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

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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 of all strains are the most common genera of probiotic organisms. 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, cheese, fermented milk, Kounniss, and yogurt. Different research studies have indicated that probiotic bacteria may lighten lactose intolerance, have a helpful impact on the gut flora of the host, stimulate/control mucosal immunity, lessen inflammatory or allergic reactions, lessen blood cholesterol, possess anti-colon cancer effects, reduce the clinical manifestations of atopic dermatitis, Crohn’s disease, candidiasis, and urinary tract infections.

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. 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 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
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 (*vanA, vanB, vanC, vanD, vanE, and vanG*). The *vanA* operon in strains presents a high-level resistance to vancomycin and teicoplanin. With minimum inhibitory concentrations between 4 and 1000 mg/mL, the *vanB* operon incites different levels of resistance to vancomycin. Only *vanA* and *vanB* have the ability to transfer vertically and horizontally as well as impart high resistance levels [53]. *vanC* 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].

<table>
<thead>
<tr>
<th>Lactobacillus strains</th>
<th>Bifidobacterium strains</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>B. bifidum</td>
<td>E. faecalis</td>
</tr>
<tr>
<td>L. amylovorus</td>
<td>B. infantis</td>
<td>E. faecium</td>
</tr>
<tr>
<td>L. brevis</td>
<td>B. lactis</td>
<td>E. durans</td>
</tr>
<tr>
<td>L. bulgaricus</td>
<td>B. breve</td>
<td>Streptococcus bouardii</td>
</tr>
<tr>
<td>L. casei</td>
<td>B. longum</td>
<td>S. thermophilis</td>
</tr>
<tr>
<td>L. casei</td>
<td>B. animalis</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>L. debrueckii</td>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>L. fermentis</td>
<td></td>
<td>Lactococcus lactis</td>
</tr>
<tr>
<td>L. gallinarum</td>
<td></td>
<td>Propionibacteria</td>
</tr>
<tr>
<td>L. helveticus</td>
<td></td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>L. infantis</td>
<td></td>
<td>Saccharomyces bouardii</td>
</tr>
<tr>
<td>L. johnsonii</td>
<td></td>
<td>S. cerevisiae</td>
</tr>
<tr>
<td>L. lactis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. plantarum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. reuteri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. salivarius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. sporogenes</td>
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<td></td>
</tr>
</tbody>
</table>

### Table 2: Different *Enterococcus* strains isolated from various sources.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Source</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecalis</em>, <em>E. casseliflavus</em></td>
<td>Raw-Milk Cheese</td>
<td>[54]</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>Tunisian cheese</td>
<td>[55]</td>
</tr>
<tr>
<td><em>E. lactis</em> sp. nov.</td>
<td>Italian raw milk cheeses</td>
<td>[56]</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Raw bovine milk</td>
<td>[57]</td>
</tr>
<tr>
<td><em>E. hiraessp. nov.</em></td>
<td>Camelus dromedaries</td>
<td>[58]</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>(Camel) milk</td>
<td>[59]</td>
</tr>
<tr>
<td><em>Enterococcus lactis</em></td>
<td>Ezine Cheese</td>
<td>[60]</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>Refrigerated poultry meat</td>
<td>[61]</td>
</tr>
<tr>
<td><em>E. faecalis</em>, <em>E. faecium</em>, <em>E. casseliflavu</em></td>
<td>Fermented meat products</td>
<td>[62]</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Sea water food</td>
<td>[63]</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Cyprinus carpio</td>
<td>Macrobrachium rosenbergii</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Psetta maxima</td>
<td>[64]</td>
</tr>
<tr>
<td><em>E. mundtii</em></td>
<td>Tunisian Fish Viscera</td>
<td>[65]</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>Retail shrimps</td>
<td>[66]</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Tunisian freshwater fishes</td>
<td>[67]</td>
</tr>
<tr>
<td><em>E. lactis</em></td>
<td>Penaeus vannamei</td>
<td>Palaemon serratus</td>
</tr>
</tbody>
</table>
vanD, 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 vanE and vanG operons, which are assumed to be acquired and inducible [85,86]. vanG 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 Enterococcus species [44]. The ability to produce this protease has been proven by the presence of geE 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], mundtici [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 mainly produced by *E. faecalis* [71].

CBB 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 as *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 *Salmonea 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

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

<table>
<thead>
<tr>
<th>Enterocin</th>
<th>Class</th>
<th>Organism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterocin AS-48</td>
<td>Class III</td>
<td><em>E. faecalis</em></td>
<td>[70]</td>
</tr>
<tr>
<td>Enterolisin</td>
<td>Class III</td>
<td><em>E. faecalis LMG 2333</em></td>
<td>[71]</td>
</tr>
<tr>
<td>Enterocin CRL35</td>
<td>Class IIa</td>
<td><em>E. mundtii</em></td>
<td>[72]</td>
</tr>
<tr>
<td>Enterocin 96</td>
<td>Class II</td>
<td><em>E. faecalis WHE96</em></td>
<td>[73]</td>
</tr>
<tr>
<td>ST4SA</td>
<td>Class IIa</td>
<td><em>E. mundtii</em></td>
<td>[74]</td>
</tr>
<tr>
<td>E 50-52</td>
<td>Class IIa</td>
<td><em>E. faecium</em></td>
<td>[75]</td>
</tr>
<tr>
<td>Hiracin JM79</td>
<td>Class II</td>
<td><em>E. hirae DCH5</em></td>
<td>[76]</td>
</tr>
<tr>
<td>E- 760</td>
<td>IIb</td>
<td><em>Enterococcus spp.</em></td>
<td>[77]</td>
</tr>
<tr>
<td>Avicin A</td>
<td>IIa</td>
<td><em>E. avium</em></td>
<td>[78]</td>
</tr>
<tr>
<td>ESL 5</td>
<td>Unknown</td>
<td><em>E. faecalis</em></td>
<td>[79]</td>
</tr>
<tr>
<td>Enterocin C</td>
<td>Class IIb</td>
<td><em>E. faecalis C901</em></td>
<td>[80]</td>
</tr>
<tr>
<td>Enterocin M</td>
<td>Unknown</td>
<td><em>E. faecium AL41</em></td>
<td>[81]</td>
</tr>
<tr>
<td>Enterocin R5</td>
<td>Class II</td>
<td><em>E. lactis RS 5</em></td>
<td>[82]</td>
</tr>
<tr>
<td>Enterocins A, B and P</td>
<td>Class II</td>
<td><em>E. lactis 4CP3</em></td>
<td>[83]</td>
</tr>
</tbody>
</table>
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 Enterococcus 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 8.3% 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 hypcholesterolemic 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. Hypcholesterolemic 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 1® 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 1® had a statistically noteworthy decline in the incidence of persistent chronic and hypertrophic sinusitis [132]. Stockert et al. (2007) researched whether therapy with laser acupuncture and probiotic (E. faecalis Symbioflor 1®) 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 meticillin-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 1® 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.

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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.

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

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REFERENCES
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.


70. Samyn B, Martínez-Bueno M, Devreese B, Maqueda M, Gálvez A,
The cyclic structure of the enterococcal peptide RS5 and purification of antibiofilm
Isolation and characterization of enterocin W, T136
AL41. J Ind et al.
Isolation and characterisation of an enterocin
88. Dobson A, Cotter PD, Paul Ross R, Hill C. Bacteriocin production:
86. Fines M, Perichon B, Reynolds P, Sahm DF, Courvalin P. VanE,
85. McKessar SJ, Berry AM, Bell JM, Turnidge JD, Paton JC.
84. Casadewall B, Courvalin P. Characterization of the vanD
81. Mareková M, Lauková A, Skaugen M, Nes I. Isolation and
78. Varankovich NV, Nickerson MT, Korber DR. Probiotic-based
75. Svetoch EA, Eruslanov BV, Perelygin VV, Mitsevich EV, Mitsevich IP, Borzenkov VN, et al.
71. Nilsen T, Nes IF, Holo H. Enterolysin A, a cell wall-degrading


