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A novel perspective for *Lactobacillus reuteri* cell-free supernatant: co-microencapsulation with propolis extracts

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Abstract
Co-microencapsulations of cell-free extract (CFE) obtained from *Lactobacillus reuteri* with propolis water and ethanolic extracts (1% of CFE) were made to investigate their enhanced antibacterial activities against four fish spoilage bacteria (*Pseudomonas luteola*, *Enterobacter cloacae*, *Photobacterium damselae*, and *Proteus mirabilis*). Minimum bactericidal (MBC) and minimum inhibitory (MIC) concentrations of the pure and microencapsulated samples were identified by the broth microdilution method, while agar well diffusion method was used to find inhibition diameter zone of extracts. Chemical content of microencapsulated samples was also detected by gas chromatography-mass spectrometer (GC–MS). GC–MS analysis results showed that microencapsulated CFE from *L. reuteri* in combination with propolis extracts (aqueous and ethanolic) contained organic acids, hydrocarbons, phenolic acids/flavonoids, and benzene derivatives. *P. mirabilis* was the most sensitive fish spoilage bacteria against CFE from *L. reuteri*. Bacteriostatic effect of CFE from *L. reuteri* against *P. mirabilis* and *P. luteola* was enhanced by microencapsulation process. The combination form of microencapsulated CFE from *L. reuteri* with aqueous propolis extract showed a MIC of 25 mg/mL on the growth of *P. mirabilis*. Microencapsulated CFE from *L. reuteri* combined with ethanolic propolis extract completely killed *P. damselae* and *P. luteola* at a level of 50 mg/mL. The study results revealed that the combined use of propolis extracts, especially ethanolic extract with CFE obtained from *L. reuteri* in microencapsulation, generally showed a higher antimicrobial effect on all tested bacteria.

Keywords *Lactobacillus reuteri* · Cell-free extract · Propolis extracts · Fish spoilage bacteria

1 Introduction

The restriction of microbial spoilage and pathogens in foods is a substantial issue because they cause a 25% loss in post-harvest food products [21]. Various synthetic preservatives such as sulfur dioxide, sodium nitrite, and sodium benzoate are generally used to limit nutritional and sensory losses in foods initiated by chemical, microbiological, or enzymatic changes. Due to the health concerns of synthetic antimicrobials [34], natural derivatives from plants, animals, or microorganisms metabolites have shown that natural substances could be used safely as an alternative sources [35, 36].

Propolis is a resin-like compound accumulated by honey bees from various plants and generally stated as safe to consume (GRAS) [5, 42, 46]. Propolis contains various biologically active compounds with antioxidant and antimicrobial properties and therefore can be used in meat products [22]. These biological activities are due to their flavonoid, phenolic acid, terpene, and sesquiterpene contents [9, 31]. Many studies have reported that propolis exhibits broad antimicrobial properties towards most food microorganisms [7, 15, 18, 20]. The antimicrobial effect of propolis depends on seasonal and regional factors, propolis concentrations, and the extraction method used [2, 40].

Lactic acid bacteria (LAB) are usually applied in meat and meat products because of their fermentation activities and probiotic properties. LAB produces bioactive substances such as hydrogen peroxide, diacetyl, organic acids, fatty acids, and bacteriocin that demonstrate antimicrobial activity against destructive and pathogenic microorganisms [45]. Probiotic bacteria are mostly used in the conventional food processing methods, but presently, novel food processing methods are gaining much more importance in the food industry [12]. In this sense, food nanotechnology applications such as nanoparticles, nanoemulsions, nanofibers, or

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nanoencapsulation of valuable biomaterials have a great potential in the fish industry [13, 14]. It has been emphasized that the addition of LAB cell-free extract (CFE) as an antimicrobial in foods could be a more efficient way to control pathogens compared to fresh probiotic extracts [38]. Because it may problematic to use live LAB members with inhibitory properties in refrigerated foods [4].

There are several studies dealing with the improvement of the potential health effects of meat products using live probiotics [10, 41]. However, some probiotic viability losses occur in dried fermented sauces because of factors such as high amounts of salt and nitrite, low pH, and water activity during fermentation and maturation [25]. In order to overcome such problems, encapsulation or immobilization of probiotic bacteria is recommended to protect food and cells in the visceral region. Spray-drying is among the most widely used encapsulation methods recently [11]. The encapsulation process is approved by the food industry in order to bring together biologically active compounds with various food types and protect them from unwanted conditions and therefore enhance the shelf life of the product [19].

It has been indicated that the co-encapsulation of LAB CFE and propolis extracts may be novel antimicrobials in controlling food pathogens since they contain several active components [8, 28]. Although quite a lot of LAB members are used in the food industry, one of the important probiotic bacteria is *Lactobacillus reuteri*, which has functional properties for human health [12]. *L. reuteri* can yield various wide-spectrum antimicrobial compounds such as reuterin, ethanol, and organic acids [32]. For this reason, it is thought that co-microencapsulation of CFE from *L. reuteri* and propolis extract will create a new potential as an antimicrobial agent. In the current study, it was aimed to investigate in vitro antibacterial effects of pure and microencapsulated CFE from *L. reuteri* together with propolis extracts on fish spoilage bacteria including *Proteus mirabilis*, *Pseudomonas luteola*, *Photobacterium damselae*, and *Enterobacter cloacae*.

2 Materials and methods

2.1 Bacterial strains

L. reuteri ATCC 55,730 was provided from American Type Culture Collection (Rockville, MD). Fish spoilage bacteria used were *Pseudomonas luteola*, *Enterobacter cloacae*, *Photobacterium damselae*, and *Proteus mirabilis.* They were obtained and determined from various spoiled fish species which including fresh anchovy, mackerel, and sardine [27]. Fish spoilage bacterial strains and *L. reuteri* were proliferated in Nutrient broth and De Man, Rogosa, and Sharpe (MRS) broth (Merck, Darmstadt, Germany), respectively.

2.2 Extract preparations

CFE from *L. reuteri* was achieved according to procedures described by Lin and Yen [29]. In brief, *L. reuteri* grown in de Man, Rogosa, and Sharpe (MRS, Merck, 1.10661, Darmstadt, Germany) broth medium for 48 h was harvested several periods in deionized water by using centrifugation (Hettich 32R, Tuttlingen, Germany) at 11200 g for 10 min at 4 °C, followed by ultrasonic mixing (Bandelin RK 100, Berlin, Germany).

Propolis was produced by *Apis mellifera* in Adana, Turkey. The extraction of propolis was followed using the method of Burgut [7]. Ethanol (96%, Merck, Darmstadt, Germany) at volume of 70% and water (100%) were added and then, they placed in daily shakeable containers in dark condition. They were stored in a dark place at 4 °C until application.

2.3 Microencapsulation

Encapsulation of CFE from *L. reuteri* and propolis extracts was made according to the procedure of Marcela, Lucía, Esther, and Elena [30] with minor modification. Encapsulation process consisted of three groups, which were the control group that alone encapsulated CFE from *L. reuteri*, and the co-microencapsulation group that encapsulated CFE from *L. reuteri* together with propolis ethanolic or aqueous extract (1%). All samples were coated with 25% maltodextrin (DE:18–20, Alfasol, Turkey) and homogenized by ultra-turrax (IKA T25, Baden-Württemberg, Germany) homogenizer for 10 min. Then, all samples were spray-dried by a mini spray-dryer (Buchi Mini Spray Dryer B-290, Switzerland). The conditions of spray-drying followed: inlet and outlet temperatures of 130 °C and 90 °C, the aspiration rate of 30 m^3/h, and the feeding rate of 20 mL/min. Dried samples were stored in dark conditions at 4 °C, until further analysis. Triplicate samples were taken for all analysis.

2.4 Morphology of microencapsulation

The morphological properties of microencapsulates were characterized by SEM (scanning electron microscope, Quanta 650, USA) in Cukurova University, Adana, Turkey. The morphological characteristics of microencapsulation were detailed in the previous study [8]. Briefly, microcapsules with spherical particles (< 18.5 μm) and irregular shapes as well as water-soluble were obtained (Fig. 1).

2.5 GC–MS analysis of microencapsulated samples

The chemical content of samples was detected by a gas chromatography-mass spectrometer (GC–MS). GC–MS

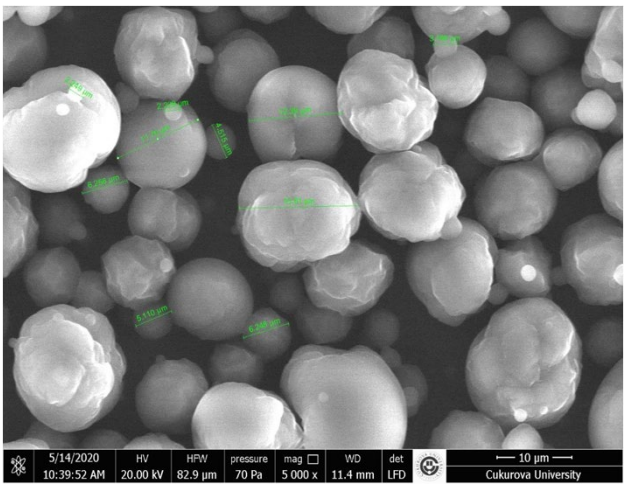


Fig. 1 Scanning electron microscope images of microencapsules of CFE from *L. reuteri*

analysis was made using an Agilent 7890 A gas chromatograph (GC) directly coupled to mass spectrometer (MS) system. A non-polar (5%-phenyl)-methylpolysiloxane column (30 m × 250 µm × 0.25 µm, Agilent 1901S-433HP-5MS) was used. Helium (Linde, Munich, Germany) was the carrier gas, and flow rate was 1.5 mL/min. The initial oven temperature at 50 °C was held for 2 min and reached at 240 °C of final temperature with rate at 2 °C/min. The injection volume was 1 µL of diluted sample in hexane. Total GC running time was 105 min. Detected peaks in GC–MS were checked with those in the commercial library of the NIST/EPA/NIH version 2.0. The relative % amount of each compound was expressed by comparing its average peak area to the total area.

2.6 Antimicrobial activities of pure and microencapsulated extracts

2.6.1 Agar well diffusion method

The antimicrobial activity of extracts was determined using the agar well diffusion method with minor modifications [33]. Control groups consisted of pure CFE from *L. reuteri* and propolis extracts were analyzed at a dose of 100% or 1% of CFE from *L. reuteri*. Microencapsulated samples were dissolved in sterile distilled water (1/2:w/v). Fish spoilage bacteria (10^8 cfu/mL) grown in nutrient broth (Merck 1.05443, Darmstadt, Germany) at 37 °C for 24 h was spread on Mueller–Hinton agar (MHA, Merck 1.05437, Darmstadt, Germany). After that, extract samples at a dose of 50 µ were introduced into the well in MHA plates. Distilled water or maltodextrin were used as control wells. The samples were incubated at 37 °C for 24 h. Triplicate samples were taken for analysis.

2.6.2 Minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of extracts

Broth microdilution method of Clinical and Laboratory Standards Institute [16] was used to determine the MIC and MBC of extracts. Briefly, extract concentrations ranging from 50 to 0.19 mg/mL were prepared in tubes with or without Mueller–Hinton broth (MHB, Merck 1.10293, Darmstadt, Germany). Then, a bacterial inoculum of 1 mL (10^8 cfu/mL) was transferred to each tube. Positive control tubes include MHB and bacterial inoculum without extract. Negative control tubes did not contain MHB. The MIC was assessed by the lowest concentration of the extract that prevented visible growth after incubation of tubes for 18–24 h at 37 °C. MBC was determined by subculturing of tubes of MIC on the MHA plates.

2.7 Statistical analysis

Data were subjected to analysis of variance and Duncan's multiple range tests using the SPSS Version 18.0 with a significant difference at $p < 0.05$.

3 Results and discussion

3.1 GC–MS analysis of microencapsulated samples

The chemical contents of microencapsulated samples are given in Table 1. Microencapsulated CFE from *L. reuteri* included a total of 5 compounds. Among these, dimethoxydimethyl silane (43.77%) and (3E, 5E, 8Z) -3,7,11-trimethyl-1,3,5, 8,10-dodecapentanene (38.11%) were identified as major compounds. Hexadecanoic and octadecanoic acid methyl esters and acetic acid [4-(1,1-dimethylethyl) phenoxy]-methyl ester were also found significant concentrations in the microencapsulated CFE from *L. reuteri*. LAB can produce antimicrobial active metabolites such as hydrogen peroxide, organic acids, and also other compounds, e.g., peptides and bacteriocins [37]. Burgut [8] reported that CFE of *L. reuteri* was rich in organic acids mainly phenols, acetic acid, and hydrocarbons.

Microencapsulated CFE from *L. reuteri* with propolis aqueous extract had a total of 9 chemical compounds, among which 2(1H)-naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha., 7.beta.) (51.47%), and cyclohexane (35.56%) were the compounds with the highest concentrations. In addition to these compounds, 3-oxapentacyclo[6.3.0 (1,3)0.0(2,6)]tetradec-13-ene (4.27%), thiophene-2-carboxylic acid [2-(5-methoxy-2-methyl-1H-indol-3-yl)-ethyl]-amide (2.56%), dimethylphosphine-D1 (2.41%), phenol, 2,2′-[(1-methyl-1,2-ethanediyl)bis(nitrilomethylidyne)] bis-(1.43%), and hexadecanoic acid methyl ester (1.21%)

Table 1 Chemical compositions of microencapsulated CFE from *L. reuteri* with or without propolis extracts

Compounds	ECFE		EPW		EPE	
	RT	%	RT	%	RT	%
Dimethylphosphine-D1	-	-	1.753	2.41	-	-
5-Phenyl-2H-tetrazole	-	-	1.884	0.34	-	-
2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.)	-	-	2.016	51.47	-	-
Cyclopentadienylphenylacetic acid	-	-	–		2.022	47.24
(3E,5E,8Z)-3,7,11-Trimethyl-1,3,5, 8,10-dodecapentanene	2.027	38.11	-	-	-	-
Cyclohexane	-	-	2.393	35.56	2.394	41.86
Dimethoxydimethyl silane	2.394	43.77	-	-	-	-
3-Oxapentacyclo[6.3.0(1,3).0(2,6)]tetradec-13-ene	-	-	2.868	4.27	-	-
Bicyclo[2.2.2]oct-5-ene-2endo,3endo-dimethanol	-	-	-	-	2.880	3.76
2,2-Dimethyl-7-methylthio-2H-chromene	-	-	25.928	0.75	-	-
Hexadecanoic acid, methyl ester	35.655	8.17	35.65	1.21	35.655	4.47
Octadecanoic acid, methyl ester	39.461	6.76	-	-	39.460	2.67
Thiophene-2-carboxylic acid [2-(5-methoxy-2-methyl-1H-indol-3-yl)-ethyl]-amide	-	-	39.609	2.56	-	-
Phenol, 2,2′-[(1-methyl-1,2-ethanediyl)bis(nitrilomethylidyne)]bis-	-	-	45.40	1.43	-	-
Acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester	50.286	3.19	-	-	-	-

RT, retention time;—not detected. *ECFE*, microencapsulated cell-free supernatant from *L. reuteri*; *EPW*, microencapsulated cell-free supernatant from *L.reuteri* in combination with propolis water extract. *EPE*, microencapsulated cell-free supernatant from *L.reuteri* in combination with propolis ethanolic extract

were identified as other important components. The chemical composition of CFE from *L. reuteri* and propolis ethanol extract mixtures was cyclopentadienylphenylacetic acid (47.24%), cyclohexane (41.86), hexadecanoic acid methyl ester (4.47%), bicyclo [2.2.2] oct-5-ene-2endo, 3endo-dimethanol (3.76%), and octadecanoic acid methyl ester (2.67%). Similarly, 2-methoxy-4-vinyl phenol and 2-propen-1-one,1-(2,6-dihydroxy-4-methoxy phenyl)-3-phenyl compounds were reported to be possible markers for the collected Turkish propolis samples [17]. The aqueous extract of propolis samples harvested in Algeria had major compounds of terpenoids, aromatic alcohols, and organic acids [6]. Propolis is known for its rich volatile components, fatty acids, phenolic acids/flavonoids, phenols, alcohols, and esters, organic acids, phenolic aldehydes, ketones, and quinone derivative components [3, 8, 24]. Kuley et al. [28] found microencapsulated cell-free supernatant from *Lactobacillus plantarum* in combination with propolis extracts including mainly alcohol, organic acids, hydrocarbons, and furanone derivatives. Heptacosane, octacosane, and episesamin 1-octacosanol were reported as newly identified compounds in ethanolic extracts of Greek propolis [23]. In this study, GC–MS analysis results showed that microencapsulated CFE from *L. reuteri* in combination with propolis extracts (aqueous and ethanolic) contained organic acids, hydrocarbons, phenolic acids/flavonoids, and benzene derivatives, as described by previous studies.

3.2 Determination of inhibition diameters of extracts

The diameters of the inhibition zone are given in Table 2. There were statistically significant differences in inhibition diameters among the groups ($p < 0.05$).

Table 2 Inhibition diameter zones (mm) of pure and microencapsulated samples against fish spoilage bacteria

Bacteria	CFE	ECFE	PW	EPW	PE	EPE
P. mirabilis	15.50 ± 0.58^{b}	15.50 ± 1.00^{b}	18.50 ± 1.29^{a}	14.00 ± 0.82^{c}	17.50 ± 0.58^{a}	16.00 ± 1.15^{b}
P. luteola	11.00 ± 0.82^{c}	10.75 ± 0.50^{c}	17.50 ± 0.58^{a}	14.00 ± 0.82^{b}	16.25 ± 0.96^{a}	17.00 ± 1.15^{a}
P. damselae	12.75 ± 1.26^{b}	12.25 ± 0.96^{b}	15.75 ± 1.50^{a}	13.50 ± 1.00^{b}	16.25 ± 0.96^{a}	16.00 ± 1.41^{a}
E. faecalis	7.00 ± 0.41^{d}	7.25 ± 0.50^{d}	8.50 ± 0.58^{b}	7.50 ± 0.58^{cd}	8.25 ± 0.50^{bc}	10.33 ± 0.94^{a}

*Mean ($n = 3$) ± standart deviation, $^{a–d}$show significant differences ($p < 0.05$) among groups in a column. *CFE*, cell-free extract from *L. reuteri*; *ECFE*, microencapsulated CFE from *L. reuteri*; *PW*, CFE from *L. reuteri* with propolis water extract (1%); *EPW*, microencapsulated CFE from *L. reuteri* with propolis aqueous extract. *PE*, *CFE from L. reuteri* with propolis ethanolic extract (1%); *EPE*, microencapsulated CFE from *L. reuteri* with propolis ethanolic extract

the antimicrobial potential of cell-free extracts obtained in various ways from the pro-

Proteus mirabilis was the most sensitive fish spoilage bacteria against CFE from *L. reuteri*. Control and microencapsulated of *L. reuteri* showed the lowest antimicrobial effect against *Enterococcus faecalis* with an inhibition diameter of 7.00 and 7.25 mm, respectively. Higher inhibition zones of CFE from *L. reuteri* were noted by Burgut [8] for gram-negative *Salmonella* Paratyphi A (13.5 mm) and *Klebsiella pneumoniae* (11.0 mm). There was no statistical difference between the control and microencapsulated CFE from *L. reuteri* in terms of antibacterial effect on all the fish spoilage bacteria. Similarly, Knysh and Martynov [26] reported the antibacterial potential of CFE of *Lactobacillus reuteri* DSM 17,938 against some antibiotic-resistant bacteria. Crude bacteriocins from CFE of *Lactobacillus acidophilus*, *Lactobacillus buchneri*, *Lactobacillus brevis*, and *Lactobacillus plantarum* had a strong inhibitory effect (16- to 22-mm inhibition zone) against most of *Salmonella* strains associated with food poisoning [39].

Antimicrobial effects of pure and microencapsulated CFE from *L. reuteri* with aqueous propolis extract were statistically different ($p < 0.05$). Microencapsulation form of *L. reuteri* supernatant in combination with aqueous propolis extract exerted lesser inhibition zone diameters compared to their pure form against all spoilage bacteria tested ($p < 0.05$). Microencapsulated CFE from *L. reuteri* combined with aqueous propolis extract exhibited the strongest antimicrobial activity on *P. mirabili*s and *P. luteola* (14.00 mm) and poor impact on *Enterococcus faecalis* (7.50 mm). As a result, microencapsulation of CFE from *L. reuteri* in combination with aqueous propolis extract exerted more antibacterial impacts against the bacteria tested than alone CFE from *L. reuteri* control group. This may be due to enhanced antimicrobial compounds from microencapsulated mixtures than alone CFE from *L. reuteri.* Microencapsulated CFE from *L. reuteri* combined with aqueous propolis extract contained a high level of 2(1H)-naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha., 7.beta.), and hydrocarbons mainly cyclohexane having antimicrobial activity unlike alone CFE from *L. reuteri.*

A similar antimicrobial effect was observed between control and microencapsulated *L. reuteri* containing ethanolic propolis extract against bacteria apart from *Proteus mirabilis* and *Enterococcus faecalis*. On the other hand, the presence of ethanolic propolis extract in the microencapsulated CFE from *L. reuteri* resulted in a higher inhibition effect against *Pseudomonas luteola* (17.00 mm) and *E. faecalis* (10.33 mm) compared to the control group without propolis extracts (16.25 and 8.25 mm). Among the groups, the highest antimicrobial activity against the growth of *Photobacterium damselae* was observed by control and microencapsulated CFE from *L. reuteri* containing 1% ethanolic propolis extract. Similarly, Kuley et al. [28] reported that co-microencapsulation of cell-free supernatant from *Lactobacillus plantarum* with propolis extract led to significant growth inhibition of *Photobacterium damselae*, with an inhibition diameter of > 15.5 mm.

3.3 Minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of extracts

Table 3 gives MIC and MBC of control and microencapsulated CFE obtained from *L. reuteri* alone or in combination with propolis extracts. Pure CFE from *L. reuteri* and ethanolic propolis extract exerted 50 mg/mL of MIC and > 50 mg/mL of MBC towards all bacteria. Lower MIC values (6.25 µg/mL) were reported for *L. reuteri* (LR12) against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Penicillium expansum* [1]. The pure water-based propolis extract exhibited low MIC values varying from 3.12 mg/mL against *Enterococcus faecalis* to 25 mg/mL against *Pseudomonas luteola*. The MBC value of the control water extract of propolis was more than 50 mg/mL. MIC of propolis ethanolic and water extract was in the range from 0.78 mg/mL for *Staphylococcus aureus* and 3.12 mg/mL for *Listeria monocytogenes* to 12.5 mg/mL for *Salmonella* Paratyphi A and *Klebsiella pneumoniae*, respectively [8].

Table 3 Minimum inhibitory (MIC) and bactericial concentration (MBC) of pure and microencapsulated samples (mg/mL)

Fish spoilage bacteria	Groups	MIC	MBC
P. damselae	PE	50	> 50
	PW	6.25	> 50
	CFE	50	> 50
	ECFE	50	> 50
	EPE	50	50
	EPW	50	> 50
P. mirabilis	PE	50	> 50
	PW	12.5	> 50
	CFE	50	> 50
	ECFE	25	> 50
	EPE	25	> 50
	EPW	25	> 50
P. luteola	PE	50	> 50
	PW	25	> 50
	CFE	50	> 50
	ECFE	25	> 50
	EPE	12.5	50
	EPW	50	50
E. faecalis	PE	50	> 50
	PW	3.12	> 50
	CFE	50	> 50
	ECFE	50	> 50
	EPE	25	> 50
	EPW	50	> 50

CFE, cell-free extract from *L. reuteri*; *ECFE*, microencapsulated CFE from *L. reuteri*; *PW*, propolis water extract; *EPW*, microencapsulated CFE from *L. reuteri* with propolis aqueous extract. *PE*, propolis ethanolic extract; *EPE*, microencapsulated CFE from *L. reuteri* with propolis ethanolic extract

The control of *L. reuteri* and its microencapsulation form give similar MIC (50 mg/mL) against the growth of *Photobacterium damselae* and *Enterococcus faecalis*, but a higher bacteriostatic effect (with MIC of 25 mg/mL) against *Pseudomonas luteola* and *Proteus mirabilis* was observed in the microencapsulated form of CFE from *L. reuteri* compared to control. This inhibitory effect may be linked to dimethoxydimethyl silane, (3E,5E,8Z)-3,7,11-trimethyl-1,3,5, 8,10-dodecapentanene, and organic acid contents of CFE from *L. reuteri* with antimicrobial properties. Control and microencapsulated CFE from *L. reuteri* exhibited more than 50 mg/mL of MBC on all bacteria tested.

The combination form of microencapsulated CFE from *L. reuteri* with aqueous propolis extract showed a MIC of 25 mg/mL on the growth of *Proteus mirabilis*. Moreover, their bactericidal effect was found at > 50 mg/mL against all groups except for *Pseudomonas luteola* which had 50 mg/mL of MBC. Lower MIC and MBC were reported for co-microencapsulated cell-free supernatants from *L. plantarum* with water extract of propolis against bacteria [28].

Among microencapsulated groups, the highest antimicrobial properties were generally observed from a combination form of CFE from *L. reuteri* with ethanolic propolis extract. The in vitro antibacterial activity of propolis ethanolic extracts on the gram-negative and gram-positive food-related was also reported in various studies [7, 43, 44]. The microencapsulated *L. reuteri* and ethanolic propolis extract combination provided MIC varied from 12.5 mg/mL against *Pseudomonas luteola* to 50 mg/mL against *Photobacterium damselae*. Microencapsulated CFE from *L. reuteri* combined with ethanolic propolis extract completely killed *Photobacterium damselae* and *Pseudomonas luteola* at a level of 50 mg/mL. However, their MBC was above 50 mg/mL against *Proteus mirabilis* and *Enterococcus faecalis*, which was in agreement with the result of Kuley et al. [28] for microencapsulated CFE from *L. plantarum* combined with ethanolic propolis extract. Unlike the work of Kuley et al. [28], in this study, the use of CFE from *L. reuteri* together with ethanolic extract showed a generally higher antibacterial activity on the fish spoilage bacteria, which is in agreement with the result of Burgut [8] for food-borne pathogens. This highest antimicrobial activity may be due to the existence of cyclopentadienylphenylacetic acid known as benzene and substituted derivatives, cyclohexane hydrocarbons, bicyclo[2.2.2] oct-5-ene-2endo,3endo-dimethanol, and organic acids in CFE from *L. reuteri* together with the ethanolic extract.

4 Conclusion

As a result of the study, it was found that although the effects of microencapsulated CFE from *L. reuteri* and propolis extract combinations on the fish spoilage bacteria varied depending on bacterial species, the growth of most bacteria was considerably inhibited. In microencapsulation, the combined use of propolis extracts, especially ethanolic extract with CFE obtained from *L. reuteri*, generally showed a higher antimicrobial effect on the bacteria. This high antimicrobial activity could be associated with their abundant bioactive compounds. This indicates that the microencapsulated product developed could have the potential to be used as alternative antimicrobial agents in foods. The bacteria used in the current study were gram-negative bacteria. In the future, more detailed studies should be carried out on different microorganism groups using different probiotic members and propolis concentrations in microencapsulation.

Author contribution Yilmaz Ucar—original draft preparation, conceptualization, software, writing, review and editing.

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Declarations

Conflict of interest The author declare no competing interests.

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