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ORIGINAL ARTICLE

Antimicrobial and prebiotic properties of *Weissella confuse B4-2* exopolysaccharide and its effects on matrix metalloproteinase genes expression

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ABSTRACT

Background and Objectives: Bacterial polysaccharides have diverse applications, including antimicrobial compounds, bio-preservatives, prebiotics, and wound-healing hydrogels. *Weissella confusa* is notable for its high polysaccharide yield among lactic acid bacteria.

Materials and Methods: The bacteria were identified via 16s rRNA and exopolysaccharide (EPS) production was performed in a 10% skim milk and 10% sucrose medium. FT-IR, SEM, and HPTLC analyzed functional groups, spatial structure, and EPS units. Moreover, MTT assay, DPPH, and Kirby-Bayer disk method assessed cell proliferation, antioxidant activity, and antimicrobial effects of EPS. Additionally, Prebiotic potential and growth kinetics of exopolysaccharide were examined using the Thitiratsakul method. Furthermore, EPS effects on MMP and TIMP gene expression in fibroblast cells were evaluated.

Results: The purified polysaccharide from *W. confusa B4-2* (Accession: KY290603), with a yield of 53 g/L, consists of glucose, fructose, and diglucuronic acid. This non-toxic polysaccharide (99-100% cell survival) exhibits 75% free radical scavenging activity along with significant antimicrobial effects against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. It also shows a high prebiotic score (0.912), accelerating wound healing in fibroblast cells while

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reducing collagen-degrading gene expression, particularly matrix metalloproteinases (MMPs). Notably, exopolysaccharides downregulated MMP1, MMP2, MMP3, and MMP9 gene expression levels by approximately 1.3, 1.2, 1.5, and 1.16 times, respectively.

Conclusion: These features highlight the commercial significance of *W. confusa* in the food, pharmaceutical, and health industries, surpassing lactobacilli with lower production yields.

Keywords: Antioxidants; Matrix metalloproteinases; Polysaccharides; Prebiotics; *Weissella confusa*

INTRODUCTION

Polysaccharides are the most important part of carbohydrates in nature and comprise monosaccharide units. These compounds are primarily derived from plants, fungi, algae, and bacteria. Natural polysaccharides have numerous biotechnological applications, including antimicrobial, antioxidant, and prebiotic activities. They also have other properties, such as increasing viscosity, stabilization, wound healing, and boosting the immune system. Additionally, polysaccharides help in bioremediation, food, pharmaceutical, petrochemical, and wastewater industries (1, 2).

Bacterial exopolysaccharides (EPSs) have distinct advantages over polysaccharides derived from plant, animal, fungal, and algal sources (3). They are produced in substantial quantities quickly, independent of seasonal or climatic variations. Additionally, their extraction and purification methods are more cost-effective. Due to their high purity, well-defined three-dimensional structures, biocompatibility, hydrophilicity, and favorable biodegradability, EPSs are promising candidates for different medical applications, including tissue engineering, wound dressings, adjuvants, and drug delivery systems (4). *Lactic acid bacteria (LAB)* are capable of synthesizing both capsular and extracellular forms of polysaccharides. They are non-pathogenic and benefit human health, so they have received significant attention. In both forms, this protective layer protects bacteria against adverse biotic and abiotic conditions and has physiochemical properties that favor its polysaccharides use in food and pharmaceutical products (5).

Our previous study examined *lactobacillus* exopolysaccharide's antioxidant, anti-elastase, and strong anti-collagenase properties. However, due to the low yield of polysaccharide production in this group of lactic acid bacteria, the researchers sought other options with high polysaccharide production yield and similar characteristics in the LAB group, thereby enhancing their industrial application's cost-effectiveness. After extensive research, the *Weissella* genus was identified as a promising candidate for this purpose (6).

Within the LAB group, the *Weissella* genus exhibits a remarkable capacity to synthesize non-digestible oligosaccharides and extracellular polysaccharides, with particular emphasis on dextran. The prebiotic and antimicrobial properties of dextran have recently garnered significant interest, leading to its widespread utilization in the bakery industry and beverage production (7, 8). Dextran, an α-glucan polymer, consists of a α (1-6) linkages, with side chains as α (1-2), α (1-3), or α (1-4) glycosidic bonds. It was first discovered during wine fermentation and used as a plasma expander. Dextran is the main component of the hydrogel covering the wounds caused by burns, facilitating rapid neovascularization and the wound-healing process (9, 10).

This study will investigate the biological activities of polysaccharides purified from *Weissella confusa* B4-2 in terms of antimicrobial, prebiotic, and antioxidant activities; scratch assay; and its effects on the level of expression of matrix metalloproteinase (MMP) and tissue inhibitors of metalloproteinase (TIMP) genes of fibroblastic cells. Also, our aim was to evaluate the potential anti-aging and skin-protective properties of the EPS, as MMPs (especially MMP-1, MMP-3, and MMP-9), as the key enzymes involved in collagen degradation and skin aging. By assessing the expression levels of these genes, we aimed to explore whether EPS could modulate their activity and thereby suggest its potential application in dermatological or cosmetic formulations. Based on established research, metabolites capable of downregulating MMPs exhibit significant potential in preventing skin aging (11, 12).

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study were A *Lactobacillus rhamnosus* ATCC7465, *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, and *Pseudomonas aeruginosa*

ATCC27853. These strains were supplied from the microbial collection maintained by the Iranian Genetic Resources Center.

Cultural media and chemicals. Man-Rogosa-Sharpe (MRS) medium, nutrient agar, and skim milk from Merck KGaA (Darmstadt, Germany) were used. EPS production medium was 10% skim milk plus 10% sucrose in PH: 6.7, at 37°C. Synthetic MRS was used to investigate the kinetics of bacterial growth by basal medium including peptone (1%), beef extract (1%), yeast extract (0.4%), sodium acetate trihydrate (0.5%), Tween 80 (0.1%), dipotassium hydrogen phosphate (0.2%), triammonium citrate (0.2%), magnesium sulfate heptahydrate (0.02%), and manganese sulfate tetrahydrate (0.005%) (13). MRS broth was prepared with 1% glucose (MRS-G). In (MRS-PS), glucose was replaced with 1% *W. confusa* exopolysaccharide, and 1% commercial inulin was added instead of glucose in (MRS-Inulin). Moreover, for preparing NB-G, 1% glucose was added to the nutrient broth, and for NB-PS, 1% *W. confusa* exopolysaccharide was applied instead of glucose. Additional chemical components, including Trichloroacetic acid (TCA) and MTT assay, were obtained from Merck, and commercial inulin was purchased as well.

Isolation and molecular identification of *Weissella*. Isolation was done after enrichment of traditional butter sample (source of *Weissella*) in MRS broth medium at 37°C, after 48 h and dilution of cell pellet, streaking on MRS agar medium. Molecular identification was performed based on the 16S rRNA gene using universal primers 27F/1492R (6). PCR products were purified via gel extraction and subsequently sequenced by Bioneer Corporation (Daejeon, Republic of Korea). The resulting nucleotide sequence was submitted to the NCBI database.

Exopolysaccharide production and isolation. For EPS production, a 10% skim milk medium supplemented with 10% sucrose was prepared. After 16 h of fermentation, following boiling, cooling, and adding trichloroacetic acid 85% (TCA), proteins were removed. The extracted polysaccharide was precipitated in cold ethanol and allowed to settle for 48 h. Subsequently, the samples were centrifuged at 8000 × g for 10 minutes at 4°C. The resulting cell pellet was rehydrated in deionized water and dialyzed against distilled water at 4°C for 48 hours using a dialysis membrane with a 12 kDa molecular weight cutoff. After dialysis, the EPS extracts were either preserved at -20°C or lyophilized for further use (6) (14). The impurity of any remaining protein was assessed using the Bradford method (15, 16). Moreover, the total carbohydrate content of the purified EPS was determined using the phenol–sulfuric acid assay (17).

Exopolysaccharide characterization. Fourier-transform infrared spectroscopy (FTIR, Aligent, USA) was applied to examine the functional groups in the lyophilized EPS in the spectral range 400-4000 cm^{-1} (18). Moreover, scanning electron microscopy (SEM, Seron Technology, AIS-2100, South Korea) was employed to visualize the three-dimensional structure of lyophilized polysaccharides at magnifications of 1000X and 5000X (19). High-performance thin-layer chromatography (HPTLC, CAMAG, Muttenz, Switzerland) was employed to identify the building blocks of EPS. The resulting spots were analyzed using Win CATS 1.4.1 software at a wavelength of 400 nm.

Antioxidant activity. The antioxidant activity assessment was examined by the capacity of the purified EPS to scavenge free radicals using the DPPH method (20). As a positive control, ascorbic acid was employed at a concentration of 1 mg/ml. The absorbance of the samples was measured using the Cytation 3 device (Bio Tek) at 517 nm, and subsequently, the scavenging activity was determined using the following formula:

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

Where Ac is the control reaction absorbance and As is the testing specimen absorbance.

Cell proliferation. To assess the cell proliferation capacity, the MTT method was applied (21). Neonatal foreskin fibroblasts were initially cultured in Dulbecco's Modified Eagle Medium (DMEM) for use in both the MTT assay and scratch wound healing test. The absorbance was recorded at 570 nm with a spectrophotometer (BioTek Instruments, Winooski, VT). In the scratch test, DMEM medium with 2% FBS was the negative control, DMEM medium with 10% FBS was the positive control, and polysaccharide treatment was added to DMEM medium with 2% FBS. The percentage of the scratch area filled by epithelial

cells was calculated using a specific formula:

$$\text{Relative viability} = \left(\frac{\text{OD samle - blank absorbance}}{\text{Mean OD control - blank absorbance}}\right)$$

Antimicrobial activity. The antimicrobial effect of EPS with 1 mg/ml concentration was performed using the Kirby-Bauer disk method (22) against *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922, and *Pseudomonas aeruginosa* ATCC27853. The concentration of index bacteria was adjusted with 0.5 McFarland ($2 \times 10^8 \frac{cfu}{ml}$, and the incubation time was carried out at 37°C for 16 h based on the CLSI protocol.

Prebiotic activity and growth kinetics. The Thitiratsakul method (23) was used to assess the prebiotic activity of the desired EPS. Also, *L. rhamnosus* was used as an indicator probiotic strain with prebiotic activity, and *E. coli* as a pathogenic strain. Commercial inulin was applied as a positive control with prebiotic activity. *L. rhamnosus* culture was inoculated at 10% in three MRS broth media: MRS broth (MRS-G), MRS-PS (EPS instead of glucose), and MRS-Inulin (Inulin instead of glucose). *E. coli* culture was inoculated at 1% in three nutrient broth media: NB-PS, NB-G, and NB-Inulin. All three media were simultaneously incubated at 37°C for 48 hours. Cfu/ml was calculated in all media at 6, 12, 18, 24, and 48 hours using the Miles and Misra method (24). This experiment was performed in three independent replicates. The score of prebiotic activity was calculated using the following formula (25):

Prebiotic activity score =

$$\left[\frac{probiotic \log \frac{CFU}{ml} \text{ on prebiotic at 48h} - probiotic \log \frac{CFU}{ml} \text{ on prebiotic at 0h}}{probiotic \log \frac{CFU}{ml} \text{ on glucose at 48h} - probiotic \log \frac{CFU}{ml} \text{ on glucose at 0h}}\right] -$$

$$\left[\frac{enteric \log \frac{CFU}{ml} \text{ on prebiotic at 48h} - enteric \log \frac{CFU}{ml} \text{ on prebiotic at 0h}}{enteric \log \frac{CFU}{ml} \text{ on glucose at 48h} - enteric \log \frac{CFU}{ml} \text{ on glucose at 0h}}\right]$$

Determination of bacterial doubling time and specific growth rate. *L. rhamnosus* was cultured in these three media: MRS-G, MRS-PS, and MRS-Inulin. Simultaneously, *E. coli* was inoculated into three media: NB-G, NB-PS, and NB-Inulin. The inoculation rate was 10% for *L. rhamnosus* and 1% for *E. coli*. The cultures were subsequently incubated for a duration of 48 hours. At designated time points (0, 6, 12, 18, 24, 36, and 48 hours), colony counts were performed on each type of culture medium. N_0 and N represent the initial bacterial count and the final count, respectively. The total culture time (T) was measured in h. The doubling time of the bacteria can be calculated using the following formula:

$$G = T/3.3 \, (\text{LogN-Log}N_0)$$

The specific growth rate refers to the rate at which the biomass of a cell population increases relative to its existing biomass concentration, which is calculated through the following formula (26):

$$(\text{Inx}_1\text{-Inx}_2)/(t_1\text{-}t_2)$$

where t_1 and t_2 are the log phase period of the bacteria growth, x_1 is the number of bacteria at t_1, and x_2 is the number of bacteria at t_2.

Gene expression of MMPs and TIMPs in response to exopolysaccharide. Real-time PCR with the program: 30 s at 90°C after 45 two-step cycles, including 15 s at 95°C and 30 s at 60°C for (MMP1, MMP3, and MMP10) and 65°C for (MMP2, MMP9, TIMP1, and TIMP2) was used in duplicate for three independent repetitions, using MMP1, MMP2, MMP3, MMP9, TIMP1, and TIMP2 primers (6), to survey the effect of the desired EPS on normal fibroblast cells. The PGM1 gene was also used as a normalizer.

Statistical analysis of experimental data. Each dataset was evaluated using three separate experimental replicates. The standard deviation was calculated using Microsoft Excel 2010. A p-value < 0.5 was considered, and the IC_{50} value was used for MTT and polysaccharide antioxidant activity.

RESULTS

***Weissella confusa* identification.** Colonies grown on MRS agar medium were convex, white, and approximately 1-3 mm in diameter. Bacteria were gram-positive, short bacilli with negative catalase activity and no spore production. The BLAST analysis of approximately 1500 base pairs from the 16s rRNA gene in the NCBI database indicated a 99% similarity with *W. confusa*. The corresponding sequence was released as KY290603.1. accession number.

Exopolysaccharide production. Exopolysaccharide production in a 10% sucrose-skim milk medium yielded approximately 53 g/l. The purified EPS contained approximately 7 mg/l of residual protein (Fig. 1).

Fig. 1. *W. confusa* B4-2 EPS precipitated in ethanol

Exopolysaccharide identification. The functional groups of the polysaccharide extracted from *W. confusa* were compared with the pattern of other polysaccharides using FTIR spectroscopy (Agilent, USA). In the FTIR spectrum of the purified polysaccharide, several peaks appeared in the range of 3444 to 544 cm^{-1}. Fig. 2 shows many hydroxyl groups with an absorption peak of about 3444 cm^{-1}, indicating that the desired molecule is a typical carbohydrate. The 2921 cm^{-1} and 2368 cm^{-1} peaks correspond to the stretching vibration of C–H bonds. The peak at 2368 cm^{-1} is associated with O=C=O stretching bonds. Furthermore, the absorption at 1641 cm^{-1} indicates the presence of the sp^2 ring of the C=C stretching double band, while the 1580-1650 range signifies N-H bonding. Peaks falling within the 1040-1050 range suggest CO-O-CO bonds and around 880 cm^{-1} indicate C-H bonds. Collectively, these observations confirm that the analyzed sample is indeed a polysaccharide (Fig. 2).

The Monosaccharide composition was analyzed using HPTLC (high-performance thin-layer chromatography) technique. EPS samples were hydrolyzed into individual monosaccharides by treatment with 10% (v/v) hydrochloric acid at 100°C. High-performance thin-layer chromatography (HPTLC) was carried out in a CAMAG ADC2 glass twin-trough chamber (CAMAG, Muttenz, Switzerland). Sample application (1 µL) was applied out with a CAMAG automatic TLC sampler 4 onto silica gel 60 F254 plates (Merck, Darmstadt, Germany). Plate development was performed at room temperature (around 25°C) up to 80 mm using a solvent mixture of chloroform, n-butanol, methanol, acetic acid, and water in a volumetric ratio of 5.5:11.0:5.0:1.5:2.0. Post-development, the plates were treated with an aniline–diphenylamine–phosphoric acid reagent and heated at 130°C for 10 minutes to visualize the spots. The resulting chromatograms were photographed and analyzed using a TLC Scanner 3 operated in reflectance mode at 400 nm with WinCATS 1.4.1 software (6). Chromatogram analysis revealed that glucose, fructose, and diglucuronic acid constitute the major units of this EPS (Fig. 3), as determined by their Rf values compared to the reference standards.

The scanning electron microscopy of the EPS surface morphology, exhibited a uniform and regular crystal structure (similar to autumn leaves) resembling dextran crystals (Fig. 4). Furthermore, the crystal derived from the purified freeze-dried polysaccharide was completely soluble in water.

DPPH radical-scavenging assay. Vitamin C or ascorbic acid, which is considered an antioxidant index, showed 74% free radical scavenging activity at a concentration of 1 mg/ml. Polysaccharide purified from *W. confusa* demonstrated 75% free radical scavenging activity at the same concentration.

MTT assay. The viability potential of the desired polysaccharide at concentrations of 1 to 10 mg/ml was between 99-100%, and it was not possible to investigate its higher concentrations due to the high viscosity of the corresponding EPS.

Antimicrobial effect. The non-growth halo at different concentrations of the extracted polysaccharide was compared to its supernatant obtained from the MRS broth culture medium (Table 1).

The antibacterial effect of the polysaccharide released in MRS medium exhibited less antibacterial activity compared to the pure polysaccharide added to the disk. Further concentration enhancement was impossible due to the gelatinization (binding) of the polysaccharide.

Prebiotic effect. To investigate the prebiotic effect of the purified polysaccharides, colony counting was employed using a dilution series on the plate, and the results were reported in cfu/ml. The number

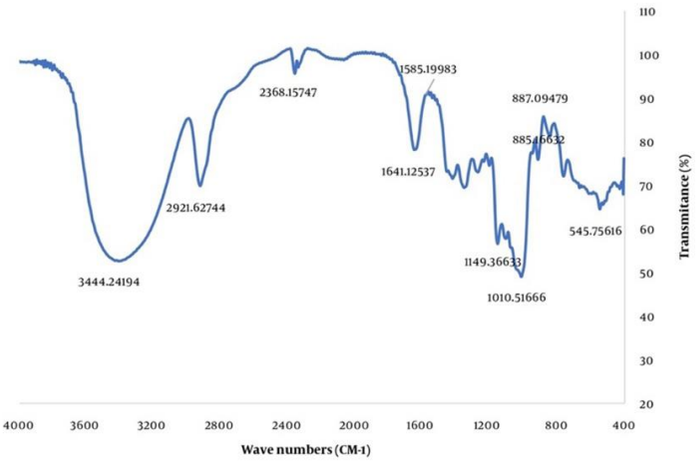
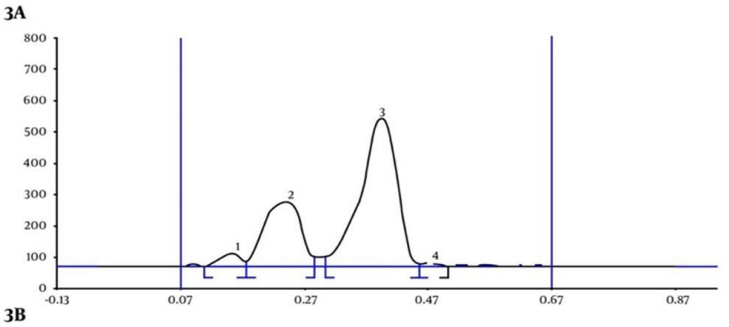


Fig. 2. FT-IR spectrum of *Weissella confusa B4-2* exopolysaccharides in the range of 400-4000 cm^{-1}



peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.11	0.1	0.15	42.8	5.81	0.17	16.5	1282.5	3.52
2	0.17	17.1	0.24	207.3	28.14	0.28	29.9	10701.1	29.35
3	0.30	33.4	0.39	475.5	64.54	0.45	8.7	24195.1	66.36
4	0.45	8.8	0.47	11.1	1.51	0.50	1.0	179.2	0.77

Fig. 3. 3A HPTLC of EPS of *W. confusa* B4-2 and 3B High-Performance Thin Layer Chromatography Rf (Retention factor) Values.

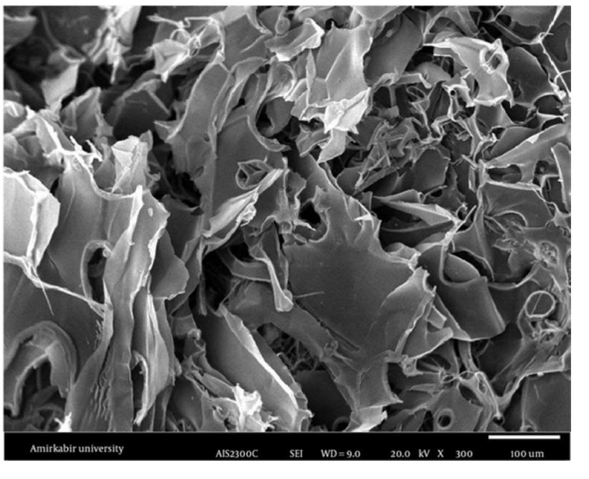


Fig. 4. Scanning electron micrograph (SEM) of *Weissella confusa B4-2* freeze-dried EPS (5000).

Table 1. Assessment of the antibacterial effects of varying concentrations of EPS derived from *Weissella confusa B4-2* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

Sample	***St. aureuse*** **(mm)**	***P. aeruginosa*** **(mm)**	***E. coli*** **(mm)**
Supernatant	8.2 ± 0.2	9 ± 0.1	8.5 ± 0.1
Polysaccharide (1mg/mL)	10 ± 0.2	10 ± 0.2	9 ± 0.1
Polysaccharide (3mg/mL)	10.5 ± 0.1	10.5 ± 0.1	9.5 ± 0.2
Polysaccharide (5mg/mL)	11.5 ± 0.3	11.5 ± 0.3	10 ± 0.2
Polysaccharide (10mg/mL)	12.5 ± 0.2	12.5 ± 0.2	10.5 ± 0.3

of colonies at zero time started from 5 ± 0.02 log cfu/ml in *L. rhamnosus* and from 6 ± 0.02 log cfu/ml in *E. coli*. As seen in chart 1, *L. rhamnosus* grew more in MRS-PS medium (8.78 log cfu/ml) than in MRS-G medium (8.24 log cfu/ml) following a 24-hour incubation period. However, it was lower than the growth of this probiotic bacteria (9.2 log cfu/ml) in MRS-Inulin medium. After 48 h, *L. rhamnosus* reached 10.57, 10, and 8.9 log cfu/ml in MRS-Inulin, MRS-PS, and MRS-G media, respectively (Fig. 5).

On the other hand, non-probiotic bacteria *E. coli* exhibited more robust growth in NB-G medium than in NB-Inulin and NB-PS media. As the plateau phase for *E. coli* occurs after 24 h, its growth in three culture media, NB-G, NB-Inulin, and NB-PS, was calculated at 9, 7.98, and 7.2 log cfu/ml, in the same order, in 24 h. The prebiotic score, based on *L. rhamnosus* and *E. coli*, was calculated 0.912 (Fig. 6).

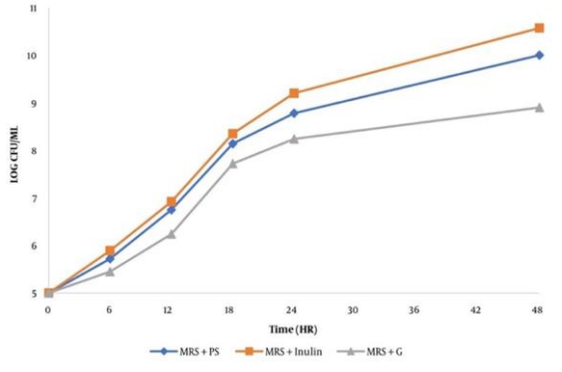


Fig. 5. kinetic growth of *L. rhamnosus* in media; MRS-PS, MRS-Inulin, and MRS-G.

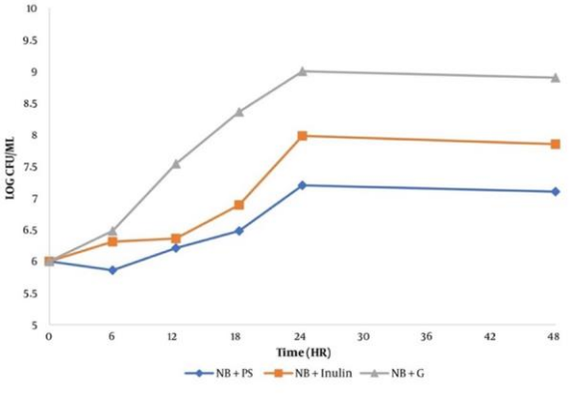


Fig. 6. Kinetic growth of *E. coli* in media; NB-PS, NB-Inulin, and NB-G

The specific growth rate and doubling time of *L. rhamnosus* with different carbon sources. According to Table 2, *L. rhamnosus* grew more slowly in a glucose-based medium, resulting in a longer doubling time. Interestingly, when the carbon source was the purified polysaccharide, the growth rate and doubling time were approximately close to the medium with the index prebiotic, inulin.

Comparative expression of MMP and TIMP genes in response to polysaccharides from *Weissella confusa.* After determining the impact of the purified polysaccharides from *W. confusa* on normal skin fibroblast cells, altered expression levels of several genes were observed. Specifically, the expression of MMP1, MMP2, MMP3, and MMP9 genes decreased by approximately 1.3, 1.2, 1.5, and 1.16 times, respectively, compared to the control gene (PGM). Conversely, the expression of TIMP1 and TIMP2 genes increased by approximately 1.4 and 1.13 times, respectively (Fig. 7).

Table 2. Comparison of doubling time and specific growth rate of *L. rhamnosus* in three media; MRS-G, MRS-Inulin, and MRS-PS.

Cultural media	Doubling time (hour)	Specific growth rate
MRS-G	3.73	0.039
MRS-Inulin	2.61	0.074
MRS-PS	2.91	0.062

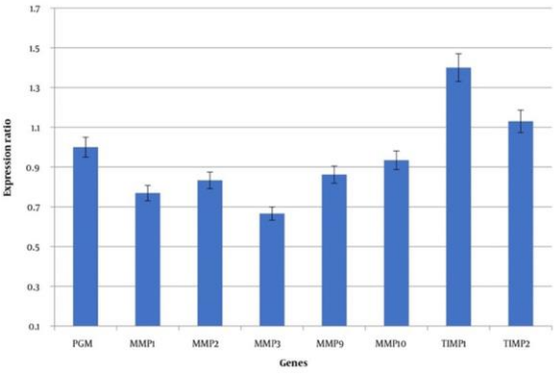


Fig. 7. Comparison of MMP gene expression levels in response to EPSs derived from *Weissella confusa B4-2*. The reference gene was used for normalization in the control group is PG.

DISCUSSION

Nowadays, using natural polysaccharides from bacterial sources has become one of the fascinating topics to researchers due to their high production yield (27), compatibility with the environment, and lack of side effects on human health (28). In our previous studies, we concluded that the EPS of lactobacilli had the potential to be used in transdermal patches, degradable scaffolds, and anti-aging skincare products because of its ability to inhibit elastase and collagenase, promote wound healing, and act as an antioxidant. Despite their remarkable capabilities, the polysaccharide production yield of EPS was very low, and their commercial use was not practically cost-effective. Therefore, we decided to look for strains with high polysaccharide production yield among different probiotic species. Among lactic acid bacteria, *W. confusa* and *W. cibaria* have gained significant interest because of their elevated yield ability of dextran, fructan, heteropolysaccharides, and non-degradable oligosaccharides (7).

In this study, we isolated *W. confusa* from a traditional fermented butter in Iran. The production of purified polysaccharides from this *W. confusa* species in a medium containing 10% skim milk and 10% sucrose reached an impressive concentration of 54 g/l. This yield was notably 168 times greater than that of *L. plantarum* and 189 times greater than that of *L. casei* (6).

The biochemical analyses of the purified polysaccharide from *W. confusa* revealed structural similarities to dextran. Additionally, its antioxidant and wound-healing properties were confirmed. Notably, the polysaccharide demonstrated a potent antimicrobial effect against typical pathogenic bacteria. Interestingly, as the polysaccharide concentration increased, its antimicrobial activity became stronger.

Furthermore, the prebiotic activity of this purified polysaccharide was strikingly similar to the widely recognized prebiotic inulin. Previous studies have explored the prebiotic effects of polysaccharides extracted from plants, often in comparison to commercial prebiotics (23, 26, 29-32).

Studies on the growth kinetics of the probiotic *L. rhamnosus* in a medium supplemented with *W. confusa* polysaccharide uncovered intriguing results. Notably, the specific growth rate of *L. rhamnosus* closely approximates that of inulin and surpasses that of glucose. Additionally, the polysaccharide reduces the doubling time of probiotics, highlighting its potential as a prebiotic source. Therefore, polysaccharides derived from bacteria can also be a good source of prebiotics. Given these characteristics, the refined polysaccharide shows as a promising candidate for diverse uses in the food industry as a biopreservative, prebiotic, and bio-stabilizing agent.

Furthermore, the wound-filling ability of this polysaccharide, coupled with its impact on reducing the expression of MMP1, MMP2, MMP3, and MMP9 genes in fibroblasts, contributes to the prevention of collagen breakdown and inhibits skin aging. Moreover, the upregulation of TIMP-1 and TIMP-2 genes in normal fibroblasts suppresses the expression of MMP promoters. Consequently, the purified polysaccharide from *W. confusa* is a valuable option for the production of anti-aging skincare products.

CONCLUSION

The findings from this study suggested that *Weissella confusa B4-2* produced 54 g/L of polysaccharide, far exceeding common strains. Its structure resembled dextran and demonstrated potent antioxidant, antimicrobial, and wound-healing properties. Importantly, it exhibited prebiotic activity nearly equivalent to inulin, promoting *L. rhamnosus* growth and reducing doubling time. Additionally, the polysaccharide downregulated collagen-degrading MMP genes and upregulated protective TIMP genes, suggesting anti-aging effects. These multifaceted properties position *W. confusa*-derived polysaccharide as an excellent potential prebiotic agent, food bio preservative, and anti-aging skincare agent.

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