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Antimicrobial Activity of Pigments Extracted from *Rhodotorula glutinis* Against Some Bacteria and Fungi

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Abstract

Background: Nowadays hazards of synthetic additives and preservatives have been identified, so researchers are looking to a natural and safe alternative for them. The aim of this study was to evaluate antimicrobial effect of carotenoids of *Rhodotorula glutinis* on the some pathogenic bacteria and fungi.

Methods: This experimental study was done in Gorgan University of Agriculture and Natural Resources. After cultivating *R. glutinis* in 50 mL YPG broth at 30°C for overnight, cells were harvested by centrifugation at 10,000 rpm for 10 minutes and were washed three times with distilled water. Cells were ruptured 3 times with 12 mL of acetone and broken using homogenizer. Then the suspension was centrifuged and the supernatant collected. The supernatant (contain pigments) was powdered using freeze-dryer. Antimicrobial activity was evaluated by disc diffusion method and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined by using the agar dilution method.

Results: Giving the results, carotenoids of *R. glutinis* was effective on the growth of all the tested bacteria, so that *Bacillus cereus* and *Salmonella enteritidis* were the lowest and highest sensitivity to this pigment, respectively. The highest MIC and MBC among the tested bacteria were observed for *S. enteritidis* and *Escherichia coli*, respectively; whereas MBC was not observed for *S. enteritidis* at concentrations of the tested pigment.

Conclusions: Gram-positive bacteria were more sensitive than Gram-negative bacteria against the antimicrobial activity of pigments of *R. glutinis*. According to the results, pigments of *R. glutinis* can be used as an inhibitor of bacterial growth.

Keywords: *Rhodotorula*, Carotenoids, Microbial Sensitivity Tests

1. Background

The need to colorants from natural sources has increased because of increasing awareness of consumers about toxicity of synthetic colors. Natural colors that are often called biocolors, because of their biological source usually extracted from vegetables, fruits, roots, and microorganisms [1].

Carotenoids are one of the main pigments, which are prepared from plant and microbial sources. The pigments that belong to the chemical group known as isoprenoid polyenes are lipid-soluble and yellow-orange-red pigments [2]. Carotenoids derived from plant have various issues such as instability to heat, light, changes in pH, low solubility, and often are non-convenient access during a year. Nowadays, researchers are seeking pigment production from microorganisms at various industries. The rapid growth of pigment-producing microorganisms, inexpensive medium, relatively easy procedure to extract pigment, independent to the weather conditions, and the wide variety of colors are the benefits of pigment production from microorganisms, in addition to benefits in human and animal health [3, 4]. Pigments producing microorganisms are completely ordinary in nature. Several microorganisms such as *Micrococcus*, *Bacillus*, *Monascus purpureus*, *Rhordosporidium*, *Rhodotorula*, *Sporobolomyces*, *Sporobolomyces* and *Phaffia* are considered as potential pigment sources [2].

Nowadays, food consumers and patients are seeking natural additives and/or traditional medicines in foods instead of synthetic types. For instance, one in three people in the US has used at least one form of alternative drug. Plants and microorganisms still represent a large source of natural bioactive compound that might serve as leads for the development of novel drugs [5, 6]. To date, the food-borne diseases and the non-performance of customary chemotherapy to obtain a reduction in the mortality rates for these problems, such as cancer and diseases caused by microbial contamination, inflammation and sensitivity, indicates a crucial need for new approaches to the control [7, 8].

Carotenoids have been studied comprehensive and verified to show different beneficial effects on human

health through serving as precursors of vitamin A, anti-inflammatory effect, antimicrobial and antioxidant activity etc. [8-12].

2. Objectives

The aim of this study was evaluation of the antimicrobial activity of pigments extracted from *R. mucilaginosa* (PER) on some food pathogenic bacteria and fungi.

3. Methods

This research study was done in Gorgan University of Agriculture and Natural Resources.

3.1. Microorganism and Materials

Rhodotorula glutinis (PTCC 5256) were obtained from Persian type culture collection (PTCC), biotechnological department of Iranian research organization for science and technology (IROST), Tehran, Iran. All chemical materials and culture media were of analytical grade and purchased from Merck (Germany), Sigma-Aldrich (UK).

3.2. Condition of Pigment Production

A single colony was transferred from the stock culture on YPG agar to 50 mL YPG broth followed by incubated at 27°C for overnight. Three mL of YPG broth was used for inoculation of 100 mL semi-synthetic medium in 500 mL flask, then stored in a shaker incubator at 150 rpm and 27°C for 72 hours [13].

3.3. Pigment Extraction

Microorganism cells were harvested by centrifugation at 10.000 rpm for 20 minutes followed by washing with distilled water and centrifuged again. To extract carotenoid pigments the method described by Bhosale and Gadre [14] was used with some modification. Briefly, cells were ruptured 2 times with 10 mL of acetone and broken by homogenizer (Scilogex D500, US). Then, the suspension was centrifuged and the supernatant was gathered. Acetone extracts were collected and carotenoid pigments were extracted with a same volume of petroleum ether.

3.4. Microorganisms and Inoculums Preparation

Antimicrobial activities of PER were studied against *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1539), *Streptococcus pyogenes* (PTCC 1447), *Escherichia coli* (PTCC 1269), *Salmonella enteritidis* (PTCC 1709), *Enterococcus faecalis* (PTCC 1393), and *Listeria monocytogenes* (PTCC 1163). Microorganisms were grown in trypticase soy broth (TSB) at 35°C for overnight. Overnight cultures were adjusted to match a 0.5 McFarland standard followed by diluting in 1: 100 with mueller-hinton broth (MHB). The dilution was used as the inoculums for antibacterial activity assay [11].

Alternaria citri and *Penicillium digitatum* were cultured on sabouraud dextrose agar (SDA). Spores and hyphae were scraped off with a sterile wire loop. The sterile tubes containing *A. citri* and *P. digitatum* were diluted by ringer solution until the solution turbidity equalizes with 0.5 McFarland standard solutions through spectrophotometrically measuring turbidity at 530 nm. The suspension must have contains 1.5×10 CFU/mL [14].

3.5. Antimicrobial Activity

3.5.1. Paper Disc Technique

Disk diffusion method was carried out to evaluate antimicrobial activity of PER. In practice, an amount of inoculums (0.1 mL) was spread on Mueller-Hinton agar (MHA) (for bacteria) and SDA (for fungi). Sterile paper disks (6 mm in diameter) were soaked into PER at concentrations of 0.5, 1.5, 2.5, 3.5 and 5 mg/mL. After incubation of plates at 37°C for 24 hours (for bacteria) and 27°C for 72 hours (for fungi), the halo of inhibition was measured. Penicillin or gentamicin disks were placed in the plates as a comparative standard. The result was obtained by measuring the microbial free zone area diameter. Three replicates were performed for each bacterium the mean values are presented [11, 15].

3.5.2. Determination of Minimum Inhibitory Concentration (MIC)

Agar dilution method was used to determine MIC of PER against some microorganisms. In practice, the pigment at concentrations of 2, 4, 8, 16, 32, 64, and 128 mg/mL was mixed with sterile MHA (for bacteria) and SDA (for fungi), followed by 0.1 mL of microbial suspension (0.5 McFarland) was plated as pour-plat. The plates were incubated at 37°C for 24 hours (for bacteria) and 27°C for 72 hours (for fungi), and then the MIC of the pigments was evaluated. The MIC was defined as the lowest concentration of PER in a plate with no visible growth. A plate contain medium and without microorganism, and a plate include medium and microorganism was considered as negative and positive control, respectively [16].

3.5.3. Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The agar dilution method was used also to determine MBC and MFC PER. With the difference that bacteria and molds were sub-cultured from the plate containing MIC of the pigment to nutrient broth (NB) and yeast extract glucose chloramphenicol (YGC) agar, respectively. If the sub-cultured microorganisms cannot grow, the MIC and MBC

and/or MFC concentrations will be equal. However in contrast to the situation, from the grown bacteria were subcultured in MHA (for bacteria) and SDA (for fungi) containing the further of the pigments concentrations (8, 16, 32, 64, and 128 mg/mL) and the MBC considered as concentration of the pigment that bacteria did not grow [11, 15].

4. Results

4.1. Disk Agar Diffusion

Table 1 show the results of disk diffusion test of PER against some bacteria and fungi. Giving the results, PER had antimicrobial activity on the all of examined microorganisms. As is obvious in Table 1, B. cereus and *A. citri* have the highest and the lowest sensitivity to PER, respectively.

4.2. Determination of MIC

Table 2 shows the MIC of PER on the examined microorganisms. The results revealed that PER had bacteriostatic and fungi static activities against all the examined microorganisms. PER had the lowest MIC (8 mg/mL) against *B. cereus* and *S. pyogenes*, and the highest MIC (128 mg/mL) against *A. citri*.

4.3. Determination of MBC

The MBC and MFC of PER against the examined microorganisms are shown in Table 3. The highest MBC (64 mg/mL) of PER was observed against *E. coli* and *S. enteritidis*. The MBC of the other bacteria was same, 32 mg/mL. However, no fungicidal activity against *A. citri* were observed for PER.

5. Discussion

It is important to study scientifically natural additives and/or traditional medicines that have been used to determine potential sources of novel antimicrobial compounds. Natural compound based antimicrobial compounds have enormous therapeutically potential as they can serve the function without any side effects that are often associated with synthetic types [17].

According to result, PER had antibacterial effect and the activity was increased by increasing concentration of PER. PER had more antibacterial activity than antifungal activity against the examined microorganisms, so that PER had 19.6 and 11.8 mm of microbial free zone area against *B. cerus* and *A. citri* at concentration of 5 mg/mL. Among bacteria, Gram-negative bacteria had less sensitively compared to Gram-positive types, which represents higher resistance of Gram-negative bacteria to PER (Table 1). The similar finding were observed by Umadevi and Krishnaveni [18]; and Manimala and Murugesan [19] for carotenoid pigment extracted from *Micrococcus luteus* KF532949 and *Sporobolomyces* sp. isolated from natural source, respectively, which reported that Gram-negative bacteria had higher resistance to the carotenoid pigments.

The results of MIC assay revealed that PER had more antibacterial effect on Gram-positive bacteria compared to Gram-negative types, so that Gram-positive bacteria such as *B. cereus* and Gram-negative bacteria such as *E.coli* and *S. enteritidis* showed lowest and highest MIC, respectively. The same finding were observed by Mohammadi et al. [17], Galindo-Cuspinera et al. [20], Smith-Palmer et al. [21], and Yolmeh et al. [11] for essential oil of *Achillea wilhelmsii*, annatto extract (2.8% norbixin), plant essential oils and essences, and annatto dye, respectively. This is probably due to presence of lipopolysaccharide in cell wall of Gram-negative bacteria. Lipopolysaccharides of cell wall can prevent influx of active compounds to cytoplasmic membrane of these bacteria [22].

It is observed that MIC of PER against the examined fungi was higher compared to the MIC measured against bacteria, which represents higher resistance of fungi compared to bacteria against PER. Ahmad and Beg [23] studied antimicrobial and phytochemical properties of 45 Indian medicinal plants against multi-drug resistant human pathogens. Their results revealed that bacteria had more sensitivity to this medicinal plants compared to fungi.

Given Table 3 PER had bactericidal activity against all examined bacteria. The highest and lowest MBC against studied bacteria were measured 32 and 64 mg/mL, respectively. At the used concentrations of PER, no fungicidal activity was observed against *A. citri*, whereas MFC of PER was measured 128 mg/mL against *P. digitatum*. Galindo-Cuspinera et al. [24] observed also the lowest MIC and MBC of 2.8% norbixin for B. cereus; in addition, they reported that 2.8% norbixin had not bactericidal effect on *S. typhimurium*. Manimala et al. [25] studied antimicrobial activity of carotenoid pigment produced from yeast *Rhodotorula mucilaginosa* YP 187 and reported that the pigment showed excellent antibacterial activity than the standard chloramphenicol. Among this, pigment showed maximum inhibition against *B. subtilis* and *S. aureus*. Lapenda et al. [26] antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398, and reported that antimicrobial activity of this pigment against Gram positive bacteria was more than Gram negative types.

In conclusion, PER is a natural pigment to use as antimicrobial agent. Giving the results, PER had more antibacterial activity compared to fungicidal activity; as well as, more antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. Therefore giving the results of this study, PER can be used as a substitution for syn-

Table 1. Average Diameter (mm) of Microbial Free Zone Area of PER[a]

Microorganism	0.5 mg/mL	1.5 mg/mL	2.5 mg/mL	3.5 mg/mL	5 mg/mL	Antibiotic Disk
B. cereus	10.6	13.0	15.9	18.0	19.6	13.5 P
E. coli	9.1	11.0	12.4	13.5	14.1	12 G
S. aureus	9.9	12.5	15.2	17.0	18.5	32 P
S. Enteritidis	8.9	11.4	12.4	13.2	13.8	10 G
L. monocytogenes	9.1	11.6	13.8	14.5	15.8	22 P
S. pyogenes	9.8	12.9	15.0	16.9	19.1	14 P
E. faecalis	9.6	12.4	14.6	16.0	17.3	13.5 P
A. citri	6.9	8.9	10.0	10.9	11.8	
P. digitatum	8.1	10.4	11.4	12.5	13.1	

[a] P, antibiotic disc of penicillin, G, antibiotic disc of gentamicin.

Table 2. MIC of PER Against Some Bacteria and Fungi[a]

Microorganism	2 mg/mL	4 mg/mL	8 mg/mL	16 mg/mL	32 mg/mL	64 mg/mL	128 mg/mL	NC	PC
B. cereus	+	+	-	-	-	-	-	-	+
E. coli	+	+	+	+	-	-	-	-	+
S. aureus	+	+	+	-	-	-	-	-	+
S. enteritidis	+	+	+	+	-	-	-	-	+
L. monocytogenes	+	+	+	-	-	-	-	-	+
S. pyogenes	+	+	-	-	-	-	-	-	+
E. faecalis	+	+	+	-	-	-	-	-	+
A. citri	+	+	+	+	+	+	-	-	+
P. digitatum	+	+	+	+	+	-	-	-	+

[a] NC, negative control; PC, positive control; +, grew; -, not grew.

Table 3. MBC and MFC of PER Against Some Bacteria and Fungi[a]

Microorganism	8 mg/mL	16 mg/mL	32 mg/mL	64 mg/mL	128 mg/mL
B. cereus	+	+	-	-	-
E. coli	+	+	+	-	-
S. aureus	+	+	-	-	-
S. enteritidis	+	+	+	-	-
L. monocytogenes	+	+	-	-	-
S. pyogenes	+	+	-	-	-
E. faecalis	+	+	-	-	-
A. citri	+	+	+	+	+
P. digitatum	+	+	+	+	-

[a] +, grew; -, not grew.

thetic colorants and preservatives. The authors suggested that the antimicrobial activity of PER along with other pigments and/or extracts or within a food system be examined on microorganisms.

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