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ORIGINAL ARTICLE

# Antimicrobial and anti-biofilm effects of carotenoid pigment extracted from *Rhodotorula glutinis* strain on food-borne bacteria

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## ABSTRACT

**Background and Objectives:** Carotenoid pigments are among the most important pigments and have many applications in various food, cosmetics, hygiene industries and biotechnology. These pigments are produced by plants and microorganisms including *Rhodotorula* spp. This research intended to study the antimicrobial and antibiofilm effects of the carotenoid pigment from *Rhodotorula glutinis* on food spoilage bacteria (*Staphylococcus aureus* and *Salmonella* Typhimurium).

**Materials and Methods:** The *R. glutinis* was isolated from milk samples of cows with mastitis and ITS sequence-based typing was performed on them. After extracting the pigment from *R. glutinis*, its purity was examined using thin-layer chromatography. Following that, the broth microdilution method was used to evaluate antimicrobial effects of the pigment and MtP assay and subsequently scanning electron microscopy were used to assess the antibiofilm effects. In addition, the sub-MIC effects of the pigment on expression of quorum-sensing (QS) genes in *S.* Typhimurium isolates (*sdiA* and *luxS*) and *S. aureus* isolates (*hld*) were studied. Finally, the degree of toxicity of the pigment was analyzed using the MTT assay.

**Results:** ITS sequence analysis of *R. glutinis* revealed that the recently separated isolates exhibited strong differences with the strains recorded in NCBI database in genetic structure. The pigment produced by *R. glutinis* had strong antimicrobial effects and its mean MIC against *S.* Typhimurium isolates (17.0 µl.ml$^{-1}$) was higher than the mean MIC against the *S. aureus* isolates (4.1 µl.ml$^{-1}$). Electron microscope images and real-time observations indicated that the sub-MIC values of the pigment suppressed biofilm formation by suppressing expression of QS genes. In addition, the mentioned pigment at high MIC concentrations did not have toxic effects on Vero cells.

**Conclusion:** This research suggests that *R. glutinis* pigment is effective in destroying the planktonic form of food spoilage bacteria and degrading food spoilage biofilm-forming bacteria. Moreover, considering the low toxicity level of *R. glutinis* pigment for eukaryotic cells, we can suggest its use as a natural antibacterial preservative in various food materials.

**Keywords:** *Rhodotorula glutinis*; Food-borne bacteria; Carotenoid pigment; Quorum sensing; Anti-biofilm

## INTRODUCTION

Foodborne diseases (FBDs) caused by microorganisms are a major and growing public health problem, and most countries that have systems for recording and reporting FBDs have documented and reported substantial increases in incidence of these diseases (1). *Staphylococcus aureus, Listeria mono-*

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*cytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* are among the important microorganisms causing FBDs (2, 3). Most of these bacterial species can be transferred to people through consumption of food materials of animal origin (dairy products, eggs and various types of meat) (4). One of the important and basic problems in the failure to control foodborne bacteria is their increased resistance to antibiotics. Many factors are involved in the development of drug resistance in microorganisms including intrinsic resistance factors such as cell wall, drug resistance genes, efflux pumps, and biofilm formation (5). A biofilm is an assemblage of bacteria on biotic or abiotic surfaces that produce a polysaccharide layer around themselves to prevent penetration of antimicrobial agents. Biofilm formation in bacteria is regulated by quorum sensing (QS). In fact, QS refers to a cell-cell communication process that includes production of diffusible extracellular molecules. This process regulates biofilm formation and also plays a role in expression regulation of virulence genes, antibiotic resistance and access to food materials (6). Consequently, this process is a suitable target for controlling microbial agents without the intervention of antibiotics, which is called anti-QS therapy (7).

In general, spread of antibiotic resistance and emergence of new diseases have prompted researchers to try to discover and identify new antimicrobial drugs. Natural compounds have attracted greater interest in researchers because they are less toxic to eukaryotic cells (8, 9). Microbial pigments are among natural compounds with various medicinal properties including antibiotic, antitumor, wound healing, and antioxidant ones (10). Compared to other biotic resources, production of pigments by using microorganisms has more advantages because their propagation is rapid and easy, requires inexpensive culture media, is not dependent on weather conditions, allows greater diversity in color, and it is more convenient to extract materials from microorganisms (10, 11). *Micrococcus, Bacillus, Phaphia, Rhodotorula* and *Monascus purpureus* are among the most important pigment-producing microorganisms (12-14). The *R. glutinis* is a very important fungal species in the production of various pigments. This species of yeast has been used at industrial scale to produce carotenoid pigments and to serve as a biological control agent against post-harvest decay in fruits (15). Carotenoids are among important and widely used pigments obtained from plant and microbial sources. They are isoprenoid polyene compounds that form lipid-soluble yellow, orange and red pigments (16, 17). These pigments also exhibit antioxidant activity, enhance immunity and prevent mutagenicity and transformation (16). Therapeutic effects of carotenoids include their use in treating cancer, cataracts and cardiovascular diseases (17, 18). Nevertheless, few and scattered studies have been conducted worldwide on antibacterial and anti-biofilm formation properties of these pigments. However, this research attempted to study the effects of this carotenoid in controlling foodborne bacteria such as *S. aureus* and *S.* Typhimurium so that, if it exhibits good efficiency as a suitable medicinal agent, further research can be conducted on it.

## MATERIALS AND METHODS

**The studied bacteria.** *Staphylococcus aureus* (ATCC 25923) and *Salmonella* Typhimurium ST38 were the two foodborne bacterial strains studied in this research to determine the antimicrobial effects of the carotenoid pigment in *Rhodotorula*. In addition, a number of clinical isolates of *S. aureus* (from milk samples in cows with mastitis) and *S.* Typhimurium (from chicken meat samples), all of which were able to form strong biofilms, were studied in this research (9, 19).

**Isolation and identification of the *Rhodotorula* species of yeast.** Twenty-five milk samples from cow with mastitis were taken and sent to the microbiology laboratory in a container with ice for isolation of the *Rhodotorula* species of yeast. The milk samples were cultured separately on Potato Dextrose Agar (PDA) and incubated for 24 h in 37ºC. The suspected *Rhodotorula* colonies were identified using a macroscopic (shape and size of colony) and microscopic methods (Lactophenol Cotton Blue Staining) and performing biochemical tests including urease, starch hydrolysis and carbohydrate fermentation tests. In addition, a sequence of the ITS in the ribosomal RNA gene was identified using PCR for the final confirmation of the *Rhodotorula* species (the procedure used was the same as in previous research) (20).

**Sequencing and phylogenetic analysis of *Rhodotorula*.** The nucleotide sequence of the ribosomal RNA gene in the *Rhodotorula* isolate was

determined and recorded in the NCBI database. Furthermore, its sequence was blasted on the Nucleotide Blast on the NCBI website to investigate the epidemiological situation of the recent isolate, and it was studied and compared with the genes recorded in the NCBI database with respect to sequence similarity. The phylogenetic tree was calculated with the maximum likelihood method with 1,000 bootstrap replicates using MEGA software (version 3.1) (21).

**Pigment extraction.** After incubation of *Rhodotorula* yeast in broth culture medium for 86 hours at 37°C, it was centrifuged at 200 rpm. The precipitated yeast cells were washed several times with PBS, added to the biomass obtained from 1N HCl and placed in a water bath at 70ºC for 90 min. The pigment was then extracted using the nonpolar solvents such as petroleum ether, n-hexane, ethanol, and acetone (25:25:50 v/v/v). Finally, the colored solution was filtrated and then concentrated using a rotary concentrator and the concentrated extract was turned into powder using a freeze dryer. The obtained pigment was stored in a lyophilized form in special vials at -18ºC until use (22).

**Pigment purification.** Thin layer chromatography (TLC) with TLC silica gel paper (Merck, 60 $F_{254}$) and ethyl acetate as the solvent was used to purify the pigment. Using a capillary tube, some of the pigment extract was put on the silica gel paper. The paper was then placed in the chromatography tank together with a mixture of the 80% ethyl acetate and 20% n-Hexane solvents. Based on capillary action, the solvents together with the pigment rise up the chromatography paper and a spot forms for each pigment (if the pigments are pure) (23).

**Determination of MIC and MBC of the pigment.** The MIC and MBC values of the pigment against the studied bacterial species were assessed based on the guidelines of the Clinical and laboratory standard institute (CLSI). In short, 100 µL bacterial suspension (0.5 McFarland turbidity standard) and 100 µL serial dilutions of the pigment in Mueller Hinton broth (0.06-61.5 mL $mL^{-1}$) were poured into each well of the ELISA microplates. There were one negative control containing dimethyl sulfoxide and one positive control containing DMSO, bacteria and norfloxacin (1 mg/mL) on the last row. The ELISA microplates were then incubated aerobically at 37°C in 5% $CO_2$ for 24 h. The lowest concentration of the pigment without any observable growth after overnight incubation was considered the MIC. To determine MBC, 5 µL solution from each of the last three wells that did not show any bacterial growth were spot inoculated on agar blood plates and incubated overnight at 37°C. MBC was reported as the minimum concentration at which no bacterial colonies were formed (24).

**Effect of the *Rhodotorula* pigment on the biofilm formation.** MtP assay was used to evaluate the effect of the pigment on biofilm formation by *Salmonella* and *Staphylococcus.* In brief, TSB (98 µL) containing MIC/2, MIC/4, MIC/4 and MIC/16 of the pigment and 2 µL of a 24-hour culture of each bacterial isolates were separately added to the wells. After incubating the plates for 24 h at 37°C, the wells were emptied and the adherent cells were stained with 200 mL crystal violet (0.2%) for 20 min. The wells were then washed, the crystal violet absorbed by the adherent cells was dissolved in 95% ethanol (100 mL) and the values of optical density (OD) were determined using a UV-Vis spectrophotometer (9, 25).

**Expression of biofilm genes.** Sub-MIC effects of the pigment on expression of the genes involved in biofilm formation were studied separately in the *S.* Typhimurium and *S. aureus* isolates using the qPCR test (26, 27). After culturing the bacteria on TBS and using the pigment at the final concentration (1/2MIC), bacterial total RNA was extracted using RNA extraction kits (SINACLON, Iran). The complementary DNA (cDNA) was then synthesized using cDNA synthesis kits (SINACLON, Iran) and the random primer and copying the synthesized RNA model. The primers, qPCR assay and the thermocycling conditions were as in previous research. The primer sequence used in this study is presented in Table 1. Also, the qPCR Program is given in Table 2.

**Cytotoxicity assay.** Cytotoxicity of *Rhodotorula* pigment was evaluated, using 3-(4, 5-dimethylthiazol-2-yl) - 2, 5-diphenyltetrazolium bromide (MTT) assay through the previously described method. Vero cells were cultured in the RPMI-1640 medium that supplemented with 10% fetal calf serum, 1% (w/v) glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin as the growth medium (All materials prepared from Gibco, UK). Then, the *Rhodotorula* pigment was added at different concentrations in 96-well plates and distributed by Vero cells. After incubation

**Table 1.** Primers used in Real-Time PCR assays

| Bacteria | Target gene | Sequence 5′- 3′ | TmºC | Reference |
|---|---|---|---|---|
| *S*. Typhimurium | 16S rRNA | F: AGGCCTTCGGGTTGTAAAGT | | (27) |
| | | R: GTTAGCCGGTGCTTCTTCTG | | |
| | *luxS* | F: ATGCCATTATTAGATAGCTT | 54 | |
| | | R:GAGATGGTCGCGCATAAAGCCAGC | | |
| | *sdiA* | F: AATATCGCTTCGTACCAC | | |
| | | R: GTAGGTAAACGAGGAGCAG | | |
| *S. aureus* | 16S rRNA | F: AGCCGACCTGAGAGGGTGA | | (26) |
| | | R: TCTGGACCGTGTCTCAGTTCC | | |
| | *Hld* | F: ATTTGTTCACTGTGTCGATAATCC | 57 | |
| | | R: GGAGTGATTTCAATGGCACAAG | | |
| *Rhodotorula* | *ITS* | F: TCCGTAGGTGAACCTGCGG | | (20) |
| | | R: TCCTCCGCTTATTGATATGC | | |

**Table 2.** qPCR Program

| Step | Time | Temperature | Cycle |
|---|---|---|---|
| Primary denaturation | 10 min | 95 | 1 |
| Denaturation | 45 sec | 95 | |
| Annealing | 45 sec | *S*. Typhimurium= 54 | |
| | | *S. aureuse*= 57 | 40 |
| | | *Rhodotorula*=57 | |
| Extension | 45 sec | 72 | |

of plates (37ºC in humidified air containing 5% $CO_2$ for 24 h) and removing of the MTT dye, fluorescence signals were measured at 490 nm using a microplate reader (ELx808, BioTek, USA).

**Statistical analysis.** Each experiment was performed at least three times, and the data are expressed as means + SD. Eventually, the results were analyzed by Student's t-test using SPSS software and $P<0.05$ was considered statistically significant.

## RESULTS

**Isolation and identification of *Rhodotorula*.** Out of 25 milk samples suspected of mastitis, one sample was infected with *Rhodotorula* spp., which was confirmed as *R. glutinis* species after microscopic and macroscopic examination and biochemical and molecular tests. Also, the color of the seeds produced by *R. glutinis* in potato dextrose agar medium is orange as shown in Fig. 1.

The 18s rRNA nucleotide sequence of *Rhodotorula* yeast was registered in the GenBank with accession number MZ323884, Also, the result of blasting determined that the identified yeast belongs to *R. glutinis* species. Phylogeny analysis and dendrogram drawing using Mega3 software created 5 different clusters. The present strain was placed in cluster II together with the LC413754.1 strain and had a 100% similarity with it. In total, the results of the dendrogram indicated the existence of a dominant type, all of which were placed in the same cluster (Fig. 2).

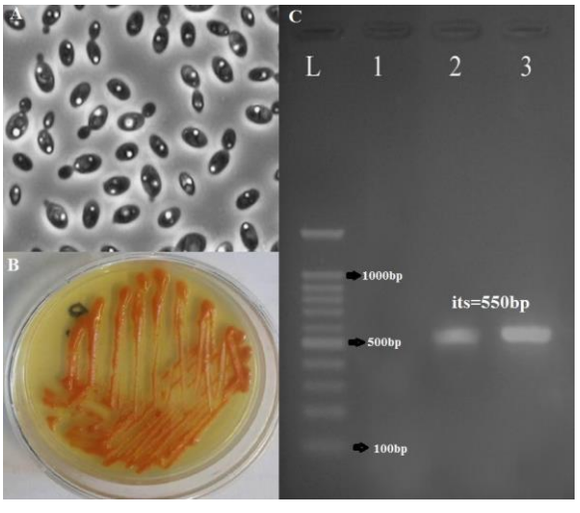


**Fig. 1.** Molecular and phenotypic identification of *Rhodotorula*:
A: Light microscope image of *Rhodotorula*, B: Production of orange pigment color in potato dextrose agar medium, C: Identification of its gene in *Rhodotorula* isolate, Lane 1: negative control (contained no template DNA), Lane 2: positive control, Lanes 3: *Rhodotorula* isolate, Lane L: a 100 bp DNA ladder

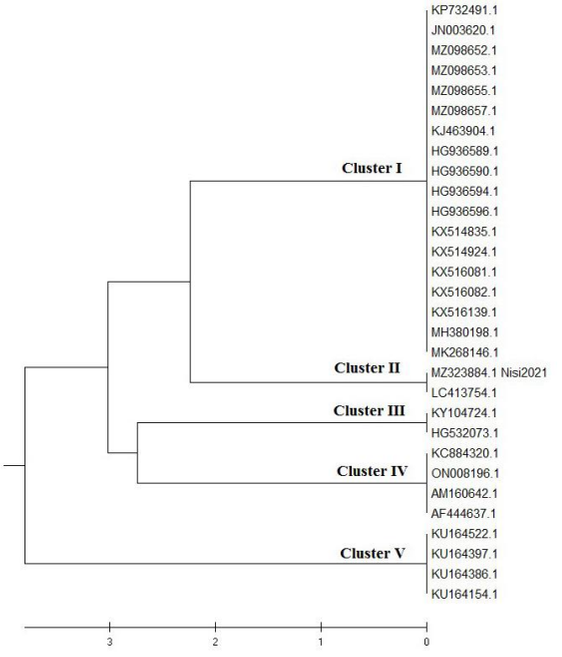


**Fig. 2.** Dendrogram drawn for 31 isolates of *R. glutinis* (recent isolates along with 30 isolates recorded in the GenBank) based on its (18s rRNA) gene sequence.

**Carotenoid pigment.** After preparation and purification, the pigment obtained was measured in terms of quality by thin layer chromatography, and the stain obtained on filter paper showed that it has high purity.

**MIC and MBC determination.** The effect of *Rhodotorula* pigment on plankton cell growth was determined by broth microdilution method. The geometric mean of MIC values for *S. aureus* and *S.* Typhimurium was 4.1 µl.ml$^{-1}$ and 17.9 µl.ml$^{-1}$, respectively. Also, the geometric mean of MBC for *S. aureus* was reported as 8.1 µl.ml$^{-1}$ and for *S.* Typhimurium as 23 µl.ml$^{-1}$. Further details are shown in Table 3.

**Effect of the pigment on the biofilm formation.** Fig. 3. shows the biofilm formation of *S.* Typhimurium and *S. aureus* isolates in the presence of sub-MIC concentrations of *Rhodotorula* pigment. In the MtP assay, 1/2MIC and 1/4MIC concentrations of the pigment played an important role in inhibiting biofilm formation by all bacteria (P<0.001).

**Electron Microscopy (SEM).** The results of SEM analysis showed that in the control slides, the bacteria were piled up and strongly stuck together and formed a strong biofilm. But the bacteria treated with *Rhodotorula* pigment are far apart and there is no strong connection between them. Also, in the groups trained with *Rhodotorula* pigment, lysed and perforated bacterial cells were observed (Fig. 4).

**Quantification of gene expression by qPCR.** The expression of quorum sensing genes of *S. aureuse* (*hld*) and *S.* Typhimurium (*sdiA* and *luxS*) gens were evaluated in the presence and absence of *Rhodotorula* pigment. The result of qPCR showed that the expression levels of these genes were significantly ($P < 0.01$) reduced in clinical and reference strains (treated by MIC/2 of pigment) Compared to negative control (non-treated by *Rhodotorula* pigment). As shown in the Table 4, the *Rhodotorula* pigment caused a significant down regulation of all genes in all isolates.

**Cytotoxicity assay.** The MTT assay is applied to measure cytotoxicity of *Rhodotorula* pigment and the results revealed that the *Rhodotorula* pigment reduced cell viability in a dose-dependent manner starting (Fig. 5). $IC_{50}$ values of *Rhodotorula* carotenoid pigment were calculated as 46.5 µL. mL$^{-1}$.

## DISCUSSION

High levels of drug resistance among microorganisms have made it difficult to control infections caused by them and have caused deep concern in human medicine and veterinary medicine (5). In addition, use of antibiotics to treat livestock diseases has led to the presence of residual antibiotics in livestock products. These drugs enter human body through foodstuffs and cause various abnormalities and diseases (28). Consequently, researchers are trying to find natural antimicrobial materials for treating infectious diseases including those caused by consuming livestock products (29). Natural compounds are good candidates for this purpose because they contain potent antimicrobial agents, have much fewer side effects for humans and livestock, and are less toxic to eukaryotic cells (9). As a result, interest in conducting research on use of microbial pigments in the framework of traditional and complementary medicine has increased substantially. Many foodborne bacterial species form biofilms and hence are beyond the reach of antimicrobial agents in food industry factories and food packaging containers and endanger the health of communities by being

**Table 3.** MIC and MBC of *R. glutinis* pigment against the *S. aureus* and *S.* Typhimurium isolates

| Isolates | Source | *Rhodotorula* pigment | | Isolates | Source | *Rhodotorula* pigment | |
|---|---|---|---|---|---|---|---|
| | | MIC µl.ml$^{-1}$ | MBC µl.ml$^{-1}$ | | | MIC µl.ml$^{-1}$ | MBC µl.ml$^{-1}$ |
| *S. aureus* ATCC25923 | - | 3.8 | 7.7 | *S.* Typhimurium ST38 | Chicken | 15.4 | 15.4 |
| *S. aureus* M1 | Milk | 3. 8 | 7.7 | *S.* Typhimurium Ch1 | Chicken | 15.4 | 15.4 |
| *S. aureus* M2 | Milk | 7.7 | 15.4 | *S.* Typhimurium Ch2 | Chicken | 15.4 | 30.7 |
| *S. aureus* M3 | Milk | 3.8 | 7.7 | *S.* Typhimurium Ch3 | Chicken | 30.7 | 30.7 |
| *S. aureus* M4 | Milk | 1.9 | 3.8 | *S.* Typhimurium Ch4 | Chicken | 15.4 | 15.4 |
| *S. aureus* M5 | Milk | 3.8 | 7.7 | *S.* Typhimurium Ch5 | Chicken | 15.4 | 30.7 |
| Geometric mean | | 4.1 | 8.1 | - | | 17.9 | 23 |

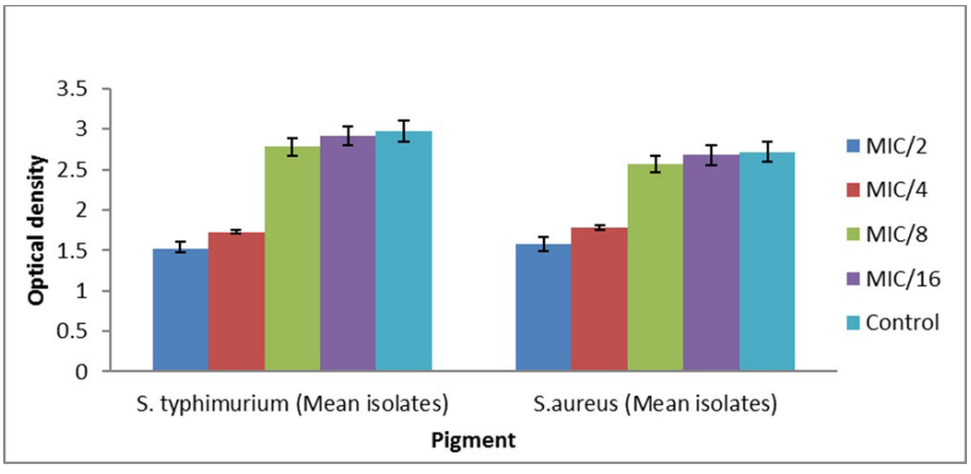


**Fig. 3.** Effect of the pigment in different concentration on the biofilm formation. The concentration of MIC/2 and 1.4 MIC of pigment has significantly reduced the expression of the constitutive gene.

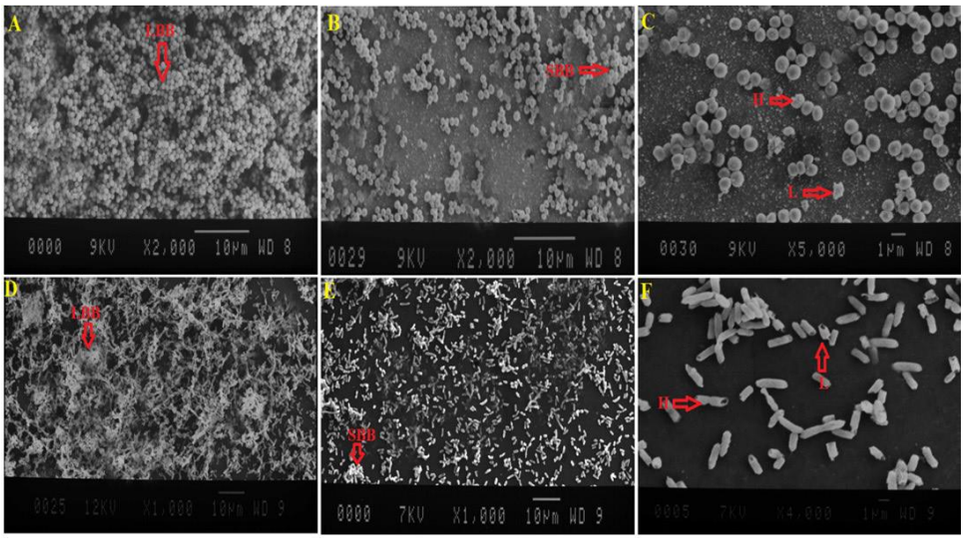


**Fig. 4.** Effect of *Rhodotorula* pigment on biofilm of *S. aureuse* and *S.* Typhimurium strains. A: Untreated group of *S. aureuse.* B and C: treated group of *S. aureuse* by *Rhodotorula* pigment at MIC/2. D: Untreated group of *S.* Typhimurium. E and F: treated group of *S.* Typhimurium by *Rhodotorula* pigment at MIC/2. LBB: Large biofilm biomass, SBB: small biofilm biomass, H: holes, L: lysis

**Table 4.** The expression of quorum sensing gens in bacterial treated with 1/2MIC of *Rhodotorula* pigment; a: significant downregulation; b: non-significant downregulation

| Bacteria | Gene | Bacteria | gene | |
|---|---|---|---|---|
| | ***Hld*** | | ***sdiA*** | ***luxS*** |
| *S. aureus* ATCC25923 | -3.86 | *S.* Typhimurium ST38 | -3.46 | -3.35 |
| *S. aureus* M1 | -4.57 | *S.* Typhimurium Ch1 | -4.13 | -3.85 |
| *S. aureus* M2 | -5.83 | *S.* Typhimurium Ch2 | -3.18 | -2.82 |
| *S. aureus* M3 | -3.13 | *S.* Typhimurium Ch3 | -3.67 | -3.42 |
| *S. aureus* M4 | -4.64 | *S.* Typhimurium Ch4 | -2.32 | -3.47 |
| *S. aureus* M5 | -3.90 | *S.* Typhimurium Ch5 | -3.34 | -3.32 |
| Geometric mean | -4.4 | Geometric mean | -3.3 | -3.4 |

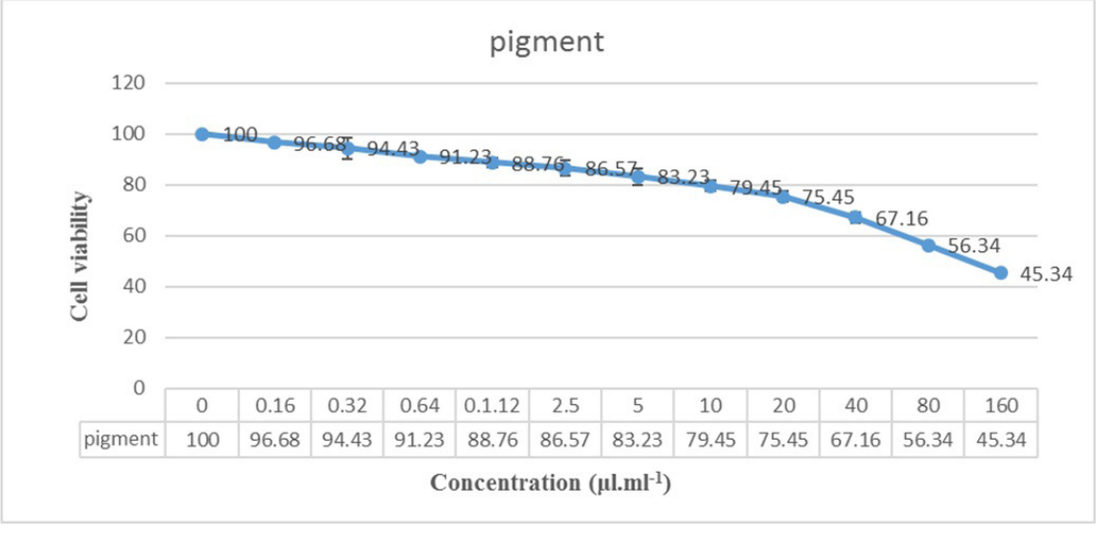


**Fig. 5.** Viability percentage of Vero cells after treatment by *Rhodotorula* pigment

transferred to food materials (11, 30). Therefore, this research intended to study the effects of the natural pigment in *Rhodotorula glutinis* on planktonic and biofilm forms of the important bacterial species that are transferred to food materials.

A *R. glutinis* strain was isolated from milk samples of cows with mastitis in this research. The milk samples infected with the species of yeast were not infected with other bacterial species causing mastitis including *Escherichia coli* and *Staphylococcus aureus.* This finding suggested that there may be competition between *R. glutinis* and bacterial species that cause mastitis that has eliminated them as agents causing mastitis. However, a synergistic relationship is usually observed in relation to bacteria causing mastitis so that sometimes several bacterial species are isolated from milk samples of cows with mastitis (31). In this study, the *R. glutinis* strain identified using the data in NCBI website based on ITS gene sequence was typed and the dendrogram results specified that it was closely similar to the *R. glutinis* strain identified in Japan (LC313754.1). However, there was one difference between it and the common strains that have become epidemic in recent years, which showed that there was a strong genetic difference between the recent strain and the internationally recorded ones. Of course, the type of milk sample and the location of isolating the species of yeast may be among the factors that caused the genetic difference between the strains.

The MIC and MBC values were first determined for the pigment in order to study its effects on *S. aureus* and *S.* Typhimurium. The results indicated that it had strong bactericidal effects on the mentioned bacterial species. In this regard, Manimala and Murugesan (2014) reported that the antimicrobial effect of

*Rhodotorula* pigment on *S. aureus* and *Bacillus subtilis* was even stronger than that of the chloramphenicol (32). The difference in the effect of *Rhodotorula* pigment in various studies can be due to the strains used in pigment production. In this relation, researchers have shown that pigments extracted from the mutated strains had greater antimicrobial and antioxidant activities compared to that extracted from the wild-type strain (33). In addition, in this research, the sensitivity of *S. aureus* as a Gram-positive bacterial species was greater than that of *S.* Typhimurium probably due to the presence of lipopolysaccharide in the cell walls of Gram-negative bacteria that prevents entry of the pigment into the bacterial cells thus making it very resistant to pigments (34). In this relation, it was reported in the research by Yolmeh et al. (2016) that resistance of intestinal bacteria to *Rhodotorula* pigment was greater than that of *Bacillus cereus* (30). In addition, it was shown in a number of other studies that Gram-negative bacteria exhibited more resistance to microbial pigments compared to Gram-positive bacteria, which is in agreement with the findings of the present research (35).

Since biofilm formation is one of the main reasons for drug resistance in bacteria, the present study studied the effect of sub-MICs of the pigment on biofilm formation by performing the MtP assay and scanning electron microscopy. The results revealed that the pigment significantly suppressed biofilm formation at 1/4 MIC and ½ MIC. In the same vein, Bin et al. (2012) studied the effect of melanin on biofilm formation by *Pseudomonas aeruginosa* and *Escherichia coli* and reported that this pigment was able to suppress biofilm formation by these bacterial species. Electron microscopy and analysis suggested that the biofilms were thinner and softer in the groups treated with melanin. Their findings are in agreement with those of the present research (36). However, electron microscopy results in the present study showed that sub-MICs completely prevented biofilm formation by strains of *Salmonella* and by *S. areus*, and in the electron microscope slides assemblages of bacteria were not observed in the groups treated with the pigment.

Biofilm formation by bacteria is usually controlled by QS systems. Since it was thought that the reason for prevention of biofilm formation by the pigment was probably its effect on QS genes, expression levels of QS genes in *S.* Typhimurium and *S. areus* after applying sub-MICs of the pigment were assessed. The results indicated that *Rhodotorula* pigment significantly decreased expression of the QS genes in both bacterial species, but this reduction among the strains of *S. areus* (geometric mean=4.4) was 10 times larger compared to *S.* Typhimurium (geometric mean=3.4). These results were compatible with those of the MtP assay and scanning electron microscopy. Researchers have used many natural compounds up to now such as algae (37, 38), extracts from medicinal plants such as the mint family (39), substances in some fruits such as carrot, tomato, pepper, and garlic (9, 40) materials extracted from fish intestine and from microorganisms (41), and materials and substances obtained from soil and sea (42) as QS suppressants. However, no research has been conducted on *Rhodotorula* pigment or on its effect on expression of QS genes. This is the first time that QS suppressing property of *Rhodotorula* pigment is reported. In a similar study, Zhu et al. (2011) reported that *A. auricula* pigments were able to suppress production of violacein, one of the factors in the QS system of the genus *Chromobacterium* (43). Although they did not study the direct effects of the pigment on biofilm formation by *Chromobacterium*, it is possible that, as in the present research, biofilms are not formed by *Chromobacterium* because of QS suppression. Ali et al. (2000) investigated the effects of *Lactobacillus plantarum* KU200656 (KU200656) supernatant on the expression of *Staphylococcus aureus* and *Salmonella* Typhimurium biofilm genes and showed that *Lactobacillus* probiotic supernatant inhibits the expression of genes involved in biofilm formation. In this case, several studies have been done on the effect of probiotics supernatant on the expression of biofilm genes. Lee, et al. (2000) investigated the effects of *Lactobacillus plantarum* KU200656 (KU200656) supernatant on the expression of *S. aureus* and *S.* Typhimurium biofilm genes and showed that the cell-free supernatant (CFS) of KU200656 inhibited biofilm formation by pathogenic bacteria. In addition, half of the minimum inhibitory concentration of the KU200656 CFS downregulated the expression of biofilm-related genes, as determined by quantitative real-time PCR (44). It is possible that if probiotic supernatant and *Rhodotorella* yeast pigment are used at the same time, prevention of biofilm formation and transmission of bacteria through food can be done more easily.

Although research on therapeutic properties of microbial pigments shows they are suitable for and efficient in medical and veterinary applications, these

pigments some undesirable properties such as toxicity for eukaryotic cells, which necessitate caution be exercised in using them (45). The present research studied toxicity of the extracted pigment on Vero cells. The results showed that these compounds at concentrations several times higher than MIC did not cause much toxicity and could be used in food products. However, extensive research must be carried out if we want to use them as drugs so that necessary attentions should be paid if they have adverse side effects such as causing allergic reactions.

## CONCLUSION

The results of isolating a *Rhodotorula* species from milk samples of cows with mastitis indicated that there may be competition between *Rhodotorula* and bacterial species causing mastitis. In addition, *R. glutinis* pigment did not have toxic effects on eukaryotic cells and was able to damage planktonic and biofilm forms of bacteria of food and livestock origins. Consequently, it is suggested that *R. glutinis* carotenoid pigment be used to control foodborne bacteria in the food industry. However, medicinal use of *Rhodotorula* pigment requires extensive research.

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