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BENEFICIAL ROLE OF QUERCETIN AGAINST OXIDATIVE STRESS-INDUCED SENESCENCE IN *Caulobacter crescentus*

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Abstract—Aging is a common complex sequential and degenerative process, and the accumulation of reactive oxygen species (ROS) leads to changes in homeostasis thereby causing oxidative stress which further leads to cellular aging. Quercetin, a plant flavonoid, has been shown to exhibit antioxidant and anti-inflammatory properties. In this study, we aimed to test the effect of quercetin on chemically induced oxidative stress in *Caulobacter crescentus* as well as to evaluate its antioxidant effect. For this, we have performed various biochemical and anti-aging assays to understand the effectiveness of quercetin in oxidative stress. In quercetin-treated bacterial cells, the activity of lipid peroxidase, catalase, superoxide dismutase, as well as total thiol content were significantly increased compared to hydrogen peroxide-treated bacterial cells, confirming the effectiveness of quercetin. In addition, quercetin showed an anti-aging effect as evidenced by collagenase and ferric-reducing/antioxidant power assay. Our study indicated that quercetin has anti-aging and antioxidant potential to prevent oxidative stress-induced cellular damage and delay cellular senescence in *C. crescentus*. The molecular docking was performed to represent the atomic level interaction between the ligand (quercetin) and the cell cycle regulatory protein, allowing us to define ligand behavior in the target protein binding sites, as well as elucidate key biochemical processes. The results of this study confirm that natural isolated active compounds can act as potent agents to delay the oxidative stress-induced aging process. However, additional extensive research is needed to confirm the anti-aging effects of quercetin in oxidative stress-caused aging.

Keywords: aging, *Caulobacter crescentus*, flavonoids, oxidative stress, quercetin, senescence

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INTRODUCTION

Aging is an unavoidable and irreversible process that can be described as a progressive deterioration of physiological functions as the organism ages [1–3]. Aging is a time-dependent mechanism, in which the cellular structural integrity is being gradually lost [4, 5]. This makes the body extremely susceptible to a number of incurable diseases [6]. Cellular aging, or senescence, is generally defined as a progressive decline in the resistance to stress and other cellular damages, causing a gradual loss of cellular functions and resulting ultimately in cell death [7, 8]. Under stress conditions, the accumulation of free radicals occurs in the cells, which cause cellular damage with time and is considered one of the common causes of aging [9, 10]. Chemicals such as superoxide anion, hydroxyl radical, hydrogen peroxide, and nitric oxide, which can provoke the generation of reactive oxygen species (ROS), are natural by-products of cell metabolism that play a vital role in homeostasis and cell signaling [11]. Excessive production of ROS results in replicative senescence and oncogene-induced senescence. Aging research opens up great prospects in the area of gerontology. It has been established that bacteria are the main subject for genetic manipulations [12]. The biological aging control mechanisms can be effectively identified in the *Caulobacter crescentus* bacteria, which are used as a model organism to study cell cycle regulation and aging [13].

Abbreviations: DMSO – dimethyl sulfoxide; DTNB – 5, 5'-dithiobis-(2-nitrobenzoic acid); EDTA– ethylene diamine tetra acetic acid; FRAP – Ferric reducing antioxidant power; GSH – glutathione; LPO – lipid peroxidation; MDA – malondialdehyde; OD_{420} – optical density at a wavelength of 420 nm; PBS – phosphate-buffered saline; PYE –peptone yeast extract; RNS – reactive nitrogen species; ROS – reactive oxygen species; SOD – superoxide dismutase; TCA – trichloroacetic acid, TBA – thiobarbituric acid.

C. crescentus is the first bacterium reported to exhibit replicative aging in which cytokinesis is intrinsically asymmetrical [14].

Natural ingredients have been used for reducing oxidative stress-induced aging both as dietary supplements and topical cosmetic formulations. Among phytochemicals, flavonoids are an excellent source of anti-oxidants that constitute a class of naturally occurring polyphenolic compounds extracted from fruits and vegetables [15, 16]. It was reported that quercetin is a potent ROS/RNS and nitric oxide scavenger, and possesses anti-inflammatory, anti-tumorigenic, and anti-proliferative properties [17]. Quercetin and its derivatives were shown to play a highly effective role against various oxidation-related abnormalities, including aging [18, 19].

Therefore, in this study, we focused on testing the antioxidant properties of quercetin and its effect to suppress the chemically (H_2O_2)-induced oxidative stress in *C. crescentus*. We emphasize the usefulness of the bacterial model in understanding how ROS and oxidative stress contribute to aging-related diseases, since there is substantial evidence that an increased ROS production and decreased ROS-scavenging ability shorten lifespan. Additionally, the ROS accumulation causes oxidative stress, which in turn leads to cellular damage. It is widely recognized that ROS play an important role in the molecular mechanisms that influence lifespan [20]. In *Caulobacter* sp., stress-induced deviation from normal cell shape parameters occurs when the sensitive cell division or cell cycle processes are stopped while cell growth continues. *Caulobacter*, like many other bacteria, responds to stress by filamentation. This process may result directly from a stress-induced damage or indirectly from stress-perception mechanisms intentionally targeted to the function of essential proteins in the interests of survival. Both division and growth arrest can be seen in response to oxidative stress in bacteria [21]. The free radical theory states that organisms age due to the accumulation of ROS-induced oxidative damage. Overall, this study demonstrates the effect of quercetin on H_2O_2-induced oxidative stress in bacteria as a model system for aging studies [22].

MATERIAL AND METHODS

Conditions for Bacteria Cultivation and Growth

A bacterial strain of *C. crescentus* (MTCC 7510T), a microbial-type culture collection, was provided by the Institute of Microbial Technology (IMTECH), Chandigarh, India.

Bacterial cells were grown in PYE medium containing in 50 mL of distilled water: peptone – 0.100 g, yeast extract – 0.50 g, NaCl – 0.010 g, at 37°C for 18 h [23]. The cells were grown on PYE agar in Petri plates at 37°C.

Purchasing and Storage of the Quercetin Compound

The quercetin compound was purchased from Sigma-Aldrich, India (catalog number Q4951 – 100 g).

Preparation of Quercetin Stock solution and Working Solution

For the stock solution, 0.151 mg of quercetin was gradually dissolved in 100 μL of dimethyl sulfoxide (DMSO), its volume was adjusted to 5 mL by adding autoclaved Milli-Q-water and the stock solution was stored at 4°C. Different working solutions of quercetin were prepared and used for further experiments.

Preparation of Hydrogen Peroxide Working Solution

The 9.8 M stock solution of H_2O_2 (100 mL, HiMedia Pvt. Ltd, India, catalog number PCT1511) was prepared as provided by the manufacturer. The working H_2O_2 solutions (2 mM and 5 mM) were freshly prepared by diluting the H_2O_2 stock solution.

Gram Staining

This procedure is most commonly used staining techniques in microbiology to distinguish bacteria based on the cell wall composition. The gram staining protocol is a sequence of four main steps. First, a heat-fixed smear of fresh bacterial culture was placed on the surface of the glass slide, and then crystal violet was applied for 1 min, followed by the addition of an iodine solution. The next step consisted of a rapid decolorization and the addition of an organic solvent of acetone or ethanol to remove the blue dye. Finally, counterstaining with Safranin was performed.

Kirby-Bauer Disk Diffusion Susceptibility Assay

Kirby–Bauer assay was performed to test the antibiotic effect of the quercetin compound. The cells were grown up to log phase and spread over PYE agar using the cotton swab technique. Paper disks infused with different concentrations of quercetin and H_2O_2 were carefully placed on the plate on top of the culture-containing agar. Then the plates were incubated at 37°C for 18 h.

Preparation of Bacterial Cell Suspension

The treated bacterial cells were centrifuged at 7155 g for 10 min at 4°C. After that, they were suspended in a lysis buffer and incubated for 30 min of in an ice bucket. Then the suspension culture was subjected to a brief sonication in the presence of ice and centrifuged at 2795 g for 15 min at 4°C. The supernatant was collected and stored at 4°C for further assays.

Experimental Groups

The experimental groups of bacterial cells were as follows:

1. Control – only bacterial cells;

2. $Q_{10\,\mu M}$ – cells treated with 10 μM quercetin;

3. $Q_{100\,\mu M}$ – cells treated with 100 μM quercetin.;

4. $H_{2\,mM}$ – cells treated with 2 mM H_2O_2.;

5. $H_{5\,mM}$ – cells treated with 5 mM H_2O_2.;

6. $Q_{10\,\mu M} + H_{2\,mM}$ – cells treated with 10 μM quercetin and 2 mM H_2O_2.;

7. $Q_{100\,\mu M} + H_{5\,mM}$ – cells treated with 100 μM quercetin and 5 mM H_2O_2.

Protein Estimation

Bradford method was used for protein estimation. In a 96-well plate, 40 μL of the cell lysate and 160 μL of Bradford reagent were added. Bovine serum albumin (BSA) was used as a standard. All reactions were performed in triplicate. Absorbance was measured at 650 nm. The protein concentration in the unknown sample was determined using the plotted standard curve.

Biochemical Assays

Lipid Peroxidation (LPO) Assay

This analysis was performed based on the protocol of Buege et al. 1978, [23] with slight modifications. Briefly, in a centrifuge tube, 100 μL of cell lysate was mixed with 200 μL of trichloroacetic acid–thiobarbituric acid–hydrochloric acid (TCA–TBA–HCl) buffer. The reaction mixture was heated for 15 min in boiling water and then cooled at room temperature. To obtain a clear supernatant, the reaction mixture was centrifuged at 252 g for 10 min. Milli-Q-water was used as a blank. The Sample absorbance was measured at 535 nm.

Catalase Activity

To estimate catalase activity, the method reported by Chance et al. 1955, [24] Banerjee A et al. 2015 was used with minor modifications. A culture solution (3 mL), 50 mM PBS, 15 mM H_2O_2, and 0.1 mL of lysate were mixed, and the absorbance of the mixture was measured on a spectrophotometer at a wavelength of 240 nm. Readings were taken up to 3 min with an interval of 30 s. The catalase activity was calculated and expressed as μmol/(mg protein min).

Total Thiol Content Assay

Glutathione (GSH) content was measured using the method in Cabiscol et al. 2000 [25] with minor modifications. Briefly, 0.65 mL of cell lysate was mixed with 0.1 mL of 4% sulfosalicylic acid and 0.5 mL of 0.1 M PBS. The mixture volume was adjusted to 2.5 ml by adding 1.25 ml of Milli-Q-water. DTNB (5, 5'-dithio-bis-(2-nitrobenzoic acid) (6.6 μL) was added to the test solution. The tubes were kept for 1 h and 15 min and then vortexed for 15 s. Absorbance at 412 nm was measured; the control did not contain DTNB.

Superoxide Dismutase (SOD) Activity

To measure the SOD activity the method of Wood et al. 2001, [26] was used with slight modifications. The analyzed mixture (1 mL) contained 0.05 M PBS (pH 8.0), 0.01 M EDTA, and 0.27 mM pyrogallol (the latter was prepared in 100 mM HCl). OD_{420} was measured against Tris-EDTA buffer at time zero 1min after the pyrogallol addition and the SOD concentration was calculated in μmol/mg protein. The enzyme activity was expressed as U/mg protein, where 1 U is the amount of enzyme required for about 50% inhibition of pyrogallol auto-oxidation.

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay consisted in measuring the potential of an antioxidant in the reaction of reducing a ferric tri-pyridyl triazine (Fe3+ TPTZ) complex to the ferrous form. The FRAP assay kit was purchased from HiMedia Ltd, India (catalog number CCK072), and used according the manufacturer's protocol with some minor modifications.

Collagenase Inhibition Assay

Collagen degradation of was measured based on a slightly modified method reported in [27]. A mixture was prepared which contained collagenase – 10 μL (0.01 U/mL in the cool equates), Tricine buffer – 60 μL (50 mM, pH 7.5, containing 10 mM $CaCl_2$ and 400 mM NaCl), and the sample – 30 μL (cell lysate, obtained by different treatments). The mixture was then incubated at 37°C for 20 min. Absorbance was measured at a wavelength of 335 nm.

MOLECULAR DOCKING OF QUERCETIN AND SIGNALING PROTEIN

Before molecular docking analysis, the construction of protein ligand and grid optimization were carried out. Information about proteins and ligands were either retrieved from the protein data bank (https://www.rcsb.org/) or they were modeled by *ab initio* using the sequence obtained from the UniProt database (https://www.uniprot.org/). As a final step in ligand preparation, the PDB structure was fed into AutoDock Vina tools where the bonds were made rotatable using a torsion tree and the ligand was extracted in PDBQT format for further analysis. The second molecular preparation (protein, in PDB format to PDBQT) was also carried out in AutoDock tools. Molecular interaction of proteins and ligands was deter-

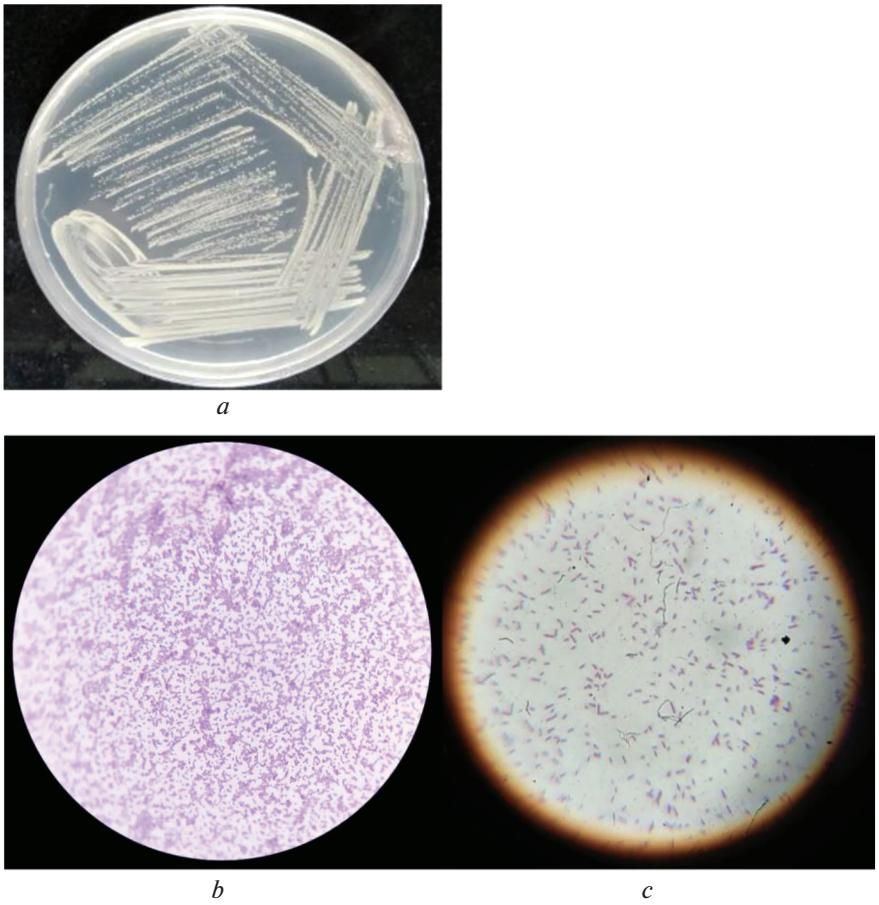

Fig. 1. (*a*) – bacterial culture of *Caulobacter crescentus* after 24-h growth on peptone yeast extract medium; (*b*) – gram staining of *Staphylococcus aureus* (gram +ve bacteria); (*c*) – gram staining of *C. crescentus* (gram – ve bacteria).

mined by AutoDock Tool 4.2 [28] and AutodockVina tools [29].

Statistical Analysis

Data were obtained in at least two to three independent experiments, each in triplicate. The results were expressed as mean ± standard deviation. All data were statistically analyzed using GraphPad 4.0 software; data at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Gram Staining

The cells of *C. cresentus* were cultured on PYE agar in Petri plates at 37 °C; the grown bacterial cultures are shown in (Fig. 1*a*).

Staining aimed at distinguishing between gram-positive and gram-negative bacteria showed that the *C. crescentus* cell wall retained the pink color after staining with Safranin dye, which indicates that these are gram – ve bacteria (Fig. 1*c*). At the same time, *S. aureus*, used as a reference of gram-negativity, was shown to retain its purple color (Fig. 1*b*). Reports state that *C. crescentus* cell wall consists of a single peptidoglycan layer, which is usually surrounded by a lipopolysaccharide wall containing phospholysaccharide and proteins.

Kirby–Bauer Disk Diffusion Susceptibility Assay

The presence or absence of growth around the disks impregnated with a certain compound is an indirect criterion of the ability of that compound to inhibit the given microorganism. No inhibition zone formation was observed around the disks with any tested concentration of quercetin, indicating that quercetin at the dose used was non-toxic for the bacteria, in other words, this dose is not lethal for them. However, broad inhibition zones were observed when using H_2O_2 or H_2O_2 in combination with quercetin as compared to the control. Moreover, the combination $Q_{100\ \mu M} + H_{2\ mM}$ displayed a smaller inhibition area than the control antibiotic disc or H_2O_2 at other doses, which shows that $Q_{100\ \mu M}$ could protect bacteria from the inhibitory effect of H_2O_2 (Fig. 2).

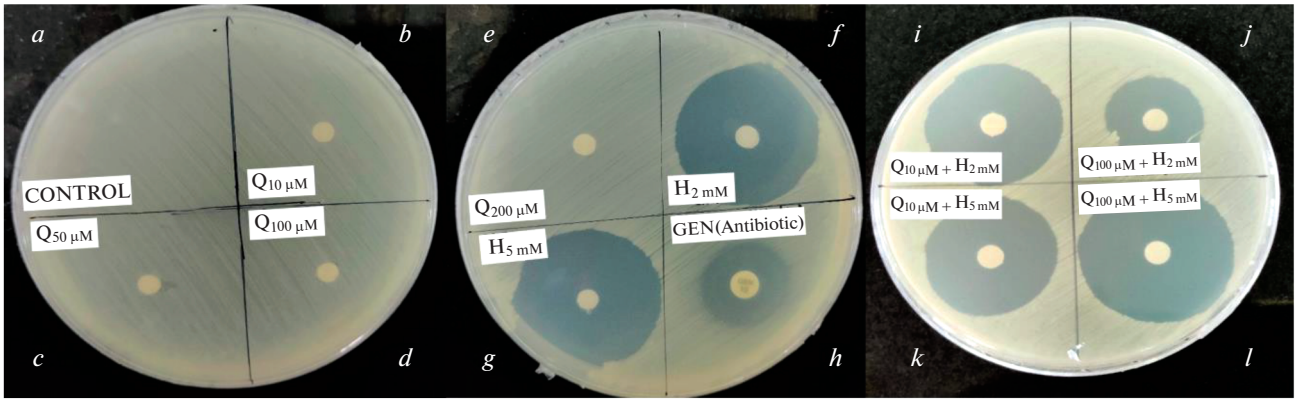

Fig. 2. Kirby–Bauer disk diffusion assay: (*a*) – control, intact bacterial cells; (*b*) – 10 μM quercetin (Q_{10}); (*c*) – 50 μM quercetin (Q_{50}); (*d*) – 100 μM quercetin (Q_{100}); (*e*) – 200 μM quercetin (Q_{200}); (*f*) – 2 mM H_2O_2 ($H_{2\,mM}$); (*g*) – 5 mM H_2O_2 ($H_{5\,mM}$); (*h*) – gramicidin antibiotic (GEN); (*i*) – 10 μM quercetin + 2 mM H_2O_2 ($Q_{10\,\mu M} + H_{2\,mM}$); (*j*) – 100 μM quercetin + + 2 mM H_2O_2 ($Q_{100\,\mu M} + H_{2\,mM}$); (*k*) – 10 μM quercetin + 5 mM H_2O_2 ($Q_{10\,\mu M} + H_{5\,mM}$); (*l*) – 100 μM quercetin + 5 mM H_2O_2 ($Q_{100\,\mu M} + H_{5\,mM}$).

Lipid Peroxidation Assay

In this study, we showed that LPO activity in cells, previously increased as a result of treatment with H_2O_2, decreased after treatment with quercetin. Options $Q_{2\,\mu m} + H_{5\,Mm}$ and $Q_{10\,\mu m} + H_{5\,Mm}$ exhibited a decrease and an increase in the lipid peroxidation activity, respectively, as compared to $H_{5\,mM}$, which indicates that quercetin was able to reduce LPO activity to some extent at a lower concentration (Fig. 3*a*).

Catalase Activity

Catalase is part of the cellular defense against reactive oxygen species. Catalases are enzymes that primarily detoxify hydrogen peroxide in bacteria. They are classified into three types, and most bacteria appear to express one or more catalases. Studying the effect of quercetin on catalase activity in both control and treated bacterial groups, we found that quercetin can boost the activity of this enzyme and thereby may enhance the cellular defense mechanism. In our experiments, H_2O_2 seemed unable to significantly increase catalase activity; however, after quercetin treatment, H_2O_2-induced stress could indeed increase catalase activity and boost the defense system. In the $Q_{100\,\mu m}$ option, quercetin caused an increase in catalase activity due to a higher dose of the *katG* gene, which is subject to multiple levels of regulation and is likely responsible for various signals. Hence, whenever it changes, the H_2O_2 production may also change [30]. Since the *C. crescentus katG* gene functions as an H_2O_2 scavenger, we assume that quercetin treatment may enhance the expression of this gene, increase catalase levels and thus strengthen cellular defense (Fig. 3*b*).

Total Thiol Content

The results show that after hydrogen peroxide treatment, the thiol content in bacterial cells was much lower than in the control group. However, quercetin treatment caused a significant increase in total thiol levels as compared to control and H_2O_2-treated cells. It was also found that the ($Q_{100\,\mu M}$+$H_{2\,mM}$)-treated group exhibited a significantly higher ($p < 0.002$) increase in total thiol content compared to $Q_{10\,\mu M} + H_{2\,mM}$ (Fig. 3*c*).

Superoxide Dismutase Activity

The level of SOD was estimated in the control and ($Q_{10\,\mu M}$ and $Q_{100\,\mu M}$)-treated group; the results show a significant increase in SOD level in the treated cells. However, in groups treated with $Q_{10\,\mu M} + H_{2\,mM}$ and $Q_{10\,\mu M} + H_{5\,mM}$, a decrease in SOD activity was observed compared to the control group. SOD activity was significantly elevated in bacterial cells, treated with quercetin alone, compared to cells treated with hydrogen peroxide. The greatest increase in SOD activity was observed in option $Q_{100\,\mu M} + H_{2\,mM}$ in comparison with the H_2O_2-treated groups (Fig. 3*d*).

FRAP Assay

In this study, the $Q_{100\,\mu M}$-treated group showed an increase in FRAP activity compared to the control. This evidence reflects a higher radical scavenging activity after quercetin treatment. However, in any other treated groups, no substantial change in FRAP activity was observed (Fig. 3*e*).

Collagenase Inhibition Assay

For this, a colorimetric assay was used. In this study, the quercetin-treated group $Q_{10\,\mu M}$ showed stronger enzyme inhibition compared to the control group. After treatment of bacteria with $Q_{10\,\mu M} + H_{2\,mM}$ and $Q_{100\,\mu M} + H_{2\,Mm}$, both cultures showed a higher inhibition of the collagenase enzyme compared to that after $H_{2\,mM}$-treatment; however $Q_{10\,\mu m}$ induced much

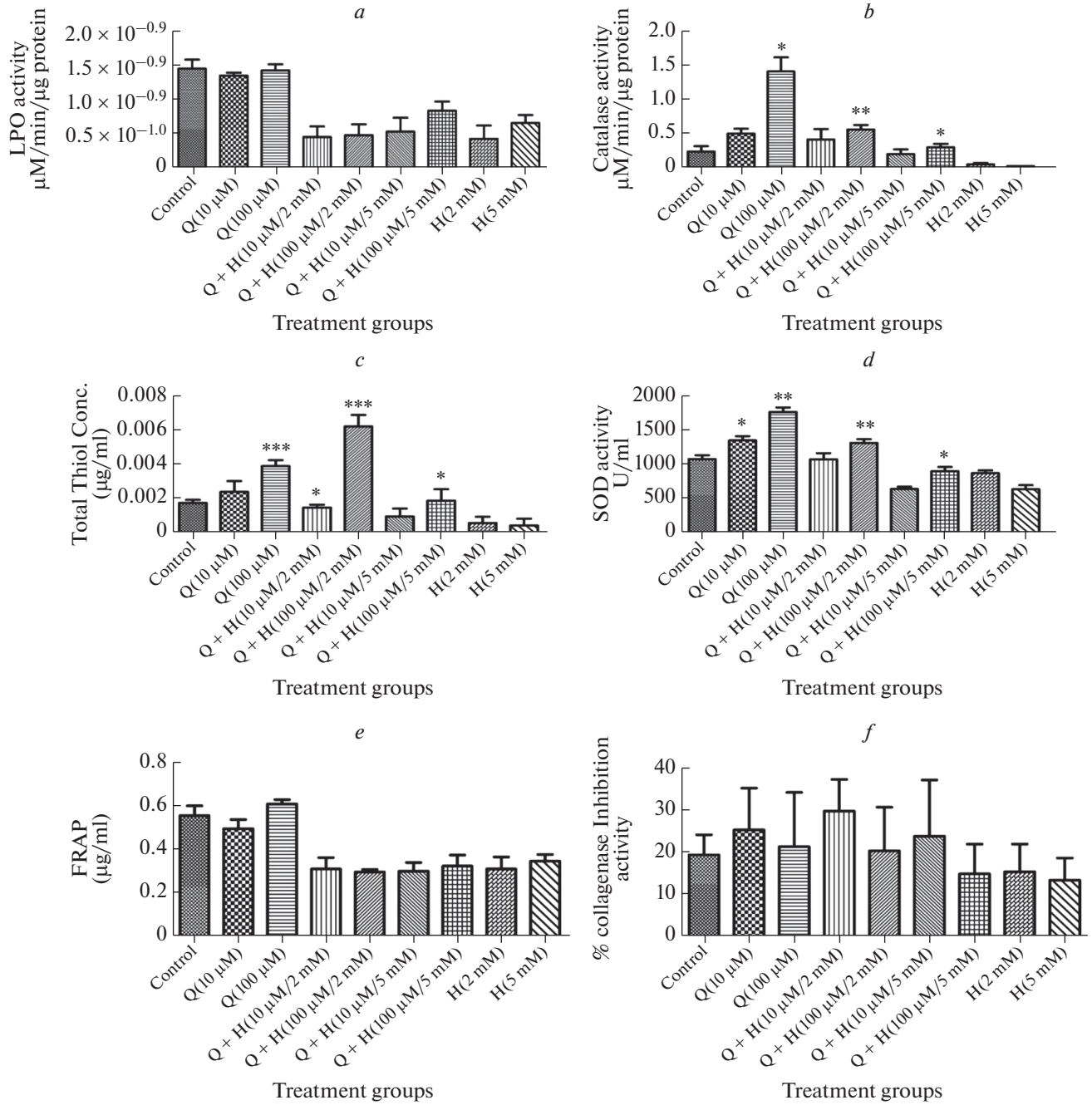

Fig. 3. Biochemical assays: (*a*) – lipid peroxidation assay; (*b*) – catalase assay; (*c*) – total thiol content; (*d*) – superoxide dismutase assay; (*e*) – ferric reducing antioxidant power (FRAP) assay; and (*f*) – collagenase inhibition activity.

stronger inhibition when used in combination with $H_{2\,mM}$ than that after combined treatment with $Q_{100\,\mu M} + H_{2\,mM}$. The same inhibition trend was also observed in the groups treated with $Q_{10\,\mu M} + H_{5\,mM}$ and $Q_{100\,\mu m} + H_{5\,mM}$ (Fig. 3*f*).

Binding Site Analysis

Docking studies were used to evaluate different bonds, including hydrogen bonds between amino acids, to analyze the potential of three proteins (GcrA, CtrA(SCIP), and CcrM), known as major interactors in cell cycle regulation. Fig. 4 shows that the major ligand (quercetin), interacting with protein GcrA, provided a highest binding affinity – 7.6 due to the formation of 6 hydrogen bonds out of 9 bonds (Fig. 4*a* (i) and 4*a* (ii)).

Quercetin was shown to contain a conventional hydrogen bond involving threonine (THR) at the 19th and arginine (ARG) at the 88th position. Protein 2,

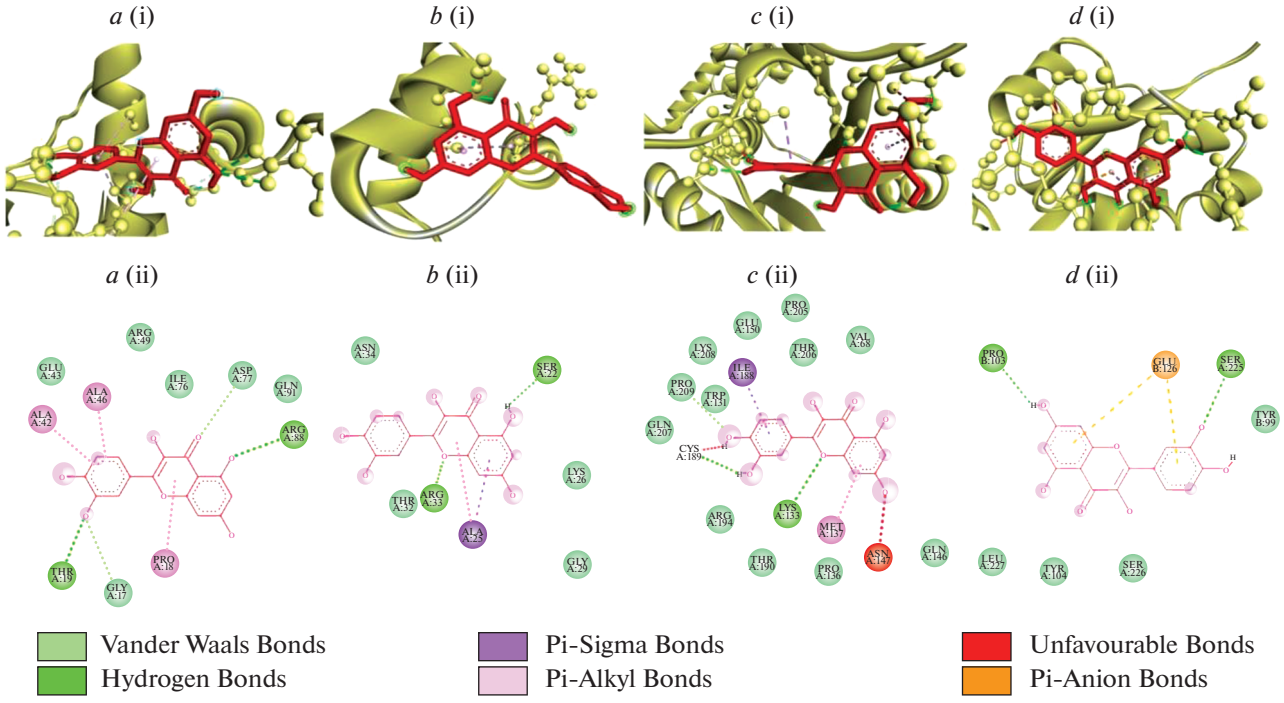

Fig. 4. Binding site analysis using flavonoid quercetin of cell cycle regulatory proteins: *a* (i, ii) – GcrA, *b* (i, ii) – CtrA(SciP), *c* (i, ii) CcrM, and *d* (i, ii) – OxyR

CtrA, had the highest binding affinity of – 6.3 and contained 4 hydrogen bonds out of 7 bonds (Fig. 4*b* (i, ii)). There was a conventional hydrogen bond between arginine (ARG) at the 33th and serine (SER) at the 22nd position. Similar to CtrA, CcrM exhibited a high binding affinity of –9.0 and had a conventional hydrogen bond involving lysine (LYS) at the 33rd position (Fig. 4*c* (i, ii)). Fig. 4*d* (i, ii) indicates the presence of 4 hydrogen, 1 electrostatic and 2 hydrophobic bonds between OxyR and quercetin. The major covalent H bonds were present between the quercetin ligand and amino acids TYR (104), LEU (227), and TYR (99) (exhibiting both O and OH bonds).

Oxidative stress is believed to be a key factor contributing to the initiation and progression of several degenerative diseases, including inflammatory diseases and age-related disorders [31, 32]. Oxidative stress is a leading cause of aging, which can be effectively reduced through the use of natural compounds, such as antioxidant-rich flavonoids [33]. In our experiments, we have used H_2O_2 as an oxidizing agent; although H_2O_2 itself is stable in culture media, it can generate toxic OH radicals in the cell through a transition metal-catalyzed reaction.

Quercetin, a plant-derived polyphenol, has a broad variety of biological functions including anti-carcinogenic, and anti-inflammatory [34]. Quercetin was found to be the most active anti-oxidant flavonoid with significant anti-inflammatory properties that can neutralize harmful free radical reactive molecules that damage cells at high concentrations, increase cell lifespan, and help maintain cell integrity [35, 36]. The accumulation of ROS in the intracellular area can lead to the macromolecule oxidation including lipid peroxidation and protein oxidation, and can further cause DNA damage [37]. In this study, the analysis of the quercetin antioxidant activity was carried out towards lysed bacterial cells exposed to different H_2O_2 doses. It was established that LPO activity increased with the course of the metabolic processes and age. In the LPO activity assay, the flavonoid quercetin, used as part of the combined treatment with $Q_{10\ \mu M} + H_{2\ mM}$, exhibited effective suppression of the free radical level, which was increased in H_2O_2 exposed bacteria. Quercetin treatment was found to suppress oxidative stress by reducing the production of free radicals in the bacterial cell.

Catalase is part of the cellular defense against ROS and it is frequently used by cells to convert H_2O_2 into less reactive gaseous oxygen and a water molecule. In this study, quercetin supplementation was able to reverse H_2O_2- induced oxidative stress by enhancing the synthesis and restoration of catalase and SOD activities, as well as total thiol content. Thiols have been reported to be a key factor determining the overall antioxidant potential of cell homogenates [38]. It is the major endogenous antioxidant produced by cells and directly involved in the neutralization of free radicals and reactive oxygen compounds. The total thiol content in quercetin-treated groups displayed a much

greater increase compared to the group, treated with $H_{2\ mM}$ alone. As a result, quercetin at the given dose was able to prevent and significantly repair cellular damage, caused by the H_2O_2-induced oxidative stress, through a significant increase in the total thiol content and FRAP activity. These findings suggest that quercetin is capable of donating electrons to convert free radicals and terminate the chain reaction. In our previous study, we reported the anti-inflammatory activity of quercetin [39].

It has been found that collagenase cleaves the amino acid bonds in collagen, which possibly leads to some consequences that enhance aging process. In this study, quercetin demonstrated great potential to inhibit collagenase and protect the collagen matrix from degradation. We showed that an increase in H_2O_2-induced oxidative stress alters anti-oxidant defense systems, whereas quercetin is highly effective in prevention oxidative stress-induced cell damage and has the potential to enhance and restore the activity of the antioxidant defense enzymatic system. Therefore, quercetin, due to its anti-oxidant properties, is a natural therapeutic agent for oxidative stress and oxidative stress-induced aging, which maintains normal cell activity and slows down the cellular aging processes.

The docking study is based on the binding proximity of docking poses. Binding site analysis showed that quercetin proved to be a suitable interactor capable of binding to the GcrA, CtrA(SciP) and CcrM cell cycle regulatory proteins with ideal affinities of – 7.6 K/Cal, –6.3 K/Cal and – 9.0 K/Cal, respectively.

GcrA, an important cell cycle regulator in *C. crescentus*, promotes target gene transcription in a fundamentally different manner. The interaction between the GcrA protein and the ligand (quercetin) was facilitated through the involvement of the following amino acids: ARG88, THR19, ILE76, GLY17, ALA42, ALA46, and GLN91. CtrA, a response regulator in *C. crescents*, plays a key role in polar morphogenesis and cell cycle transition by regulating sciP expression. CcrM (cell cycle-regulated DNA methyltransferase) methylates adenine in hemimethylated GANTC after replication. The amino acid interaction with CtrA(SciP) involves ARG33, SER22 and ASN34, while the amino acid interacting with CcrM is LYS133. Additionally, the docking study was to clarify whether OxyR and quercetin could interact directly or not. KatG is the only catalase-peroxidase enzyme in *C. crescentus*, induced by OxyR, an H_2O_2-sensitive transcriptional regulator. Our study revealed a strong interaction between the ligand (quercetin) and the OxyR alpha chain. Computational molecular docking analysis allowed us to predict that the studied 3 main cell cycle regulatory proteins, GcrA, CtrA(SciP), and CcrM, can interact with quercetin, and therefore, this compound may be one of the most important therapeutic targets that we can use in future studies related to aging or the cell cycle.

CONCLUSIONS

Our results confirmed that in *C. crescentus*, H_2O_2-induced toxicity leads to lipid peroxidation, alteration of the cellular collagenase composition, and degradation of catalase, SOD, and thiols. We demonstrated that the flavonoid quercetin can reverse the adverse effects of H_2O_2 treatment to some extent. Complementary approaches using natural isolated active compounds proved that they can act as agents to slow down the oxidative stress-induced aging. We propose to conduct further studies of the effect of induced toxicity on membrane biogenesis and membrane asymmetries for oxidative stress signaling. The used flavonoid-rich plant extract may be a potential candidate for a novel natural anti-aging ingredient. However more research is needed to analyze the anti-aging effects of the flavonoid treatment to confirm the results of the study and the proposed hypothesis.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest.

This article does not contain any studies involving animals performed by any of the authors.

This article does not contain any studies involving human participants performed by any of the authors outside the scope of people's normal professional activities.

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Полезная роль кверцетина при старении, индуцированном окислительным стрессом, у *Caulobacter crescentus*

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Старение – это всеобщий комплекс последовательных дегенеративных процессов, при котором накопление активных форм кислорода (АФК) приводит к изменениям в гомеостазе, тем самым вызывая оксидативный стресс и дальнейшее клеточное старение. Было показано, что растительный флавоноид кверцетин проявляет антиоксидантную и противовоспалительную активность. В данной работе мы поставили целью изучить эффект кверцетина на химически индуцированный окислительный стресс у бактерий *Caulobacter crescentus,* а также оценить его антиоксидантное действие. Чтобы понять какова эффективность кверцетина при окислительном стрессе, мы исследовали различные биохимические характеристики и процессы, связанные со старением. Показано, что в клетках, обработанных кверцетином, значительно повышена активность липидпероксидазы, каталазы, супероксиддисмутазы, а также общее содержание тиолов по сравнению с бактериальными клетками, обработанными перекисью водорода, что указывает на эффективность изучаемого флавоноида. Кроме того, с использованием исследования железоредуцирующей/антиоксидантной способности было показано, что кверцетин обладает активностью агента против старения. В нашей работе было установлено, что кверцетин может предотвращать повреждения клеток *C. crescentus*, вызванные окислительным стрессом, и замедлять клеточное старение. Молекулярный докинг был использован для изучения на атомарном уровне взаимодействия между лигандом (кверцетином) и белком, регулирующим клеточный цикл, что позволило нам изучить поведение лиганда в центрах связывания с целевым белком, а также пролить свет на происходящие ключевые биохимические процессы. Результаты данной работы подтверждают, что изолированные активные натуральные соединения могут быть потенциальными агентами против старения, индуцированного оксидативным стрессом. Однако для подтверждения роли кверцетина как агента против старения, вызванного окислительным стрессом, требуются дополнительные масштабные исследования.

Ключевые слова: старение, *Caulobacter crescentus*, фловоноиды, окислительный стресс, кверцетин