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Antioxidant and Antimicrobial Activities of Exopolysaccharides from Yoghurt Starter

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Abstract: The *in vitro* antimicrobial and antioxidant activities of the exopolysaccharide (EPS) extracted from yoghurt starter were investigated. Antimicrobial activity tests were carried out using disc diffusion methods with *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* D, *Proteus* spp and the yeast of *Candida albicans*. The antioxidant properties were evaluated using the test of DPPH free radical trapping. The results show that the EPS has weak antimicrobial activity against the tested strains with inhibitions zones ranging from 9 to 13 mm. EPS could reduce the free radical (DPPH) to diphenylpicrylhydrazine a yellow-colored at 24.25% showing antioxidant activity less than that of ascorbic acid which was 69.79%.

Keywords: EPS, Yoghurt Starter, Antimicrobial, Antioxidant Activities

1. Introduction

Antimicrobial resistance is one of the world's most serious public health problems. Many of the microbes (bacteria, viruses, protozoa) that cause infectious disease no longer respond to common antimicrobial drugs. There is an urgent need to find new efficient antimicrobial drugs [1]. In other hand, It has been found that free radicals and other reactive oxygen species play a cardinal role in oxidative damage to cellular constituents which leads to cell injury and death. This has been associated with pathogenesis of various chronic disease, e.g. carcinomas, coronary heart disease, and many other hearth problems related to advancing age [2]. Therefore, there is a need to develop new and safe antioxidants from natural sources to minimize the oxidative damages in living cells. Many species of bacteria possess the ability to synthesize and excrete extracellular polysaccharides (exopolysaccharides, EPS) [3]. The bacterial exopolysaccharides are widely used in food, pharmaceutical and chemical industries as bioflocculants, bioabsorbents, drug delivery agents, etc [4]. Lactic acid bacteria (LAB) are able to produce exopolysaccharides (EPSs) in the surrounding medium as a slime or on the surface of bacterial cells to form a capsule [5]. Some of the EPSs produced by LAB may confer health benefits such as immune-modulatory, anti-tumor, anti-bio film and antioxidant activities [6]. The aim of this study was to evaluate the *in vitro* antioxidant and antimicrobial properties of the exopolysaccharides extracted from yoghurt starter.

2. Material and Methods

2.1. Yoghurt Starter

Freeze dried mixed yoghurt culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*) was obtained from GIPLAIT dairy unit of Tizi (Mascara).

2.2. Inoculum Preparation

The inoculum for the experiments was prepared by growing 2% of yoghurt starter strain in 50 mL flasks containing 25 mL of the sterilised standard medium BMM (basal minimal medium) [7]. The flask was incubated at 30°C for 24 h.

2.3. Extraction of Exopolysaccharides

Exopolysaccharides were extracted according to the method of Zhang et al (2011) [8] with modifications. After incubation, the medium inoculated with the yoghurt starter was maintained at 100°C for 15 min and refrigerated at 4°C for 24 h. After the refrigeration period, the samples were

centrifuged at 9950 x g for 30 min at 4°C and the supernatant containing the EPS were precipitated with 3 volumes of cold ethanol 95%. The samples were stored at 4°C for 3 h then centrifuged at 8000 x g for 20 min at 4°C. The precipitate was re-suspended by dialysis in distilled water for 24 h, then dried using a Christ lyophilizer until a constant weight was observed. The lyophilized samples were dissolved again in trichloroacetic 10%, centrifuged at 5000 rpm for 15 min, dialysed for 5 days and lyophilized.

2.4. Micro-organisms

The organisms used in the microbiological assays were obtained from Laboratory of Medical Analysis at Meslem Tayeb hospital in Mascara city. The antimicrobial activity was individually tested against a panel of microorganisms, including *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* D, *Proteus* spp and the yeast of *Candida albicans*. The organisms were identified by cellular, cultural and biochemical characteristics. Bacterial strains were cultured overnight at 37°C in Mueller Hinton broth. Yeast was cultured at 30°C in Sabouraud dextrose broth.

2.5. Antimicrobial Screening

The Exopolysaccharides were examined for their antimicrobial activity by agar disc diffusion method [9]. Briefly, each suspension of the tested microorganism (1×10^6 CFU/ml) was spread on the solid media plates. Filter paper discs (5 mm in diameter) were individually impregnated with 10 µl of undiluted EPS (0.12, 0.25, 0.5, and 1 mg/ml) and placed on the incubated plates. After 30 min at room temperature, the dishes were incubated at 37°C for 24 h (for bacteria strains) and 30°C for 48 h (for yeast). At the end of the incubation period the antimicrobial activity was evaluated by measuring the clear inhibition zones formed around the discs. The antibiotic Gentamycin was used as positive control.

2.6. Antioxidant Activity

DPPH-radical scavenging activity of the EPS and control was measured according to the method described by Younes et al. (2012) [10] with some changes. 50 µl of various concentrations (0.02-0.1 mg/ml) of EPS was added to 1.950 ml of methanolic solution containing DPPH (1,1- Diphenyl-2-picryhydrazyl) radicals (2.4 mg DPPH was dissolved in 100 ml of methanol). The mixture was shaken vigorously and allowed standing for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$I\% = 100x\ (A_{Control} - A_{Sample})\ A_{Control}$$

Ascorbic acid was used as a control.

3. Results

The values for the diameter of the growth inhibition zones of the EPS for the different micro-organisms tested in the present study are shown in Table below. There was no activity observed against all organisms by the concentration of EPS ranged between 0.12 and 0.5 mg/ml. In contrary, at 1 mg/ml all tested micro-organisms showed sensitivity to EPS but their efficiency in inhibition was varied from one microorganism to another. The diameter of inhibition zones ranged between 9 and 13 mm. The large zone of inhibition was observed against *E. coli*, while the lowest was recorded against the yeast *C. albicans*. *S. aureus* and *Streptococcus* D presented the same susceptibility to the EPS (10mm). Interestingly, the EPS was more active against Gram-negative bacteria (*E. coli* and *Proteus* Sp) than Gram-positive bacteria (*S. aureus* and *Streptococcus* D) and all bacteria are more susceptible than the yeast.

Table 1. *Antimicrobial activity (zone of inhibition, mm) of Exopolysaccharides against clinical pathogens.*

		Diameter of the zone inhibition in mm				
		S.aureus	***E.coli***	***Streptococcus D***	***C.albicans***	***Proteus. sp***
[EPS] mg/ml	1	10	13	10	9	12
	0,5	-	-	-	-	-
	0,25	-	-	-	-	-
	0,12	-	-	-	-	-
Gen	10µg	15	16	15	17	27

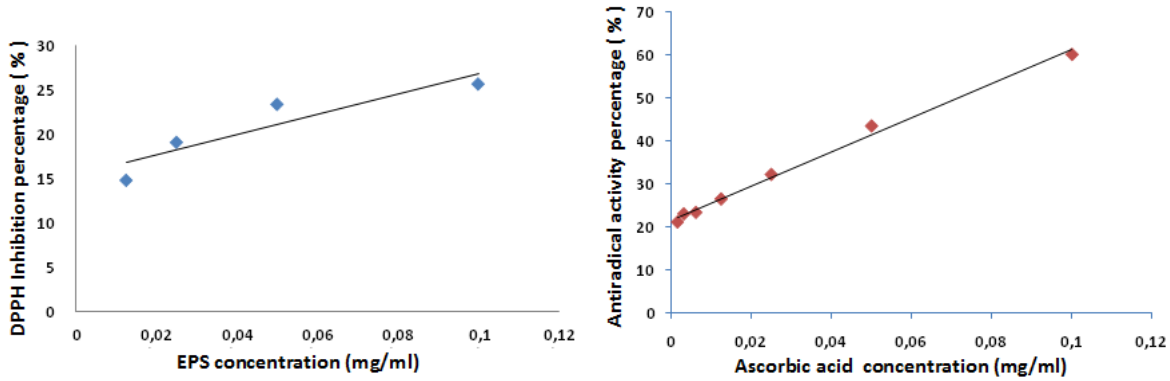

Figure 1. *DPPH radical scavenging activity of standard antioxidant compound ascorbic acid and EPS from yoghurt starter.*

The percentage inhibition of DPPH radical scavenging capacity of EPS from yoghurt starter in comparison of ascorbic acid has been shown in Figure 1. The DPPH free radical scavenging activity of EPS and ascorbic acid was determined at concentration level ranged between 0.02 mg/ml and 0.1 mg/ml.

In this study, the EPS showed dose-dependent DPPH radical scavenging activity as did by ascorbic acid (standard). The scavenging activity directly increased with the promoting concentration. The maximum scavenging rate of EPS was 24.25% at 0.1 mg/mL, whereas that of ascorbic acid was 69.79% at the same concentration.

4. Discussions

Many researchers have also shown that the Exopolysaccharides obtained from different source inhibited the growth of various microorganisms at different concentrations.

For example, Ezeronye et al. (2005) [1] investigated the antibacterial activities of crude polysaccharide of *Pleurotus tuber-regium* on some bacterial pathogens using the agar cup diffusion and disc diffusion methods. They found that ethanolic polysaccharide extracts of *P tuber-regium* fruitbody showed mean inhibition zones of 19.33, 20.67, 23.00 and 26.67 mm on *Enterococcus feacalis*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* respectively. The Minimum Inhibitory Concentrations (MIC) determined by the agar cup diffusion techniques for the ethanolic polysaccharide were 6.25 mg mL^{-1}, 12.5 mg mL^{-1} and 12.5 mg mL^{-1} and 25 mg mL^{-1} for *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Enterococcus feacalis* respectively. Challouf et al. (2011) [11] also screened the antibacterial activities of different extracts of the EPS (Methanol, Hexane, Petroleum ether, Ethanol, Acetone, and Water) of Cyanobacterium *Arthrospira platensis* against a collection of Gram+/- bacteria by the paper disk agar diffusion method, the determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC). The methanol extract exhibited a more potent activity than the other organic extracts, whereas the aqueous extract was active against *Staphylococcus epidermis* (Gram+) and *Salmonella typhimurium* (Gram-). Recently, Majolagbe et al. (2013) [12] conducted in vivo studies on antibacterial properties of the crude exopolysaccharides of *Lentinus subnudus* injected intraperitoneally in aqueous solution (20mg ml^{-1}) into groups of Swiss albino rats infected with pathogenic strains of *Escherichia coli* and *Pseudomonas aeruginosa*. The prophylactic activities of the exopolysaccharides were monitored when administered 7 and 2 days before bacteria induction while therapeutic activities were monitored by administering the exopolysaccharides 1 hour after the bacterial induction. Nisha and Thangavel, (2014) [13] assessed the activity of EPS from Halomonassp (biofilm) against *Escherichia coli*, *Klebsiella spp*., *Salmonella typhi* and *Staphylococcus* sp, using the disc diffusion method. The EPS showed activity against the four microorganisms with diameter zone of inhibition ranged between 8 and 12 mm. The same authors reported that Exopolysaccharide producing *Micrococcus* sp isolated from Arabian Sea had a weak antibacterial activity (zone of inhibition, 8 - 12 mm) against the same tested clinical isolates in the previous work [14]. Li et al. (2014) [15] reported that exopolysaccharides isolated from *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* R315 exhibited antimicrobial activities against tested pathogens such as *Cronobacter sakazakii, Escherichia coli, Listeria monocytogenes, Staphyloccocus aureus, Candida albicans, Bacillus cereus, Salmonella typhimurium*, and *Shigella sonnei* at 300 μg/mL. In another Study, Li and Shah (2014) [16] reported that exopolysaccharides from *Streptococcus thermophilus* ASCC 1275 showed an inhibition zone of 9.8, 14.5 and 10.8 mm to *E. coli* ATCC 25922, *S. aureus* CMCC 26003 and *L. monocytogenes* CMCC 54001 respectively. Wang et al. (2015a) [17] also found that the exopolysaccharide (EPS) produced by *Lactobacillus plantarum* YW32 exhibited a concentration-dependent inhibitory effect on the formation of biofilms by several pathogenic bacteria, including *Escherichia coli* O157, *Shigella flexneri* CMCC (B), *Staphylococcus aureus* AC1 and *Salmonella typhimurium* S50333.

Similarly EPS from different microorganisms also showed DPPH free radical scavenging activity in a dose-dependent manner [18, 19, 20, 21].

Overproduction of free radicals can cause oxidative damage to biomolecules, (lipids, proteins, DNA), eventually leading to many chronic diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in humans [22]. EPSs have been proved to have antioxidant and free radical scavenging properties in several studies. Kishk and Al-Sayed. (2007) [23] observed the antioxidant and free radical scavenging activities of exopolysaccharide isolated from *Rhizobium meliloti*. A moderate antioxidant activity of methanol extract of exopolysaccharide from Cyanobacterium *Arthrospira platensis* using the Trolox Equivalent Antioxidant Activity assay was determined by Challouf et al. (2011) [11]. A strong scavenging ability of exopolysaccharide from *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* R315 was observed against DPPH and superoxide radicals at high concentration [15]. The EPS produced by *Lactobacillus plantarum* YW32 at a dose of 5mg/ml had strong scavenging abilities toward hydroxyl and superoxide radicals [17]. Water - soluble exopolysaccharides of *Lactococcus lactis* NCR112 isolated in *Phyllanthus urinaria* were capable of scavenging DPPH free radical [24].

In recent years, there has been an increasing interest in exploiting the EPS-producing lactic acid bacteria for their biological and therapeutic potential to be promising natural antioxidants and antimicrobials as well as food additives or

functional food ingredients with both health and economical benefits [19]. Several studies have shown that microbial polysaccharides act as an effective antioxidant [25, 15, 17, 24], but very limited information is available on the antioxidant mechanisms of the exopolysaccharides action at molecular level [6, 26].

5. Conclusion

The EPS extracted from yoghurt starter was found to possess antioxidant and antimicrobial activities. The in vitro antioxidant study shows that EPS has the ability to scavenge DPPH free radicals. This study revealed also that the EPS exhibit antimicrobial property against the five tested organisms at a concentration of 1 mg/ml. There is a need to conduct in vivo studies to ascertain the safety and acceptability of these products for their exploitation in food and pharmaceutical industries.

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