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Original article

Study the effect of nanoparticle probiotic from *Enterococcus faecium* as antibacterial and antibiofilm

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ABSTRACT

Background: Enterococci, known for their importance effect, these bacteria are commonly found in food, environment, as well as the animal and human digestive systems. Nanoparticles (NPs) are increasingly used to target bacteria as an alternative to antibiotics. Probiotic bacteria were increase used as antimicrobial effect so this study were aimed to use the probiotic nanoparticle from strains of *Enterococcus faecium* as antibacterial and antibiofilm. **Methods:** *Enterococcus* were isolated from dairy product after biochemical characterization and vitek identification the isolate includes *Enterococcus faecium*. The isolate underwent for probiotic production and examined as antibacterial effect against *E.coli*, *pseudomonas aeruginosa*, *staphylococcus*, and result declared the inhibitory effect against these bacteria. **Results:** The silver nanoparticles, or AgNPs, such as their optical, electrical, and antibacterial properties. AgNPs have significant antimicrobial activity, prevent resistance from developing, and have a broad synergistic effect when paired with other medications, making them viable candidates in the current antibiotic crisis, which is characterized by a rise in antimicrobial resistance (AMR) and a decline in newly made medications so the result declared their inhibitory effect against *klebsiella* and *Enterococcus*. **Conclusion:** Antibiofilm, on the other hand, were tested against both gram-positive and gram-negative bacteria, and result show more effect against Gram positive than Gram negative bacteria.

Introduction

Probiotics, which contain live bacteria and are marketed as food, drugs, or nutritional supplements, have drawn the attention of both scientists and consumers in recent years. The public's renewed interest in living healthier lives and physicians' acceptance of complementary therapies have brought attention back to the role of human microbiota in illness prevention and treatment [1].

Probiotic bacteria provide several health benefits, such as the capacity to interact with the immune system, alter the host's gut microbiota momentarily, and directly or indirectly affect the autochthonous microflora. Numerous in vitro and in vivo investigations using both conventional and molecular biologic methods [2].

The gastrointestinal system's numerous spaces are home to colonies of microorganisms. The majority of important bacterial communities are found in the colon, where a true symbiotic

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interaction with the host is crucial for general health and wellbeing [3].

The effects of certain probiotic strains on health have recently been thoroughly studied. The efficacy of several probiotics to lessen rotavirus diarrhea and decrease the symptoms of lactose allergy is now well acknowledged. There is a lot of promise in several areas, such allergies treatment and prevention [4].

Supplements containing probiotics may change the gut microbiota's composition and provide a practical means of increasing the gut and systemic immune response, which has been shown to be beneficial by reducing the risk of infection in vulnerable groups. [5;6]. Probiotics are live bacteria that have beneficial medicinal effects [7].

Probiotics are live microbial feed additives that colonize animal intestines, competing with harmful bacteria for nutrition and space while generating antimicrobial compounds to prevent their growth[8]. The predominant intestinal flora in humans and most animals' intestines is *Enterococcus*[9]. Several Enterococcus species have been utilized as probiotics in various nations due to their great capacity to produce bacteriocins [10].

According to [11] Bacteriocins are tiny, ribosomally generated, extracellularly released bacterial peptides or protein molecules that can kill some closely related bacteria. Ribosomal proteins (RPs), in addition to bacteriocin, has antibacterial qualities [12].

Enterococcus faecium, has been identified from a variety of sources, including dairy products, shrimp, and the gastrointestinal systems of mammals [13]. Among its many biological traits are the ability to withstand environments with high bile salt concentrations and strong acids as well as the ability to stop the formation of harmful microorganisms[14].

On the other hand, nanotechnology enables new dietary supplements for particular medicinal applications. Microencapsulating probiotic bacteria in chitosan and Na-alginate nanoparticles improved their capacity to survive and operate in gastrointestinal settings while also increasing their tolerance to gastric conditions (pH 2.0) [15].

Antibiotics, both natural and synthetic, can be become ineffective by bacteria through a variety of methods, requiring to the creation of novel treatments. However, because creating a novel antibiotic is costly and difficult, only a small number of new antibiotics have been marketed recently[16]. Microbial biofilms are of grave health concern worldwide owing to their ability to be resistant to antibiotics, resist host immune response, and combat extreme environmental stress, and their association with persistent infections [17]. This study was aimed to examine the effect of nanobacterial probiotics against different bacterial infections.

Methods:

In the city of Baghdad, numerous samples of regional dairy products were collected.

After being homogenized and diluted in peptone water, product samples were inoculated on the top of chocolate agar and blood agar at 37 °C for 24 hours each, in anaerobic and aerobic environments. Colonies were subjected to the catalase reaction and Gram staining and catalase reaction. Biochemical tests, such as Gram stain, catalase, citrate, generation of H2S and indole, and the isolates' motility, were used to identify the bacterial strains, and VP (Voges Proskauer)[18].

The strain was grown for 24 to 72 hours at 37C in de Man Rogosa and Sharpe (MRS) medium in order to produce probiotics. Following the culture process, the cell-free supernatant (CFS) was obtained by centrifugation and subsequently passed through a 0.22 μm syringe filter to exclude any remaining cells. To quantify the antimicrobial activity, bacterial colony was serially diluted, and 10 μL of each dilution was added to the wells. The highest dilution showing inhibition of the indicator strain. Thus, the arbitrary unit (AU) of antimicrobial activity per milliliter was defined as [19]. Lastly, the clear zones surrounding the wells that contained bacterial growth were used to analyze the antibiotic activity. The clear zones were measured in millimeters and classified as inhibitory zones.

AgNP Synthesis; Making a Silver Nitrate (AgNO3) Solution Extracts from the bacterial production of Enterococcus faecium were added to a 1 mmol/L AgNPs solution and left to incubate for 48 hours. A 1 mM solution was created by dissolving 16.98 g of silver nitrate (AgNO3) in 100 ml of de-ionized distilled water (DW) and adding 1 ml of the obtained AgNO3 to 1000 ml of DW. AgNo3 solution storage. The *Enterococcus faecium* isolate was cultured for three days at 36°C in the laboratory using appropriate fermentation media. Biomass Enterococcus faecium culture washed by centrifugation for 20 minutes at 4500 rpm. One

milliliter of supernatant was utilized to create silver bio nanoparticles, and the biomass was disposed of. A milliliter of the 99 milliliters of AgNO3 solution (1 mM) were mixed with the previous supernatant. By adding enough NaOH, the pH was brought down to 8. Next, for three days at 35°C in the dark, all of the flasks were kept in a shaking water bath at 150 rpm. The emergence of a pale yellowish-brown hue served as an indicator for the generation of Ag bio-NPs. using a spray drier, the Ag bioNPs solution was dried. In order to facilitate further characterization, the dried molecules were finally gathered and conserved (figure 1) [20;21] .

According to [22;23] the most popular technique for evaluating the antibacterial efficacy of probiotic nanoparticles was the agar well diffusion experiment.

The influence of *Enrococcus faisium* extract on each representative strain's ability to build biofilms. The strains that were examined were produced as a cell suspension, and 100 µL of this was put into each 96-well plate. Positive controls in the form of PBS and extract-free media were employed, along with a blank control. The supernatants were disposed of after a 24-hour incubation period at 37 °C and were then PBS-washed three times. The formation of biofilms was measured. Utilizing the formula [1 − (A570 of the test/A570 of the non-treated control)] × 100.

Result and Discussion:

Numerous bacterial species, including Clostridium butyricum, Escherichia coli, Lactococcus lactis, Pediococcus mesenteroides, Enterococcus faecium, Streptococcus thermophilus, Bacillus subtilis, Bacillus coagulans, and Bacillus subtilis, were classified as probiotics[24].

By using biochemical identification, the bacterial isolates were further determined to be *Enterococcus faecium* such as Gram-positive, catalase and oxidase negative, then demonstrated by the Vitek procedure figure (2).

After Lactobacillus and Streptococcus, Enterococcus was the third-largest genus [25]. *Enterococcus* has been found in dairy products, meat, and unleavened vegetables [26; 27; 28]. Enterococcus can be added purposefully or found in fermented foods. It can be used to treat animal or human gastroenteritis and to improve the balance of microorganisms in the gut[28;29; 30].

This study looked at the antibacterial properties of *Enterococcus faecium* probiotics against a variety of bacteria, including *Klebsiella pneumonia, Enterococcus fecalis, Pseudomonas aeruginosa, Actinobacter, Escherichia coli.* The bacterial probiotic exhibits varying inhibitory effects against multiple microorganisms at different concentrations (table 1) when tested using the well diffusion method.

According to [31,32;33] declared that because it contains product against bacteria properties (bacteriocins, fatty acids, and water love biosurfactants) in addition to other potential antimicrobial effects like tryptophan, polyamine, and glutathione metabolites. This is the reason behind the probiotic isolate's antimicrobial effect against various bacterial strains.

Synthesis of silver nanoparticles AgNPs: Extract of *streptococcus faecalis* was used as a reducing agent in conjunction the biological reduction method of the synthesis process. First clue is the development of the yellow hue, which is indicative of the production of silver nanoparticles, as seen in Figure (3). biological reduction is one of the most widely used processes for creating AgNPs because of its simplicity, affordability, and high yield [34].

Transmission Electron Microscope (TEM) analysis: The typical TEM pictures of AgNPs generated using the Green synthesis of nanoparticles method are shown in Figure (4). The TEM images demonstrated the spherical form and sizes of AgNPs, which were 20 nm and10 nm. According to the analysis, the technique can be used to prepare silver nanoparticles with spherical or semi-spherical shapes and a range of sizes depending on the material concentrations used. These particles can overlap to become larger and even microscopic when used with water-polarized electrodes[35].

Spectroscopic Characterization (UV-analysis: Sharp excitation peaks, indicative of nanoparticles energy levels arising from are visible in the UV absorption spectra of AgNPs at 400nm. These intriguing peaks' location and intensity can reveal details about the size, size distribution, and crystallinity of AgNPs Figure (5) [35].

Result (table 2) declared that the nanoparticle probiotic had inhibitory activity against *Klebsiella pneumonia, Enterococcus fecalis, Pseudomonas aeruginosa, Actinobacter, Escherichia coli* with different concentration with high effect at concentration 100 mg as shown in figure (6).

Bacteriocins, a type of antibacterial peptide, may be the primary cause of the inhibitory activity. According to studies by [36] and the supernatant of *E. faecium* strains showed inhibitory action against L. monocytogenes, according to [36].

According to the biofilm results, probiotic nanoparticles exhibited a primary influence on gram positive and gram negative bacteria, with a greater effect on gram positive bacteria as shown in figure(7)table(3).

The outcomes demonstrated that both gram-positive and gram-negative bacteria might be inhibited by this probiotic. It's important to note that a large number of enterococci are capable of producing bacteriocins, which have anti-gram-positive and anti-gram-negative bacterial activity [37,38].

Table 1, Inhibtion zone of different concentration of probiotic agaist different bacterial strain.

Bacterial spp.	Conc.25µg/ml	Cons:50 µg/ml	Conc:100µg/ml
Klebsiella pneumonia	10mm	16mm	18mm
Enterococcus fecalis	9mm	15mm	18mm
Pseudomonas aeruginosa	8mm	13mm	17mm
Actinobacter.	7mm	10mm	14mm
Escherichia coli	8mm	15mm	17mm

Table 2. Antibacterial activity of AgNPs against pathogenic strain (inhibition zone diameter in (mm).

Bacterial strain	Inhibition zone (mm) by (25 µgmL^{-1} AgNP)	Inhibition zone (mm) by (50 µgmL^{-1} AgNP)	Inhibition zone (mm) by (70 µgmL^{-1} AgNP)	Inhibition zone (mm) by (100 µgmL^{-1} AgNP)	**Control+**
Klebsiella pneumonia	12	18.8	24.2	31.2	0
Enterococcus fecalis	11.1	25.9	36	37.6	0
Pseudomonas aeruginosa	12	24	22	33.5	0
Actinobacter	13	25	22	23.5	0
Escherichia coli	14	22	22	34.6	0

Table 3. Anti-biofilm effect of AgNPs (MIC) (50 and 25 µg. mL^{-1}) against biofilm forming by pathogenic bacteria strains using microtiter plate 96 wells.

Treatment Bacteria strain	AgNPs (50 µg.mL^{-1})	AgNPs (25 µg.mL^{-1})	Without AgNPs
Kleibsiella pneumoniae	None (100%)	None (33%)	Strong (100%)(
Enterococcus faecalis	Weak (100%)	Weak (100%)	Strong (100%)
Pseudomonas aeruginosa	None (100%)	None (33%)	Strong (100%)(
Actinobacter	None (100%)	None (33%)	Strong (100%)(
Escherichia coli	Weak (100%)	Weak (100%)	Strong (100%)

Figure 1: Stages of synthesis of nano silver by reducing silver with the bacterial extract of bacteria.

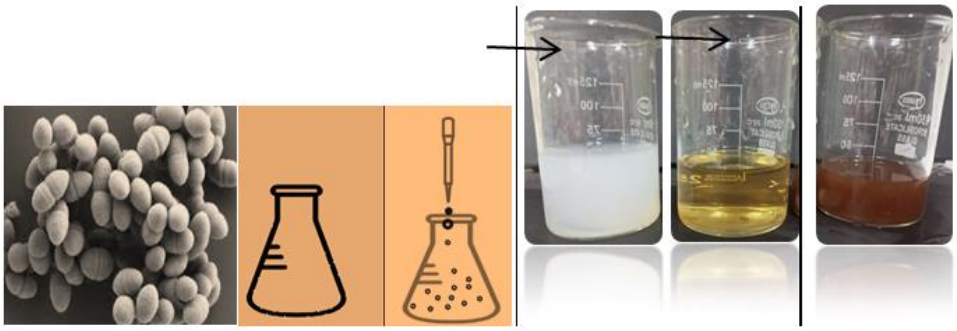

Figure 2. Vitek test for bacterial identification.

Organism Quantity:
Selected Organism : Enterococcus faecium

Source: Collected:

Comments:	

Identification Information	Analysis Time:	4.83 hours	Status:	Final
Selected Organism	94% Probability Bionumber:	Enterococcus faecium 0120020657414231		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
12	APPA	-	14	CDEX	+	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	-	51	dMAN	+	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH1s	-
64	OPTO	+															

Figure3. Synthesis of silver nanoparticles.

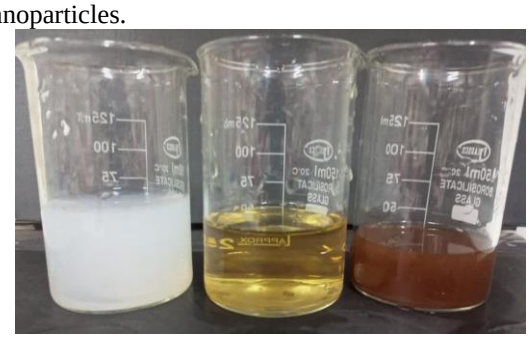

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Figure 4. TEM of AgNPs.

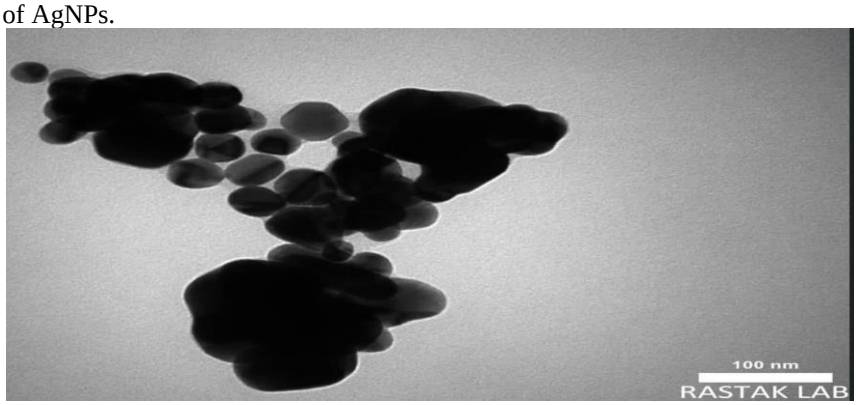

Figure 5. UV spectroscopy of AgNPs.

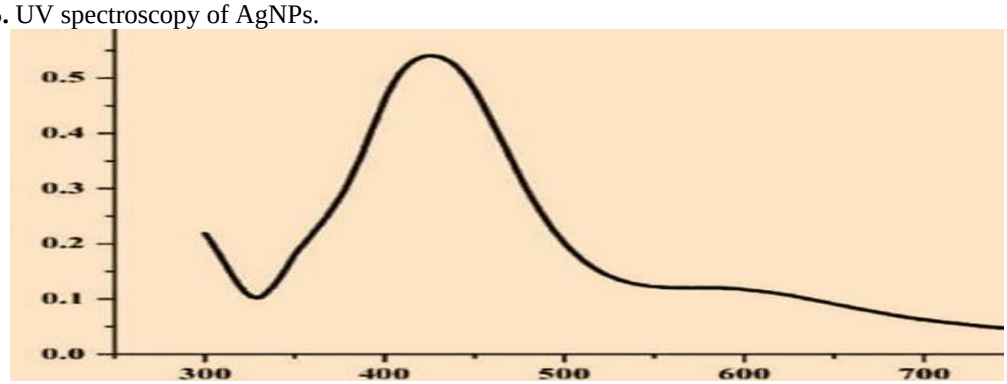

Figure 6. Antibiotic sensitivity of different concentration of probiotic nanoparticle.

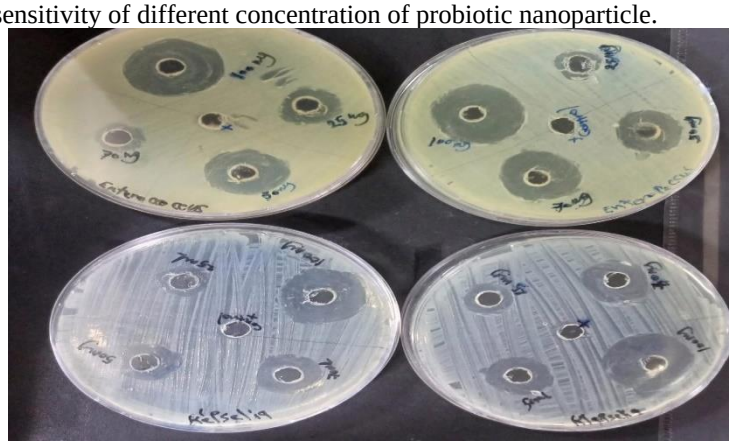

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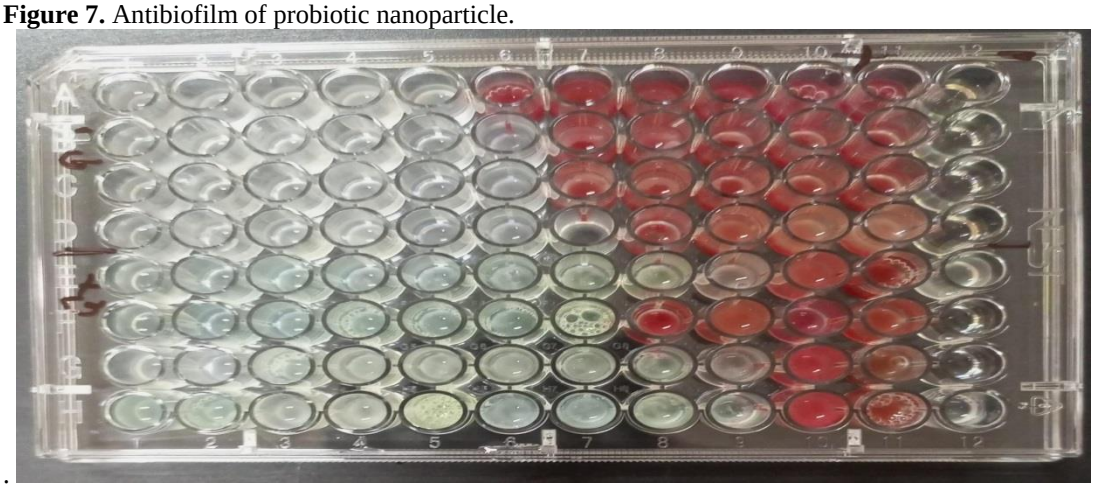
Figure 7. Antibiofilm of probiotic nanoparticle.



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Conflict of interest

The authors report no conflicts of interest

Financial disclosure

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Data availability

All data generated or analyzed during this study are included in this published article.

Authors' contribution

All authors contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data.

References

1- Giovanna B, Davide F, Roberto Di M, Probiotics: overview of microbiological and immunological characteristics. Expert Review of Anti-infective Therapy" 2024; 6(4): 497-508 .

2- Michael de V, Schrezenmeir J(2008). "Probiotics, prebiotics, and synbiotics".Adv Biochem Eng Biotechno.2008;111:1-66.doi: 10.1007/10_2008_097.

3- Marcel R, Glenn R, Lesley H, Anne L, McCartney, Robert Rl, Ian Rowland, Danielle W., et al."Prebiotic effects: metabolic and health benefits". Br J Nutr. 2010; 104 (2):S1-63..doi: 10.1017/S0007114510003363.

4- Ouwehand AC, Salminen S, Isolauri E.Probiotics: an overview of beneficial effects. Antonie van Leeuwenhoek", 2002; 82(1-4):279-289.https://doi.org/10.1023/a:1020620607611 PMID: 12369194 .

5- Nagata S, Asahara T, Wang C, Suyama Y, Chonan O, Takano K, Daibou M, et al."The Effectiveness of Lactobacillus Beverages in Controlling Infections among the Residents of

an Aged Care Facility: A Randomized Placebo-Controlled Double-Blind Trial". Ann. Nutr. Metab. 2016; 68:51–59. doi: 10.1159/000442305.

6- Wang Y, Li X, Ge T, Xiao Y, Liao Y, Cui Y, Zhang Y, et al. "Probiotics for prevention and treatment of respiratory tract infections in children: A systematic review and meta-analysis of randomized controlled trials". Medicine. 2016;95:e4509. doi: 10.1097/MD.0000000000004509.

7- Hiba MN. Maha HT. Ali II. Study the bacterial infection of chronic supportive otitis media from Iraqi patients. Journal of Global Pharma Technology.2018;10(8):146-151.

8- Watson AK. Kespar H. Lategan MJ. Gibson L." Probiotics in aquaculture: the need principles and mechanism of action and screening processes". Aquaculture. 2008;274:1–14.

9- Lebreton F. Manson AL. Saavedra JT. Straub TJ. Earl AM. Gilmore MS."Tracing the Enterococci from paleozoic origins to the hospital". Cell.2017; 169:849–861. doi: 10.1016/j.cell.2017.04.027.

10- [10]Franz CM. Stiles ME. Schleifer KH. Holzapfel WH."Enterococci in food—a conundrum for food safety". Int J Food Microbiol. 2003; 88:105–122. doi: 10.1016/s0168-1605(03)00174-0

11- Ahmadova A. Todorov SD. Choiset Y. Rabesona H. Zadi, TM. Kuliyev A. de Melo Franco BD. "Evaluation of antimicrobial activity, probiotic properties and safety of wild strain Enterococcus faecium AQ71 isolated from Azerbaijani Motal cheese". Food Control 2013;30, 631–641.

12- Ma Z. Qu B. Yao L. Gao Z. Zhang S."Identification and functional characterization of ribosomal protein S23 as a new member of antimicrobial protein". Dev. Comp. Immunol.2020; 103730.

13- Liu YA. Li XR. Zhou JH. Ma LN. Liu YS. "Isolation, identification and drug resistance analysis of pathogenic Enterococcus faecium of sheep". China Anim Husb Vet Med. 2017;44:530–537. doi: 10.16431/j.cnki.1671-7236.2017.02.032.

14- Saelim K. Sohsomboon N. Kaewsuwan S. Maneerat S."Probiotic properties of Enterococcus faecium CE5-1 producing a bacteriocin-like substance and its antagonistic effect against antibiotic-resistant enterococci in vitro". Czech J Anim Sci. 2012;57:529–539. doi: 10.17221/6386-cjas.

15- Chávarri, M. "Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions". Int. J. Food Microbiol. 2010;142, 185–189.

16- Ozdal M. and Gurkok, S. "Recent advances in nanoparticles as antibacterial agent". ADMET DMPK. 2022;10(2): 115–129.Published online 2022 Feb 2. doi: 10.5599/admet.1172.

17- Vasudevan R. "Biofilms: Microbial cities of scientific significance". J. Microbiol. Exp. 2014; 1:84–98.m doi: 10.15406/jmen.2014.01.00014.

18- Dimitrios V. Eugenia B. Chrysa V. Athanasios A. Vasiliki M.Biochemical and antimicrobial profile of Enterococcus faecium and E. faecalis isolated from traditional dairy products and infant intestinal microbiota .Microbial Ecology in Health and Disease. 2009; 241-250.

19- Yamamoto Y. Togawa Y. Shimosaka M. Okazaki M. Purification and characterization of a novel bacteriocin produced by Enterococcus faecalis strain RJ-11. Appl Environ Microbiol. 2003;69:5746–53. doi:

10.1128/AEM.69.10.5546-5553.2003. [DOI] [PMC free article] [PubMed] [Google Scholar]

20- Nawaz H. Arshad U. Ansari F. Shahzadi R. Anjum FR. Bashir F. "Biogenic Synthesis of Silver Nanoparticles and Valuation of their Antimicrobial Activity against Dengue Larvae". J. Pla. Pathol. & Microb.2017; 8(8) 1-5. DOI: 10.4172/2157- 7471.1000418

21- Reverberi AP. Vocciante M. Lunghi E. Pietrelli L. Fabiano B. "New trends in the synthesis of nanoparticles by green methods". Chem. Eng. Trans. 2017; 61 667–72. https://doi.org/ 10.3303/ CET1761109.

22- Torres-Sánchez A. Pardo-Cacho J. López-Moreno A. Ruiz-Moreno Á. Cerk K. Aguilera M."Antimicrobial Effects of Potential Probiotics of Bacillus spp. Isolated from Human Microbiota: In Vitro and In Silico Methods". Microorganisms. 2017;9:1615. doi: 10.3390/microorganisms9081615.

23- Schifano E. Tomassini A. Preziosi A. Montes J.Aureli W., Mancini P., Miccheli A., Uccelletti D. (2021)."Leuconostoc mesenteroides Strains Isolated from Carrots Show Probiotic Features". Microorganisms. 9:2290. doi: 10.3390/microorganisms9112290.

24- Fijan S. Kocbek P. Steyer A. Vodičar PM. Strauss M. "The Antimicrobial Effect of Various Single-Strain and Multi-Strain Probiotics, Dietary. To quantify the antimicrobial activity, CFS was serially diluted, and 10 µL of each dilution was added to the wells. The highest dilution showing inhibition of the indicator strain. Thus, the arbitrary unit (AU) of antimicrobial activity per milliliter was defined as [19]. Supplements or Other Beneficial Microbes against Common Clinical Wound Pathogens". Microorganisms. 2022;10:2518. doi: 10.3390/microorganisms10122518.

25- Atya AK. Drider-Hadiouche K. Ravallec R. Silvain A. Vachee A. Drider D. "Probiotic potential of Enterococcus faecalis strains isolated from meconium". Front. Microbiol. 2015; 6, 227.

26- Gaaloul N. Ben Braiek O. Berjeaud JM. Arthur T. Cavera VL. Chikindas ML. Hani K. et al."Evaluation of Antimicrobial Activity and Safety Aspect of E nterococcus Italicus GGN 10 Strain Isolated from T unisian Bovine Raw Milk". J. Food Saf.. 2014;34, 300–311.

27- Pieniz S. Andreazza R. Okeke BC. Camargo FA. Brandelli A. "Antimicrobial and antioxidant activities of Enterococcus species isolated from meat and dairy products". Braz. J. Biol. 2015;75, 923–931.

28- Saelim K. Sohsomboon N. Kaewsuwan S. Maneerat S. Probiotic properties of Enterococcus faecium CE5-1 producing a bacteriocin-like substance and its antagonistic effect against antibiotic-resistant enterococci in vitro. Czech J Anim Sci; 2012.57:529–539. doi: 10.17221/6386-cjas.

29- Tamang JP. Watanabe K. Holzapfel W."Review: Diversity of microorganisms in global fermented foods and beverages". Front. Microbiol. (2016);7, 377.

30- Giraffa G."Functionality of enterococci in dairy products". Int. J. Food Microbiol. 2003; 88, 215–222.

31- Bhardwaj A. Malik RK. Chauhan P."Functional and safety aspects of enterococci in dairy foods".2008; Indian J. Microbiol. 48, 317–325.

32- Fijan S.Microorganisms with Claimed Probiotic Properties: An Overview of Recent Literature. Int. J. Environ. Res. Public Health.

2014;11:4745–4767. doi: 10.3390/ijerph110504745.

33- Sudan S. Flick R. Nong L. Li J."Potential Probiotic Bacillus subtilis Isolated from a Novel Niche Exhibits Broad Range Antibacterial Activity and Causes Virulence and Metabolic Dysregulation in Enterotoxic E. coli. Microorganisms". 2021;9:1483. doi: 10.3390/microorganisms9071483.

34- Tenea GN. Gonzalez GL. Moreno JL.Probiotic Characteristics and Antimicrobial Potential of a Native Bacillus subtilis Strain Fa17.2 Rescued from Wild Bromelia sp. Flowers. Microorganisms.2022;10:860. doi: 10.3390/microorganisms10050860.

35- Pinzaru I. et al. "Stable PEG-coated silver nanoparticles – A comprehensive toxicological profile," Food and Chemical Toxicology.2018; 111: 546–556, 2018.

36- Riaz M. Mutreja V. Sareen S. Ahmad B. Faheem M. Zahid, N. Jabbour G. et al. "Exceptional antibacterial and cytotoxic potency of monodisperse greener AgNPs prepared under optimized pH and temperature". Scientific Reports.2012; 11(1). https://doi.org/10.1038/s41598-021-82555-z.

37- Kang JH. Lee MS." Characterization of a bacteriocin produced by Enterococcus faecium GM-1 isolated from an infant". J Appl Microbiol.2005; 98:1169–1176.

38- Belguesmia Y. Choiset Y. Prévost H. Dalgalarrondo M. Chobert JM. Drider D."Partial purification and characterization of the mode of action of enterocin S37: a bacteriocin produced by Enterococcus faecalis S37 isolated from poultry feces". J Environ Public Health. 2010;1–8.

39- Suhad HA. Ekhlas SF. Suhaib k I. Biofilm Formation of Staphylococcus Aureus in Multiple Sclerosis Patients and its Essential Role in the Pathogenicity of the Disease. Journal of Techniques, 2022; 4(3), 14–18. https://doi.org/10.51173/jt.v4i3.511.