT as natural inhabitants, which is a significant trait for their usage as probiotics [102]. Antibiotics have raised concerns about antimicrobial resistance, thus bacteriocins are a promising alternative [103].

E. lactis, which generates the enterocins A, B, and/or P, was isolated from fresh shrimps (*Penaeus vannamei* and *Palaemon serratus*) and identified as *E. lactis* [104]. For the reason of the presence of enterocins, *E. lactis* showed antibacterial activity ($P < 0.05$) against Gram-positive and Gram-negative foodborne pathogens (*Listeria monocytogenes* and *Pseudomonas aeruginosa*) and some filamentous fungi (*Aspergillus niger* A79) [105]. This might be employed as a safe natural preservative or as a novel probiotic strain in food and feed.

The genus also comprises a variety of strains that can be used as starter cultures and help to create the unique organoleptic qualities of many fermented foods, such as meat, dairy, and vegetable products [26]. Furthermore, the ability of *Enterococcus* strains' bacteriocins to kill competitors is regarded as a successful method for maintaining population and decreasing competitor numbers [105]. These

bacteriocins are being considered promising drug candidates for replacing antibiotics to treat multiple drugs resistant pathogens and maintain human health. Bacteriocins are shown to have synergistic or additive effects when used in combination with other antimicrobial drugs, opening up new possibilities for more effective pathogen control in human and veterinary medicine [103].

By saying that, in the case of feed or novel food, the safety evaluation of candidates at the species level is obligatory before commercialization. The recent molecular biology progressions have revealed that Enterococcal food strains are safe and differentiable by the virulence and antibiotic gene resistance from nosocomial strains [106]. This will enable the safety evaluation of *Enterococci* used in food and feed to be improved.

5. PROBIOTIC POTENTIAL OF *ENTEROCOCCUS*

Many studies have been conducted to assess the probiotic qualities of Enterococcal strains, with clear evidence of positive and significant health effects. *Enterococci* were employed as probiotic drugs for a diversity of applications, including in pharmaceutical, human, veterinary, and food industries. The various probiotic applications of *Enterococcus* spp. are reviewed in the following sections.

Administration of *E. faecium* L3 and *B. animalis* subsp. *lactis* BB12 (iNatalPed®) to atopic children significantly reduced rhinitis, watery eyes, and cough/bronchospasm with the use of oral antihistamines, and inhaled corticosteroids and oral corticosteroids [107]. In a randomized study involving 94 healthy children, probiotic formulations containing *B. animalis* subspecies *lactis* BB-12 and *E. faecium* L3 reduced the incidence and duration of acute gastroenteritis (AGE) by 82% and 45%, respectively, and the onset of upper respiratory tract infections (URTIs) by 84% and 50%. Salivary IgA levels were shown to be higher in a subset of 34 healthy, treated children [108].

5.1. Antibiotic-associated Diarrhea (AAD) and Acute Diarrhea

Borgia *et al.* (1982) were one of the pioneering groups to accomplish a controlled clinical study on the effectiveness of probiotics having *Enterococcus* spp., as one of the constituents. Only 3% of the people in the study group reported the development of AAD in contrast to 18% who reported AAD after receiving a placebo [109]. Wunderlich *et al.* (1989) did a similar placebo-controlled double-blind clinical study on the effectiveness of lactic acid-producing strain *E. faecium* SF 68 in treating AAD and Acute diarrhea. The limitation of this study was a lack of a considerably high success rate, with a success rate of only 27.2% in the verum group as opposed to the 8.7% in the study group receiving the placebo among 78 patients multi-centered across 10 locations treated thrice a day for 7 days [110].

E. faecium SF68 was also the subject of a meta-analysis study by D'Souza *et al.* (2002) in testing the efficacy of probiotic treatment for preventing diarrhea. An odds ratio of 0.37 was reported in this study supporting probiotic treatment against placebo administration [111]. The "Biothree" probiotic combination contains distinct probiotic strains *E. faecalis*, *Clostridium butyricum,* and *Bacillus mesentericus*. It was proven to be beneficial in reducing the intensity and length of diarrhea in children (n = 304) aged 3 months to 6 years [112].

Recently, a growing number of investigations are being conducted on the effect of *Enterococcus* on piglets. One such double-blind placebo-controlled study by Zeyner and Boldt concluded that *E. faecium* DSM 10663 administered orally reduced the occurrence of diarrhea by 40% as opposed to only 14.8% in the placebo group [113].

5.2. Irritable Bowel Syndrome (IBS)

The administration of probiotics is known to change the gastrointestinal micro population. Fan *et al.* (2006) reported that this change could potentially lower the symptoms of IBS by competitively excluding the causative food pathogens based on the outcomes of a clinical study among 85 test subjects [114]. However, a major drawback of this study was that it was not being placebo-controlled and double-blinded. A well-known freeze-dried *E. faecium* containing probiotic called "Paraghurt" was clinically checked in a placebo-controlled double-blind study among 54 patients in a 4-week span and found to be an effective therapeutic for IBS [115].

A similar but more extensive study by Enck *et al.* (2008) tested the efficacy of ProSymbioflor® containing *E. faecalis* DSM 16440 along with *E. coli* DSM 17252 as an autolysate of cells and cell fragments in the treatment of IBS among 297 patients in 8 weeks and yielded sufficiently positive results [116]. A meta-analysis on the effectiveness of *Enterococcus* over placebo in treating IBS by McFarland and Dublin has also featured both of the above-stated clinical studies [117]. Medilac DS® containing *E. faecium* along with *Bacillus subtilis* has also been analyzed in a placebo-controlled and double-blind study among 40 patients by Kim *et al.* (2006) and has shown encouraging results against IBS [118]. Suvorov *et al.* (2010) have recently studied the efficacy of treating 76 IBS patients with auto-probiotic *Enterococcus* strains and examining their stool samples with that of the healthy volunteers for the presence of Enterococcal strains. The outcomes of the investigation conclusively proved that the auto-probiotic Enterococcal strains not only cured IBS but also acted on the dysbiotic microbiome of the patients and restored autochthonous colonies that were comparable to the samples from the healthy volunteers [119]. *E. durans* strain M4 along with its metabolic butyrate was found to have significant anti-inflammatory effects mediated by the regulation of the anti-inflammatory cytokine IL-10 and pro-inflammatory immune factors (IL-8, IL-6, and TNF-α) as well as the preservation of intestinal epithelial integrity. This signifies that it could be a beneficial prophylactic treatment strategy to alleviate inflammatory bowel diseases (IBDs) [120].

5.3. Cholesterol Reduction/Assimilation

In Denmark, fermented milk containing *E. faecium* SF 68 (Gaio®; MD Foods, Aarhus, Denmark) was used for several years due to its hypocholesterolemic influence on individuals. Agerbaek *et al.* (1995) conducted a placebo-controlled, 6-week randomized, and double-blind trial on male volunteers and found a significant reduction in cholesterol (−0.37 mmol/l, confidence interval: [−0.51]–[−0.23]) in the group given biologically fermented milk (GIAO, Denmark), whereas no changes (−0.02 mmol/l) ($P < 0.01$) were witnessed in the placebo group. The drop in cholesterol was attributed to a 10% fall in LDL cholesterol. However, HDL-cholesterol and triglyceride remained unaffected in both groups [121]. In another 8-week, a placebo-controlled, randomized, and double-blind study led by Agerholm-Larsen *et al.* (2000) a yogurt fermented with one strain of *E. faecium* and two strains of *Streptococcus thermophilus* (CAUSIDO culture), Gaio® was administered to one among five groups. The CAUSIDO®, the culture showed reduction in LDL-cholesterol and increased fibrinogen overnight at a 450 mL of yogurt given daily for 8 weeks [122]. The long-term effect of a probiotic was studied in a new placebo-controlled and double-blind trial after 50 weeks, *E. faecium* M-74 indicated a comprehensive cholesterol-lowering effect, notably on LDL cholesterol, while there was no variation in HDL cholesterol, triglycerides, or the placebo group [123]. Through its ability to reduce

human blood cholesterol levels, *E. durans* KLDS 6.0930 has been proposed as a probiotic candidate [124].

E. lactis BT 16 and *E. faecium* VC 223 were two of the 58 strains isolated from traditional Italian cheeses that reduced cholesterol levels in broth, implying that these strains could be potential candidates for novel probiotic-containing formulations [125]. Oral treatment of *E. faecalis* ATCC19433 altered the composition of gut microbiota and raised the counts of *Lactobacillus*, *Bifidobacterium,* and *Akkermansia*. Hypocholesterolemic impact on the hypercholesterolemic mouse was also exerted through enhancing ATP-binding ABCG5 and ABCG8 transport carriers [126]. A strain of *E. faecium* isolated from rhizospheric soils possessed the *bsh* gene (Bile salt hydrolase), reduced cholesterol *in vitro*, and possessed necessary and desired probiotic properties [127]. Similarly, a BSH positive strain of *E. lactis* (Assigned reference number - MTCC 25438) was isolated from goat milk and has shown significant cholesterol reduction *in vitro* (data not published yet).

5.4. Antioxidant, Anti-inflammatory, and Anticancer Properties of *Enterococcus* Species

E. faecium was tested for antioxidant and anti-inflammatory activities in a study by Prapulla *et al.* (2015). In lyophilized cell-free supernatant (LPS)-stimulated macrophage cell lines, a combination of *E. faecium* CFR 3003*, Lactobacillus rhamnosus* GG MTCC 1408, and LPS demonstrated anti-inflammatory impact through negative modulation of TNF-α and upregulation of IL-10 which confirmed the strain possessed anti-inflammatory activity. In both animal models and humans, pathogenic microorganisms in the intestine have been shown to play a role in the worsening of IBDs, such as Crohn's disease and ulcerative colitis [128]. Live probiotic bacteria and their metabolites, such as organic acids, are beneficial in the treatment of IBD [73,74]. Probiotic microorganisms have been shown in the past to be effective anti-inflammatory agents in chronic inflammatory diseases [129]. The LPS of *E. faecium* CFR 3003 strain exhibited antioxidant activity which was evaluated by 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity [130].

LCS exhibited the highest reducing ability which indicates that *E. faecium* has the ability to degrade hydroperoxides into hydroxyoctadecadienoic acids [131]. A 6-month, placebo-controlled, and double-blind trial was conducted with the probiotic using *E. faecalis* Symbioflor I® which is used for the treatment of chronic recurrent bronchitis, and it was found that the probiotic was substantially more effective than the placebo in terms of clinical efficacy. In the group which has received the probiotic, that is, verum group, the time for relapses was much longer and less frequent, relapses took much longer, and the frequency and severity of relapses were much lower [132]. A further double-blind, placebo-controlled, and multicenter research established that patients provided with *E. faecalis* Symbioflor I® had a statistically noteworthy decline in the incidence of persistent chronic and hypertrophic sinusitis [132]. Stockert *et al.* (2007) researched whether therapy with laser acupunction and probiotic (*E. faecal* Symbioflor I®) as a therapeutic routine would enhance therapeutic effectiveness compared to traditional asthma medical treatment of many school children further by randomized, placebo-controlled, and double-blind study. The outcomes demonstrated that combining laser acupuncture with probiotics had a beneficial influence on bronchial hyperactivity in sporadic or slightly chronic asthma, and hence could be useful to treat acute respiratory disorders.

The antioxidant and effectiveness ability of *E. lactis* Q1 and 4CP3 against the development of biofilms generated by methicillin-resistant *Staphylococcus aureus* (MRSA) strains were also examined, with encouraging findings [134]. The protective impact of probiotic *E.lactis* IITRHR1 toward APAP (an antipyretic/analgesic medication that has been associated with toxicity in overdose) induced liver damage in male Wistar rats was investigated. The probiotic was shown to affect critical apoptotic/anti-apoptotic proteins such as cytochrome-c, Bcl2, Bax, caspase production, and DNA damage [135]. The supernatant of *E.lactis* IW5, a probiotic isolated from human feces, substantially inhibited the development of numerous pathogenic bacteria and lowered the viability of various cancer cells, including HeLa, MCF-7, AGS, HT-29, and Caco-2 [136].

6. CONCLUSION AND FUTURE ASPECTS

Due to safety concerns and a lack of safety expertise, and different requirements, only a small number of Enterococcal probiotics are in the market. The GRAS status for *Enterococcus* has yet to be granted [26]. However, given its positive characteristics, certain Enterococcal strains in the food and/or probiotic industries are often used as starter cultures, cocultures, or protective cultures. The combined feature of being a good probiotic candidate and opportunistic pathogens remains a controversial subject. Pathogenicity, which is dependent on virulence factors and AR genes, is the fundamental issue with *Enterococcus* spp. as probiotics. The most interesting and significant information is that *Enterococci* are not considered food-borne pathogens [136,87]. The rapid evolution of antibiotic resistance in *Enterococci* is likely a factor in their emergence as frequent nosocomial infections, raising the question of how to treat food-borne *Enterococci*. Although molecular typing approaches are still unable to separate food from clinical isolates, no link has yet been established between *Enterococci* consumption and infection. Furthermore, *Enterococci* in cheeses appear to be resistant to most medicines of concern, including gentamycin and vancomycin. There have been no instances of illness caused by any of the *Enterococci* probiotic organisms. *E. faecium* SF68 and *E. faecalis* Symbioflor I® probiotics that are presently available on the market indicate the safety of these Enterococcal organisms [138]. A thorough examination of antibiotic resistance transferability from food *Enterococci* to human *Enterococci* and other pathogens should be part of the future safety concerns. In addition, research is needed to distinguish between pathogenic and non-pathogenic strains, by understanding the mechanisms involved in Enterococcal pathogenesis. Industry, health professionals, and consumers should accept these strains as potential candidates for beneficial applications based on current scientific technology, up-to-date knowledge about *Enterococci* and their properties, appropriate guidance, and relevant legislation to distinguish between pathogenic and safe Enterococcal strains.

7. ACKNOWLEDGMENTS

Dr. Alok Malaviya is thankful to the Vision Group on Science and Technology (VGST), Government of Karnataka, for the generous research grant on Probiotic development (VGST/RGS-F/GRD-894/2019-20/2020-21/198).

8. AUTHORS CONTRIBUTION

All authors made substantial contributions with respect to the design, data acquisition, analysis, and interpretation of the manuscript. The final version of the paper has been approved by all authors to be published.

9. CONFLICT OF INTEREST

There are no conflicting interests declared by the authors in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

12. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 2008;111:1-66.
2. Palaniyandi SA, Damodharan K, Suh JW, Yang SH. Probiotic characterization of cholesterol-lowering Lactobacillus fermentum MJM60397. Probiotics Antimicrob Proteins 2020;12:1161-72.
3. Aloğlu HS, Özer ED, Öner Z. Assimilation of cholesterol and probiotic characterisation of yeast strains isolated from raw milk and fermented foods. Int J Dairy Technol 2016;69:63-70.
4. Guan X, Xu Q, Zheng Y, Qian L, Lin B. Screening and characterization of lactic acid bacterial strains that produce fermented milk and reduce cholesterol levels. Braz J Microbiol 2017;48:730-9.
5. Ahn YT, Kim GB, Lim KS, Baek YJ, Kim HU. Deconjugation of bile salts by *Lactobacillus acidophilus* isolates. Int Dairy J 2003;13:303-11.
6. Pan DD, Zeng XQ, Yan YT. Characterisation of Lactobacillus fermentum SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. J Sci Food Agric 2011;91:512-8.
7. El-Shafie HA, Yahia NI, Ali HA, Khalil FA, El-Kady EM, Moustafa YA. Hypocholesterolemic action of *Lactobacillus plantarum* NRRL-B-4524 and *Lactobacillus paracasei* in mice with hypercholesterolemia induced by diet. Aust J Basic Appl Sci 2009;3:218-28.
8. Kim HS, Gilliland SE. *Lactobacillus acidophilus* as a dietary adjunct for milk to aid lactose digestion in humans. J Dairy Sci 1983;66:959-66.
9. Mustapha A, Jiang T, Savaiano DA. Improvement of lactose digestion by humans following ingestion of unfermented acidophilus milk: Influence of bile sensitivity, lactose transport, and acid tolerance of *Lactobacillus acidophilus*. J Dairy Sci 1997;80:1537-45.
10. Spanhaak S, Havenaar R, Schaafsma G. The effect of consumption of milk fermented by Lactobacillus casei strain Shirota on the intestinal microflora and immune parameters in humans. Eur J Clin Nutr 1998;52:899-907.
11. Salminen S, Isolauri E, Salminen E. Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. Antonie Van Leeuwenhoek 1996;70:347-58.
12. Di Felice G, Barletta B, Butteroni C, Corinti S, Tinghino R, Colombo P, *et al.* Use of probiotic bacteria for prevention and therapy of allergic diseases: Studies in mouse model of allergic sensitization. J Clin Gastroenterol 2008;42 Suppl 3 Pt 1:S130-2.
13. Park YH, Kim JG, Shin YW, Kim SH, Whang KY. Effect of dietary inclusion of *Lactobacillus acidophilus* ATCC 43121 on cholesterol metabolism in rats. J Microbiol Biotechnol 2007;17:655-62.
14. Singh TP, Malik RK, Katkamwar SG, Kaur G. Hypocholesterolemic effects of Lactobacillus reuteri LR6 in rats fed on high-cholesterol diet. Int J Food Sci Nutr 2015;66:71-5.
15. De Preter V, Coopmans T, Rutgeerts P, Verbeke K. Influence of long-term administration of lactulose and Saccharomyces boulardii on the colonic generation of phenolic compounds in healthy human subjects. J Am Coll Nutr 2006;25:541-9.
16. Lee DK, Jang S, Baek EH, Kim MJ, Lee KS, Shin HS, *et al.* Lactic acid bacteria affect serum cholesterol levels, harmful fecal enzyme activity, and fecal water content. Lipids Health Dis 2009;8:21.
17. Blanchet-Réthoré S, Bourdès V, Mercenier A, Haddar CH, Verhoeven PO, Andres P. Effect of a lotion containing the heat-treated probiotic strain *Lactobacillus johnsonii* NCC 533 on *Staphylococcus aureus* colonization in atopic dermatitis. Clin Cosmet Investig Dermatol 2017;10:249-57.
18. Won TJ, Kim B, Lim YT, Song DS, Park SY, Park ES, *et al.* Oral administration of Lactobacillus strains from Kimchi inhibits atopic dermatitis in NC/Nga mice. J Appl Microbiol 2011;110:1195-202.
19. Guslandi M, Mezzi G, Sorghi M, Testoni PA. Saccharomyces boulardii in maintenance treatment of Crohn's disease. Dig Dis Sci 2000;45:1462-4.
20. Campieri M, Rizzello F, Venturi A, Poggioli G, Ugolini F. Combination of antibiotic and probiotic treatment is efficacious in prophylaxis of post-operative recurrence of Crohn's disease: A randomized controlled study VS mesalamine. Gastroenterology 2000;118:A781.
21. Appleyard CB, Cruz ML, Isidro AA, Arthur JC, Jobin C, de Simone C. Pretreatment with the probiotic VSL#3 delays transition from inflammation to dysplasia in a rat model of colitis-associated cancer. Am J Physiol Gastrointest Liver Physiol 2011;301:1004-13.
22. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 2007;104:13780-5.
23. Falagas ME, Betsi GI, Athanasiou S. Probiotics for prevention of recurrent vulvovaginal candidiasis: A review. J Antimicrob Chemother 2006;58:266-72.
24. Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. FEMS Immunol Med Microbiol 2001;30:49-52.
25. Hanchi H, Mottawea W, Sebei K, Hammami R. The genus *Enterococcus*: Between probiotic potential and safety concerns-an update. Front Microbiol 2018;9:1791.
26. Franz CM, Huch M, Abriouel H, Holzapfel W, Gálvez A. Enterococci as probiotics and their implications in food safety. Int J Food Microbiol 2011;151:125-40.
27. Ben Braïek O, Smaoui S. Enterococci: Between emerging pathogens and potential probiotics. Biomed Res Int 2019;2019:5938210.
28. Oprea SF, Zevros J, Krishna KV, Koujalagi K, Namratha MP, Ulhas RS, et al. *Enterococcus* and foodborne illness. In: Infectious Disease. Totowa, New Jersey: Humana Press; 2007. p. 157-84.
29. Aarestrup FM, Butaye P, Witte W. Nonhuman Reservoirs of Enterococci. Washington, DC: ASM Press. 2015. p. 55-99.
30. Fanaro S, Chierici R, Guerrini P, Vigi V. Intestinal microflora in early infancy: Composition and development. Acta Paediatr 2003;91:48-55.
31. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010;107:11971-5.
32. Gerald W. Tannock GC. Enterococci as members of the intestinal microflora of humans. In: The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance. Washington, DC: ASM Press; 2002. p. 101-32.
33. Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, *et al.*

Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA J 2017;15:4664.
34. Ogier JC, Serror P. Safety assessment of dairy microorganisms: The Enterococcus genus. Int J Food Microbiol 2008;126:291-301.
35. Mundy LM, Sahm DF, Gilmore M. Relationships between enterococcal virulence and antimicrobial resistance. Clin Microbiol Rev 2000;13:513-22.
36. Bhavnani SM, Drake JA, Forrest A, Deinhart JA, Jones RN, Biedenbach DJ, *et al.* A nationwide, multicenter, case-control study comparing risk factors, treatment, and outcome for vancomycin-resistant and -susceptible enterococcal bacteremia. Diagn Microbiol Infect Dis 2000;36:145-58.
37. Jones ME, Draghi DC, Thornsberry C, Karlowsky JA, Sahm DF, Wenzel RP. Emerging resistance among bacterial pathogens in the intensive care unit a European and North American surveillance study (2000-2002). Ann Clin Microbiol Antimicrob 2004;3:14.
38. Coque TM, Willems RJ, Fortín J, Top J, Diz S, Loza E, *et al.* Population structure of *Enterococcus faecium* causing bacteremia in a Spanish University Hospital: Setting the scene for a future increase in vancomycin resistance? Antimicrob Agents Chemother 2005;49:2693-700.
39. Treitman AN, Yarnold PR, Warren J, Noskin GA. Emerging incidence of *Enterococcus faecium* among hospital isolates (1993 to 2002). J Clin Microbiol 2005;43:462-3.
40. Bouza E, San Juan R, Muñoz P, Voss A, Kluytmans J, Co-operative Group of the European Study Group on Nosocomial Infections. A European perspective on nosocomial urinary tract infections I. Report on the microbiology workload, etiology and antimicrobial susceptibility (ESGNI-003 study). European Study Group on Nosocomial Infections. Clin Microbiol Infect 2001;7:523-31.
41. Huycke MM, Sahm DF, Gilmore MS. Multiple-drug resistant enterococci: The nature of the problem and an agenda for the future. Emerg Infect Dis 1998;4:239-49.
42. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. Clin Microbiol Rev 1994;7:462-78.
43. Wells CL, Jechorek RP, Erlandsen SL. Evidence for the translocation of *Enterococcus faecalis* across the mouse intestinal tract. J Infect Dis 1990;162:82-90.
44. Huycke MM, Spiegel CA, Gilmore MS. Bacteremia caused by hemolytic, high-level gentamicin-resistant *Enterococcus faecalis*. Antimicrob Agents Chemother 1991;35:1626-34.
45. Edmond MB, Ober JF, Dawson JD, Weinbaum DL, Wenzel RP. Vancomycin-resistant enterococcal bacteremia: Natural history and attributable mortality. Clin Infect Dis 1996;23:1234-9.
46. Pelz RK, Lipsett PA, Swoboda SM, Diener-West M, Powe NR, Brower RG, *et al.* Vancomycin-sensitive and vancomycin-resistant enterococcal infections in the ICU: Attributable costs and outcomes. Intensive Care Med 2002;28:692-7.
47. Caballero-Granado FJ, Cisneros JM, Luque R, Torres-Tortosa M, Gamboa F, Díez F, *et al*. Comparative study of bacteremias caused by *Enterococcus* spp. with and without high-level resistance to gentamicin. The Grupo Andaluz para el estudio de las Enfermedades Infecciosas. Clin Microbiol 1998;36:520-5.
48. Mathews FP. Enterococcal endocarditis. Northwest Med 1948;47:581.
49. McDonald JR, Olaison L, Anderson DJ, Hoen B, Miro JM, Eykyn S, *et al.* Enterococcal endocarditis: 107 cases from the international collaboration on endocarditis merged database. Am J Med 2005;118:759-66.
50. Kaçmaz B, Aksoy A. Antimicrobial resistance of enterococci in Turkey. Int J Antimicrob Agents 2005;25:535-8.
51. Endtz HP, van den Braak N, Verbrugh HA, van Belkum A. Vancomycin resistance: Status quo and quo vadis. Eur J Clin Microbiol Infect Dis 1999;18:683-90.
52. Shepard BD, Gilmore MS. Antibiotic-resistant enterococci: The mechanisms and dynamics of drug introduction and resistance. Microbes Infect 2002;4:215-24.
53. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin Microbiol Rev 2000;13:686-707.
54. Gelsomino R, Vancanneyt M, Cogan TM, Condon S, Swings J. Source of enterococci in a farmhouse raw-milk cheese. Appl Environ Microbiol 2002;68:3560-5.
55. Ghrairi T, Manai M, Berjeaud JM, Frère J. Antilisterial activity of lactic acid bacteria isolated from rigouta, a traditional Tunisian cheese. J Appl Microbiol 2004;97:621-8.
56. Morandi S, Cremonesi P, Povolo M, Brasca M. Enterococcus lactis sp. nov., from Italian raw milk cheeses. Int J Syst Evol Microbiol 2012;62:1992-6.
57. Gaaloul N, ben Braiek O, Hani K, Volski A, Chikindas ML, Ghrairi T. Isolation and characterization of large spectrum and multiple bacteriocin-producing *Enterococcus faecium* strain from raw bovine milk. J Appl Microbiol 2015;118:343-55.
58. Kadri Z, Spitaels F, Cnockaert M, Praet J, El Farricha O, Swings J, *et al. Enterococcus bulliens* sp. nov., a novel lactic acid bacterium isolated from camel milk. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 2015;108:1257-65.
59. El Hatmi H, Jrad Z, Oussaief O, Nasri W, Sbissi I, Khorchani T, *et al.* Fermentation of dromedary camel (*Camelus dromedarius*) milk by *Enterococcus faecium*, *Streptococcus macedonicus* as a potential alternative of fermented cow milk. LWT Food Sci Technol 2018;90:373-80.
60. Tezel BU. Preliminary *in vitro* Evaluation of the probiotic potential of the bacteriocinogenic strain *Enterococcus lactis* PMD74 isolated from ezine cheese. J Food Qual 2019;2019:4693513.
61. Barakat RK, Griffiths MW, Harris LJ. Isolation and characterization of Carnobacterium, Lactococcus, and Enterococcus spp. from cooked, modified atmosphere packaged, refrigerated, poultry meat. Int J Food Microbiol 2000;62:83-94.
62. Barbosa J, Ferreira V, Teixeira P. Antibiotic susceptibility of enterococci isolated from traditional fermented meat products. Food Microbiol 2009;26:527-32.
63. Cai Y, Suyanandana P, Saman P, Benno Y. Classification and characterization of lactic acid bacteria isolated from the intestines of common carp and freshwater prawns. J Gen Appl Microbiol 1999;45:177-84.
64. Campos CA, Rodríguez Ó, Calo-Mata P, Prado M, Barros-Velázquez J. Preliminary characterization of bacteriocins from *Lactococcus lactis*, *Enterococcus faecium* and *Enterococcus mundtii* strains isolated from turbot (*Psetta maxima*). Food Res Int 2006;39:356-64.
65. Sarra M, Taoufik G, Patrick LC, Benjamin B, Yannick F, Khaled H. Isolation and characterization of enterococci bacteriocinic strains from Tunisian fish viscera. Food Nutr Sci 2013;4:701-8.
66. Chajecka-Wierzchowska W, Zadernowska A, Łaniewska-Trokenheim Ł. Virulence factors, antimicrobial resistance and biofilm formation in *Enterococcus* spp. isolated from retail shrimps. LWT 2016;69:117-22.
67. El-Jeni R, El Bour M, Calo-Mata P, Böhme K, Fernández-No IC, Barros-Velázquez J, *et al. In vitro* probiotic profiling of novel *Enterococcus faecium* and Leuconostoc mesenteroides from Tunisian freshwater fishes. Can J Microbiol 2016;62:60-71.
68. Ben Braïek O, Morandi S, Cremonesi P, Smaoui S, Hani K, Ghrairi T. Biotechnological potential, probiotic and safety properties of newly isolated enterocin-producing *Enterococcus lactis* strains. LWT Food Sci Technol 2018;92:361-70.
69. Navarro F, Courvalin P. Analysis of genes encoding D-alanine-D-alanine ligase-related enzymes in *Enterococcus casseliflavus* and Enterococcus flavescens. Antimicrob Agents Chemother 1994;38:1788-93.
70. Samyn B, Martinez-Bueno M, Devreese B, Maqueda M, Gálvez A,

Valdivia E, *et al.* The cyclic structure of the enterococcal peptide antibiotic AS-48. FEBS Lett 1994;352:87-90.
71. Nilsen T, Nes IF, Holo H. Enterolysin A, a cell wall-degrading bacteriocin from *Enterococcus faecalis* LMG 2333. Appl Environ Microbiol 2003;69:2975-84.
72. Salvucci E, Saavedra L, Hebert EM, Haro C, Sesma F. Enterocin CRL35 inhibits Listeria monocytogenes in a murine model. Foodborne Pathog Dis 2012;9:68-74.
73. Izquierdo E, Wagner C, Marchioni E, Aoude-Werner D, Ennahar S. Enterocin 96, a novel class II bacteriocin produced by *Enterococcus faecalis* WHE 96, isolated from Munster cheese. Appl Environ Microbiol 2009;75:4273-6.
74. Knoetze H, Todorov SD, Dicks LM. A class IIa peptide from Enterococcus mundtii inhibits bacteria associated with otitis media. Int J Antimicrob Agents 2008;31:228-34.
75. Svetoch EA, Eruslanov BV, Perelygin VV, Mitsevich EV, Mitsevich IP, Borzenkov VN, *et al.* Diverse antimicrobial killing by *Enterococcus faecium* E 50-52 bacteriocin. J Agric Food Chem 2008;56:1942-8.
76. Sánchez J, Diep DB, Herranz C, Nes IF, Cintas LM, Hernández PE. Amino acid and nucleotide sequence, adjacent genes, and heterologous expression of hiracin JM79, a sec-dependent bacteriocin produced by *Enterococcus hirae* DCH5, isolated from Mallard ducks (*Anas platyrhynchos*). FEMS Microbiol Lett 2007;270:227-36.
77. Line JE, Svetoch EA, Eruslanov BV, Perelygin VV, Mitsevich EV, Mitsevich IP, *et al.* Isolation and purification of enterocin E-760 with broad antimicrobial activity against gram-positive and gram-negative bacteria. Antimicrob Agents Chemother 2008;52:1094-100.
78. Varankovich NV, Nickerson MT, Korber DR. Probiotic-based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. Front Microbiol 2015;6:685.
79. Kang BS, Seo JG, Lee GS, Kim JH, Kim SY, Han YW, *et al.* Antimicrobial activity of enterocins from *Enterococcus faecalis* SL-5 against Propionibacterium acnes, the causative agent in acne vulgaris, and its therapeutic effect. J Microbiol 2009;47:101-9.
80. Maldonado-Barragán A, Caballero-Guerrero B, Jiménez E, Jiménez-Díaz R, Ruiz-Barba JL, Rodríguez JM. Enterocin C, a class IIb bacteriocin produced by *E. faecalis* C901, a strain isolated from human colostrum. Int J Food Microbiol 2009;133:105-12.
81. Mareková M, Lauková A, Skaugen M, Nes I. Isolation and characterization of a new bacteriocin, termed enterocin M, produced by environmental isolate *Enterococcus faecium* AL41. J Ind Microbiol Biotechnol 2007;34:533-7.
82. Shastry RP, Arunrenganathan RR, Rai VR. Characterization of probiotic *Enterococcus lactis* RS5 and purification of antibiofilm enterocin. Biocatal Agric Biotechnol 2021;31:3-9.
83. Ben Braïek O, Cremonesi P, Morandi S, Smaoui S, Hani K, Ghrairi T. Safety characterisation and inhibition of fungi and bacteria by a novel multiple enterocin-producing *Enterococcus lactis* 4CP3 strain. Microb Pathog 2018;118:32-8.
84. Casadewall B, Courvalin P. Characterization of the vanD glycopeptide resistance gene cluster from *Enterococcus faecium* BM4339. J Bacteriol 1999;181:3644-8.
85. McKessar SJ, Berry AM, Bell JM, Turnidge JD, Paton JC. Genetic characterization of vanG, a novel vancomycin resistance locus of *Enterococcus faecalis*. Antimicrob Agents Chemother 2000;44:3224-8.
86. Fines M, Perichon B, Reynolds P, Sahm DF, Courvalin P. VanE, a new type of acquired glycopeptide resistance in *Enterococcus faecalis* BM4405. Antimicrob Agents Chemother 1999;43:2161-4.
87. Kanemitsu K, Nishino T, Kunishima H, Okamura N, Takemura H, Yamamoto H, *et al.* Quantitative determination of gelatinase activity among enterococci. J Microbiol Methods 2001;47:11-6.
88. Dobson A, Cotter PD, Paul Ross R, Hill C. Bacteriocin production: A probiotic trait? Appl Environ Microbiol 2012;78:1-6.
89. Izquierdo E, Marchioni E, Aoude-Werner D, Hasselmann C, Ennahar S. Smearing of soft cheese with *Enterococcus faecium* WHE 81, a multi-bacteriocin producer, against Listeria monocytogenes. Food Microbiol 2009;26:16-20.
90. Cotter PD, Hill C, Ross RP. Bacteriocins: Developing innate immunity for food. Nat Rev Microbiol 2005;3:777-88.
91. Cox CR, Coburn PS, Gilmore MS. Enterococcal cytolysin: A novel two component peptide system that serves as a bacterial defense against eukaryotic and prokaryotic cells. Curr Protein Pept Sci 2005;6:77-84.
92. Sawa N, Wilaipun P, Kinoshita S, Zendo T, Leelawatcharamas V, Nakayama J, *et al.* Isolation and characterization of enterocin W, a novel two-peptide lantibiotic produced by *Enterococcus faecalis* NKR-4-1. Appl Environ Microbiol 2012;78:900-3.
93. Aymerich T, Artigas MG, Garriga M, Monfort JM, Hugas M. Effect of sausage ingredients and additives on the production of enterocins A and B by *Enterococcus faecium* CTC492. Optimization of *in vitro* production and anti-listerial effect in dry fermented sausages. J Appl Microbiol 2000;88:686-94.
94. Kawamoto S, Shima J, Sato R, Eguchi T, Ohmomo S, Shibato J, *et al.* Biochemical and genetic characterization of mundticin KS, an antilisterial peptide produced by Enterococcus mundtii NFRI 7393. Appl Environ Microbiol 2002;68:3830-40.
95. Saavedra L, Minahk C, de Ruiz Holgado AP, Sesma F. Enhancement of the enterocin CRL35 activity by a synthetic peptide derived from the NH2-terminal sequence. Antimicrob Agents Chemother 2004;48:2778-81.
96. Cintas LM, Casaus P, Herranz C, Håvarstein LS, Nes IF, Holo H, *et al*. Biochemical and genetic evidence that *Enterococcus faecium* L50 produces enterocins L50A and L50B, the sec-dependent enterocin P, and a novel bacteriocin secreted without an N-terminal extension termed enterocin Q. J Bacteriol 2000;182:6806-14.
97. Casaus P, Nilsen T, Cintas LM, Nes IF, Hernández PE, Holo H. Enterocin B, a new bacteriocin from *Enterococcus faecium* T136 which can act synergistically with enterocin A. Microbiology (Reading) 1997;143 Pt 7:2287-94.
98. Maqueda M, Gálvez A, Bueno MM, Sanchez-Barrena MJ, González C, Albert A, *et al.* Peptide AS-48: Prototype of a new class of cyclic bacteriocins. Curr Protein Pept Sci 2004;5:399-416.
99. Pieniz S, Andreazza R, Anghinoni T, Camargo F, Brandelli A. Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18s. Food Control 2014;37:251-6.
100. Zhang F, Jiang M, Wan C, Chen X, Chen X, Tao X, *et al.* Screening probiotic strains for safety: Evaluation of virulence and antimicrobial susceptibility of enterococci from healthy Chinese infants. J Dairy Sci 2016;99:4282-90.
101. Gupta H, Malik RK. Incidence of virulence in bacteriocin-producing enterococcal isolates. Dairy Sci Technol 2007;87:587-601.
102. Lauková A, Kandričáková A, Buňková L, Pleva P, Ščerbová J. Sensitivity to enterocins of biogenic amine-producing faecal enterococci from ostriches and pheasants. Probiotics Antimicrob Proteins 2017;9:483-91.
103. Hammami R, Fernandez B, Lacroix C, Fliss I. Anti-infective properties of bacteriocins: An update. Cell Mol Life Sci 2013;70:2947-67.
104. Ben Braïek O, Ghomrassi H, Cremonesi P, Morandi S, Fleury Y, Le Chevalier P, *et al.* Isolation and characterisation of an enterocin P-producing Enterococcus lactis strain from a fresh shrimp (Penaeus vannamei). Antonie Van Leeuwenhoek 2017;110:771-86.
105. Yang SC, Lin CH, Sung CT, Fang JY. Antibacterial activities of bacteriocins: Application in foods and pharmaceuticals. Front Microbiol 2014;5:1-10.
106. Montealegre MC, Singh KV, Murray BE. Gastrointestinal tract colonization dynamics by different *Enterococcus faecium* clades.

J Infect Dis 2016;213:1914-22.
107. Di Pierro F, Basile I, Danza ML, Venturelli L, Contini R, Risso P, *et al.* Use of a probiotic mixture containing *Bifidobacterium* animalis subsp. lactis BB12 and *Enterococcus faecium* L3 in atopic children. Minerva Pediatr 2018;70:418-24.
108. DI Pierro F, Lo Russo P, Danza ML, Basile I, Soardo S, Capocasale G, *et al.* Use of a probiotic mixture containing *Bifidobacterium* animalis subsp. lactis BB-12 and *Enterococcus faecium* L3 as prophylaxis to reduce the incidence of acute gastroenteritis and upper respiratory tract infections in children. Minerva Pediatr (Torino) 2021;73:222-9.
109. Borgia M, Sepe N, Brancato V, Borgia R. A controlled clinical study on *Streptococcus faecium* preparation for the prevention of side reactions during long-term antibiotic treatments. Curr Ther Res 1982;31:265-71.
110. Wunderlich PF, Braun L, Fumagalli I, D'Apuzzo V, Heim F, Karly M, *et al.* Double-blind report on the efficacy of lactic acid-producing Enterococcus SF68 in the prevention of antibiotic-associated diarrhoea and in the treatment of acute diarrhoea. J Int Med Res 1989;17:333-8.
111. D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: Meta-analysis. Br Med J 2002;324:1361-4.
112. Wu P, Wan D, Xu G, Wang G, Ma H, Wang T, *et al.* An unusual protector-protégé strategy for the biosynthesis of purine nucleoside antibiotics. Cell Chem Biol 2017;24:171-81.
113. Zeyner A, Boldt E. Effects of a probiotic *Enterococcus faecium* strain supplemented from birth to weaning on diarrhoea patterns and performance of piglets. J Anim Physiol Anim Nutr (Berl) 2006;90:25-31.
114. Fan YJ, Chen SJ, Yu YC, Si JM, Liu B. A probiotic treatment containing Lactobacillus, *Bifidobacterium* and Enterococcus improves IBS symptoms in an open label trial. J Zhejiang Univ Sci B 2006;7:987-91.
115. Gade J, Thorn P. Paraghurt for patients with irritable bowel syndrome. A controlled clinical investigation from general practice. Scand J Prim Health Care 1989;7:23-6.
116. Enck P, Zimmermann K, Menke G, Müller-Lissner S, Martens U, Klosterhalfen S. A mixture of *Escherichia coli* (DSM 17252) and *Enterococcus faecalis* (DSM 16440) for treatment of the irritable bowel syndrome a randomized controlled trial with primary care physicians. Neurogastroenterol Motil 2008;20:1103-9.
117. McFarland LV, Dublin S. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. World J Gastroenterol 2008;14:2650-61.
118. Kim YG, Moon JT, Lee KM, Chon NR, Park H. The effects of probiotics on symptoms of irritable bowel syndrome. Korean J Gastroenterol 2006;47:413-9.
119. Suvorov A, Yermolenko E, Tsapieva A, Sundukova Z, Simanenkov V. Potential of Enterococi Used as Probiotics or Auto-probiotics for the Treatment of Intestinal Disorders. In: GME 2010 Gut Micro-Ecology, Research Gate; 2010. p. 36-7.
120. Avram-Hananel L, Stock J, Parlesak A, Bode C, Schwartz B. E durans strain M4-5 isolated from human colonic flora attenuates intestinal inflammation. Dis Colon Rectum 2010;53:1676-86.
121. Agerbaek M, Gerdes LU, Richelsen B. Hypocholesterolaemic effect of a new fermented milk product in healthy middle-aged men. Eur J Clin Nutr 1995;49:346-52.
122. Agerholm-Larsen L, Raben A, Haulrik N, Hansen AS, Manders M, Astrup A. Effect of 8 week intake of probiotic milk products on risk factors for cardiovascular diseases. Eur J Clin Nutr 2000;54:288-97.
123. Hlivak P, Odraska J, Ferencik M, Ebringer L, Jahnova E, Mikes Z. One-year One-year application of probiotic strain *Enterococcus faecium* M- 74 decreases serum cholesterol levels. Bratisl Lek Listy 2005;106:67-72.
124. Liu F, Li B, Du J, Yu S, Li W, Evivie SE, *et al.* Complete genome sequence of *Enterococcus durans* KLDS6.0930, a strain with probiotic properties. J Biotechnol 2016;217:49-50.
125. Albano C, Morandi S, Silvetti T, Casiraghi MC, Manini F, Brasca M. Lactic acid bacteria with cholesterol-lowering properties for dairy applications: *In vitro* and *in situ* activity. J Dairy Sci 2018;101:10807-18.
126. Zhu Y, Li T, Din AU, Hassan A, Wang Y, Wang G. Beneficial effects of *Enterococcus faecalis* in hypercholesterolemic mice on cholesterol transportation and gut microbiota. Appl Microbiol Biotechnol 2019;103:3181-91.
127. Singhal N, Maurya AK, Mohanty S, Kumar M, Virdi JS. Evaluation of bile salt hydrolases, cholesterol-lowering capabilities, and probiotic potential of *Enterococcus faecium* isolated from rhizosphere. Front Microbiol 2019;10:1567.
128. Balish E, Warner T. *Enterococcus faecalis* induces inflammatory bowel disease in interleukin-10 knockout mice. Am J Pathol 2002;160:2253-7.
129. Baroja ML, Kirjavainen PV, Hekmat S, Reid G. Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients. Clin Exp Immunol 2007;149:470-9.
130. Divyashri G, Krishna G, Muralidhara, Prapulla SG. Probiotic attributes, antioxidant, anti-inflammatory and neuromodulatory effects of *Enterococcus faecium* CFR 3003: *In vitro* and *in vivo* evidence. J Med Microbiol 2015;64:1527-40.
131. Gardner HW. Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. J Agric Food Chem 1975;23:129-36.
132. Habermann W, Zimmermann K, Skarabis H, Kunze R, Rusch V. Einfluß eines bakteriellen immunstimulans (humane *Enterococcus faecalis*-Bakterien) auf die rezidivhäufigkeit bei patienten mit chronischer bronchitis. Arzneimittelforschung 2011;51:931-7.
133. Habermann W, Zimmermann K, Skarabis H, Kunze R, Rusch V. Verminderung der rezidivhäufigkeit bei patienten mit chronisch rezidivierender hypertrophischer sinusitis unter behandlung mit einem bakteriellen immunstimulans (*Enterococcus faecalis*-Bakterien humaner Herkunft). Arzneimittelforschung 2011;52:622-7.
134. Ben Braïek O, Merghni A, Smaoui S, Mastouri M. *Enterococcus lactis* Q1 and 4CP3 strains from raw shrimps: Potential of antioxidant capacity and anti-biofilm activity against methicillin-resistant *Staphylococcus aureus* strains. LWT 2019;102:15-21.
135. Sharma S, Chaturvedi J, Chaudhari BP, Singh RL, Kakkar P. Probiotic *Enterococcus lactis* IITRHR1 protects against acetaminophen-induced hepatotoxicity. Nutrition 2012;28:173-81.
136. Nami Y, Haghshenas B, Haghshenas M, Abdullah N, Yari Khosroushahi A. The prophylactic effect of probiotic Enterococcus lactis IW5 against different human cancer cells. Front Microbiol 2015;6:1317.
137. Su YA, Sulavik MC, He P, Makinen KK, Makinen PL, Fiedler S, *et al.* Nucleotide sequence of the gelatinase gene (gelE) from *Enterococcus faecalis* subsp. liquefaciens. Infect Immun 1991;59:415-20.
138. Domann E, Hain T, Ghai R, Billion A, Kuenne C, Zimmermann K, *et al.* Comparative genomic analysis for the presence of potential enterococcal virulence factors in the probiotic *Enterococcus faecalis* strain Symbioflor 1. Int J Med Microbiol 2007;297:533-9.

How to cite this article:
Krishna KV, Koujalagi K, Surya RU, Namratha MP, Malaviya A. *Enterococcus* species and their probiotic potential: Current status and future prospects. J App Biol Biotech. 2023;11(1):36-44.
DOI: 10.7324/JABB.2023.110105-1