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Research Article

Eugenol Exhibits Antimutagenic Activity in Model Yeast *Schizosaccharomyces pombe* by Regulating DNA Damage Repair System: A Transcriptomic Analysis

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ABSTRACT

Free radicals that accumulate in cells can cause oxidative imbalance and trigger oxidative stress at the cellular level. Such conditions lead to damage to genetic material that accelerates cellular aging. Plant secondary metabolites have been reported to exhibit antioxidant activities that have the potential to overcome oxidative stress. Eugenol, derived from cloves (*Syzygium aromaticum*), has been shown to possess antioxidant activity *in vitro*. However, there is no information on the antioxidative activity of eugenol at the cellular level. Our study focused on the effect of eugenol as an antigenotoxic agent in preventing DNA damage caused by oxidative stress treatment. Further analysis on the cellular levels of eugenol was done through a transcriptomics study in the model yeast *Schizosaccharomyces pombe*. Treatment with eugenol may increase the cell viability of yeast exposed to mutagenic UV and EMS, suggesting that eugenol can act as an antigenotoxic agent. Indeed, eugenol treatment can repair DNA damage as indicated by the DNA nicking assay. Eugenol showed potential as an antiaging agent by regulating the cell cycle of *S. pombe*, specifically by slowing down at the G1 phase and progressing towards the S phase. Transcriptomics analysis revealed that eugenol treatment can regulate genes related to DNA damage response, specifically mismatch repair, base excision repair, and Nucleotide excision repair, in the yeast *S. pombe*. Our data suggest that eugenol has the potential to regulate the oxidative stress-induced DNA damage response in yeast *S. pombe*, which supports cell longevity.

1. Introduction

Currently, degenerative diseases are among the leading causes of death globally. According to the World Health Organization (WHO), out of 57 million deaths worldwide, approximately two-thirds are caused by degenerative diseases. One of the degenerative diseases with a particularly high mortality rate is cancer. The etiology of cancer is related to exposure to mutagens, which trigger DNA damage, commonly referred to as DNA adducts or DNA lesions (Basu 2018). DNA damage in cancer-related genes (oncogenes) due to mutagen exposure is a key factor in the onset of cancer (Steen 2000). In healthy individuals, the DNA Damage Repair (DDR) system plays a crucial role in repairing DNA damage caused by mutagen exposure (Chatterjee & Walker 2017). However, with advancing age, the DDR mechanism becomes less efficient, leading to an increased rate of mutations and the accumulation of DNA adducts in oncogenes, which can trigger cancer development (Li *et al.* 2021)

Mutagens that cause DNA mutations can originate from various sources, including external sources (such as toxins in food, water, or air), UV, and radioactive radiation, and internal sources (metabolic or inflammatory byproducts) (Basu 2018). Mutagen exposure has been

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reported to increase the accumulation of reactive oxygen species (ROS) (Srinivas *et al.* 2019). One of the ROS molecules formed due to mutagen exposure is the hydroxyl ion (Francés-Monerris *et al.* 2014). This ion has a high affinity for DNA molecules, leading to the formation of DNA adducts, where the DNA undergoes single-strand breaks (nicking) (Azzouz & Palaniyar 2023). Continuous exposure to mutagens can lead to the accumulation of DNA adducts that overwhelm the DNA Damage Repair (DDR) system, thereby promoting the formation of cancer cells (Rosendahl Huber *et al.* 2021).

One strategy to combat mutagen-induced mutations is through the use of antimutagens. Antimutagens are compounds capable of reducing or even eliminating the mutagenic effects of mutagens (AbdelHakem *et al.* 2020). The actions of antimutagens can be classified into desmutagens and bioantimutagens. Exploring bioactive compounds with dual actions, including desmutagenic and bioantimutagenic activity, is essential. Naturally derived antimutagenic compounds, which are easily sourced and extracted from nature, can ensure the sustainable availability of these compounds to the public. These natural antimutagenic compounds are expected to serve as therapeutic agents for both the prevention and treatment of cancer. In our previous study, eugenol from cloves exhibits antioxidant activity at the cellular level, as demonstrated in studies using the yeast models *Schizosaccharomyces pombe* (Fauzya *et al.* 2019; Anwar *et al.* 2021;) and *Saccharomyces cerevisiae* (Astuti *et al.* 2019; Lesmana *et al.* 2021). Additionally, eugenol has been shown to induce apoptosis, which is crucial for inhibiting the progressive growth of cancer cells (Ariybah *et al.* 2021; Amini *et al.* 2022). It also helps maintain cellular viability when cells are exposed to carcinogenic compounds (Afrendi *et al.* 2023).

In this study, the ability of eugenol to suppress DNA damage caused by mutagen exposure is investigated. Eugenol's antimutagenic potential was evaluated at the cellular level to confirm its *in vitro* antimutagenic activity, including cell cycle analysis and transcriptomic analysis. The cellular level antimutagenic analysis was conducted using the yeast model *Schizosaccharomyces pombe. S. pombe* has contributed significantly to the understanding of essential biological processes such as cellular metabolism, DNA replication, recombination, cell cycle, cell death, protein folding, and organelle biogenesis in higher organisms, including humans (Nielsen 2019; Kachroo *et al.* 2022; Tungmunnithum *et al.* 2022). Therefore, understanding the potential of eugenol in a yeast model would bring essential insight into the cellular mode of action of eugenol in higher organisms.

2. Materials and Methods

2.1. Yeast Strain

Yeast *Schizosaccharomyces pombe* was cultured in YES medium (yeast extract with supplements) at 30°C for one to three days. Eugenol (Sigma Aldrich) was supplemented in liquid YES medium for treatment.

2.2. Antioxidant Test using DPPH Method (2,2-diphenyl-1-picrylhydrazyl)

The antioxidant content of eugenol was measured using the DPPH method. Antioxidant activity was tested in vitro with DPPH (2,2-diphenyl-1-picrylhydrazyl) as a radical agent. A DPPH solution (125 µM) was mixed with eugenol at various concentrations. The solution was incubated at room temperature in the dark for 30 minutes, and then the absorbance was measured at a wavelength of 514 nm. Ascorbic acid was measured as a standard. The percentage of inhibition was then calculated using a linear regression equation to obtain the Inhibitory Capacity (IC_{50}) value (Astuti *et al.* 2021).

The percentage inhibition activity of the sample was calculated using equation 1.

$$\% \text{ Inhibition} = 1 - \frac{\left(\begin{matrix}\text{Absorbance} \\ \text{of sample}\end{matrix} - \begin{matrix}\text{Absorbance} \\ \text{of control}\end{matrix}\right)}{\left(\begin{matrix}\text{Absorbance} \\ \text{of blank}\end{matrix} - \begin{matrix}\text{Absorbance} \\ \text{of control}\end{matrix}\right)} \times 100\%$$

Sample absorbance is the absorbance of eugenol + DPPH, blank absorbance is the absorbance of ethanol + DPPH, while the control absorbance is the absorbance of ascorbic acid.

2.3. UV Antimutagenic Test

This test was conducted to determine the effect of eugenol on mutagenic agents. UV toxicity analysis on yeast was conducted in accordance with a previous study (Asfahani *et al.* 2023). The culture of *S. pombe* yeast was grown on liquid YES media (OD_{600} = 1) and treated by adding eugenol with concentrations of 10, 20, and 40 µg. mL^{-1} into the cell culture. Control and eugenol-treated yeast cells were irradiated with UV-C light with a wavelength of 200-260 nm for 15 minutes as a mutagenic agent. The viability of yeast cells was analyzed after UV exposure. Cell cultures at hours 0 and 12 were taken and diluted in physiological solution to a

concentration of 10^{-4}, which was then spread by the pour plate method using 1,000 µL from each dilution onto a petri dish containing solid YES medium. The medium was then incubated at 30°C for 72 hours. The colony of yeast cells was then calculated.

2.4. Antimutagenic Test with Ethyl Methanesulfonate (EMS)

The antimutagenic test was conducted to determine the effect of eugenol on mutagenic agents. EMS toxicity analysis was conducted according to the previous study (Marques *et al.* 2011). *S. pombe* culture (OD_{600} = 1) was given the addition of 10, 20, 40 µg mL^{-1} eugenol. Cell cultures at hours 0 and 12 were serially diluted in physiological solution up to 10^{-4}, and 1,000 µL from each dilution was plated onto petri dishes containing solid YES medium supplemented with EMS at 0%, 0.25%, and 0.5% using the pour plate method. The medium was then incubated at room temperature (30°C) for 72 hours. The colony of yeast cells was then calculated.

2.5. Cell Cycle Assay

The cell cycle assay method followed the Abcam Propidium Iodide Flow Cytometry Kit protocol as previously described (Lesmana *et al.* 2021). The initial yeast culture had an optical density at 600 nm (OD_{600}) of 0.05 in 15 mL of YES medium. The treatment involved adding 15 µL of eugenol at a concentration of 10 µg/mL, whereas the control culture did not receive eugenol. The yeast cultures were incubated for 16-18 hours, after which they were centrifuged at 4,000 rpm for 5 minutes in a 15 mL Falcon tube. The supernatant was removed, and the pellet was washed with 1 mL of 1x PBS solution and gently resuspended with a micropipette. The pellet suspension was transferred to a 1.5 mL microtube, centrifuged again at 4,000 rpm for 5 minutes, and the supernatant was discarded. The pellet in the microcentrifuge tube was placed on ice, and 300 µL of 1x PBS, along with 700 µL of 99.8% alcohol, were added as a fixation solution. The fixation solution was gently resuspended using a 1,000 µL micropipette to prevent clumping. The cell fixation solution was incubated for 16 hours at 4°C. A propidium iodide dye mixture was prepared by mixing 945 µL of 1x PBS solution, 50 µL of propidium iodide (1 mg/mL), and 5 µL of RNAse (110,000 U/mL). The cell fixation solution was centrifuged at 4,000 rpm for 5 minutes, and the supernatant was discarded. Flushing was performed twice with the addition of 1x PBS solution, and the cell solution was then centrifuged at 4,000 rpm for 5 minutes. Next, the cell solution was added to a propidium iodide mixture in a dark room and incubated for 1 hour at 37°C. Analysis was performed using a flow cytometer (NovoCyte flow cytometer).

2.6. Antigenotoxic Assay: DNA Nicking Assay

This method aimed to determine the effect of treatment on maintaining the pBR322 plasmid in the presence of Fenton reagent stress. The method was performed as described by a previous study (Soumya *et al.* 2019). A mixture of 1 µL of plasmid DNA (50 µg/100 µL), 10 µL of Fenton reagent (2 mM $FeCl_3$, 30% H_2O_2, 50 mM Ascorbic acid, and phosphate buffer), and eugenol with concentrations of 5, 10, 20, and 40 µg. mL^{-1}, and distilled water. The How mixture was incubated for 60 minutes at 37°C. Positive controls or ROS were generated by adding plasmid DNA and the Fenton reagent. For negative controls, only 1 µL of plasmid DNA (50 µg/100 µL) was added. The mixture was loaded into electrophoresis wells containing 1% agarose and electrophoresed for 50 minutes at a voltage of 50V.

2.7. Transcriptomic Analysis, Gene Ontology Analysis, and KEGG Pathway Analysis

RNA extraction and transcriptomic analysis were performed using the method described in a previous study with slight modifications (Geng *et al.* 2019). Sample preparation for RNA extraction was carried out by inoculating S. pombe on YES medium that had been supplemented with the optimal concentration of eugenol (as determined in the previous viability test) and incubated for 24 hours. The incubated samples were collected in volumes of up to 1 mL and then centrifuged at 5000 rpm for 5 minutes at 4°C to harvest the cells. RNA was extracted using the RneasyMini Kit (Qiagen, USA). The quality of the extracted RNA was then examined using a Nanodrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) at wavelengths of 280 nm and 230 nm. The RNA used as a sample has a value between 1.8 and 2 in the reading results at a wavelength of 280:230 nm.

The RNA-seq technique performed transcriptomic analysis. RNA-seq libraries were prepared using the NEBNext® Ultra TM RNA Library Prep Kit for Illumina® (NEB, USA). RNA libraries were then analyzed using an Agilent Bioanalyzer 2100 (Life Technologies, USA) to determine their quality and quantity. The cDNA samples were then sequenced with the Illumina HiSeq 2500. The sequencing results were then analyzed bioinformatically.

Gene functions were annotated using seven databases, including NCBI Nucleotide sequences (Nt), Protein family (Pfam), NCBI non-redundant protein sequence (NR), SwissProt, eukaryotic orthologous groups of proteins (KOG), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Ontology (GO). The resulting data were grouped into three main categories: genes for biological processes (BP), genes for cellular components (CC), and genes for molecular functions (MF). Gene expression data from ontology analysis was then compared with S. pombe gene expression data that was not treated (control).

2.8. Transcriptome Data Analysis

Transcriptomic data were processed using three methods: Principal Component Analysis (PCA) with the help of the RStudio program version 4.0.5, gene ontology analysis was carried out using the Gene Ontology page (http://geneontology.org/), and the Pombase database (https://www.pombase.org). The analysis results grouped genes into three ontology groups: biological process (BP), cellular component (CC), and molecular function (MF), as analyzed on the KEGG Pathway page (https://www.kegg.jp/). The results of the analysis grouped genes into two groups: those that experience increased regulation and those that experience decreased regulation due to the addition of eugenol. The analysis was conducted by analyzing genes related to DNA damage response in cells (DNA Damage Response). The results of the analysis using the KEGG Pathway were re-analyzed using the PCA method.

3. Results

3.1. Antioxidant Activity

The antioxidant assay revealed that eugenol exhibits significant antioxidant activity, with an IC_{50} value of 26±0.04 µg/mL against DPPH (Table 1). This strong antioxidant activity helps counteract the harmful effects of free radicals, as antioxidant compounds can donate hydrogen atoms through a process of electron transfer. The efficacy of these compounds is indicated by a color change in the test solution from purple to yellow due to oxidation by DPPH radicals. Nonetheless, eugenol's IC_{50} value is lower than that of ascorbic acid.

Table 1. Antioxidant activity (IC_{50}) of eugenol against DPPH radicals

Sample	IC_{50} value (µg. mL^{-1})	Category
Eugenol	26±0.04	Very strong
Ascorbic acid	21±0.50	Very strong

3.2. Antimutagenic Assay with UV and EMS

The observation of cell viability of *S. pombe* showed the addition of eugenol into the yeast culture can increase yeast cell viability exposed to UV (Figure 1A). In yeast cultures without eugenol, cell viability was lowest at 0 hours, but increased slightly at 12 hours, with a relative cell concentration of 1.9 × 10^6±0.05 CFU/mL at 0 hours and 2.5 × 10^6±0.13 CFU/mL at 12 hours. The yeast cells showed only a minor increase in viability at 12 hours, likely due to metabolic stress from UV exposure. In contrast, the addition of eugenol enhanced cell viability across all test concentrations. The most significant increase was observed with eugenol at 40 µg mL^{-1}, which led to a 1.94-fold increase compared to the control at 0 hours of UV treatment. In addition, the control with a value of 3.8 × 10^6±0.13 CFU/mL at 0 hours incubation and for cells with 12 hours incubation time increased 3.4 × with a value of 7.8 × 10^6±0.01 CFU/mL. These results align with previous research [34], which demonstrated increased viability in *S. cerevisiae* treated with endophytic bacterial extracts from cloves. In their study, *S. cerevisiae* showed increased viability from a concentration of 3 × 10^4 CFU/mL at 0 hours to 6 × 10^4 CFU/mL after 12 hours.

Antimutagenic assay using EMS indicated that adding eugenol to the yeast culture appears to have antigenotoxic effects by reducing mutations caused by EMS stress, as evidenced by increased cell viability (Figure 1B). In the absence of eugenol and EMS, cell viability in *S. pombe* increased to a relative value of 1, with a count of 1.31 × 10^6±0.05 × 10^6 CFU/mL. When eugenol (10 µg/mL) was added to yeast cultures with 0%, 0.25%, and 0.5% EMS media, cell viability increased by factors of 4.5×, 3.21×, and 2.51×, respectively, compared to the control. *S. pombe* with the addition of 20 µg. mL^{-1} eugenol into yeast cultures grown on EMS media 0%, 0.25%, and 0.5%. respectively had viability increased by 4.7×; 4.2×; and 3.2× compared to the control and had cell viability values of 6.3 × 10^6± 0.02 CFU/mL; 55 × 10^6±0.03 CFU/mL; and 42 × 10^6±0.03 CFU/mL. *S. pombe* with the addition of 40 µg. mL^{-1} eugenol to the yeast culture grown on EMS media 0%, 0.25%, and 0.5%. respectively had viability increased by 9.2×; 3.4×; and 2.6× compared to the control and had cell viability

values of 120 × 10^6±0.02 CFU/mL; 44.3 × 10^6±0.04 CFU/mL; and 35 × 10^6±0.05 CFU/mL.

3.3. Cell Cycle Analysis on S. pombe

The results of the cell cycle analysis showed that the frequency of yeast cells in the G1 phase increased from 18.83 to 58.22% after eugenol treatment. In addition, the treatment of eugenol alters the majority of the yeast life cycle, causing it to transition from the S phase to the G2 phase (Figure 2).

3.4. Antigenotoxic Assay: DNA Nicking Assay

Eugenol with a concentration of 40 µg. mL^{-1}, has the best results characterized by high plasmid band thickness compared to other eugenol concentrations (Figure 3). Additionally, most of the plasmid is in

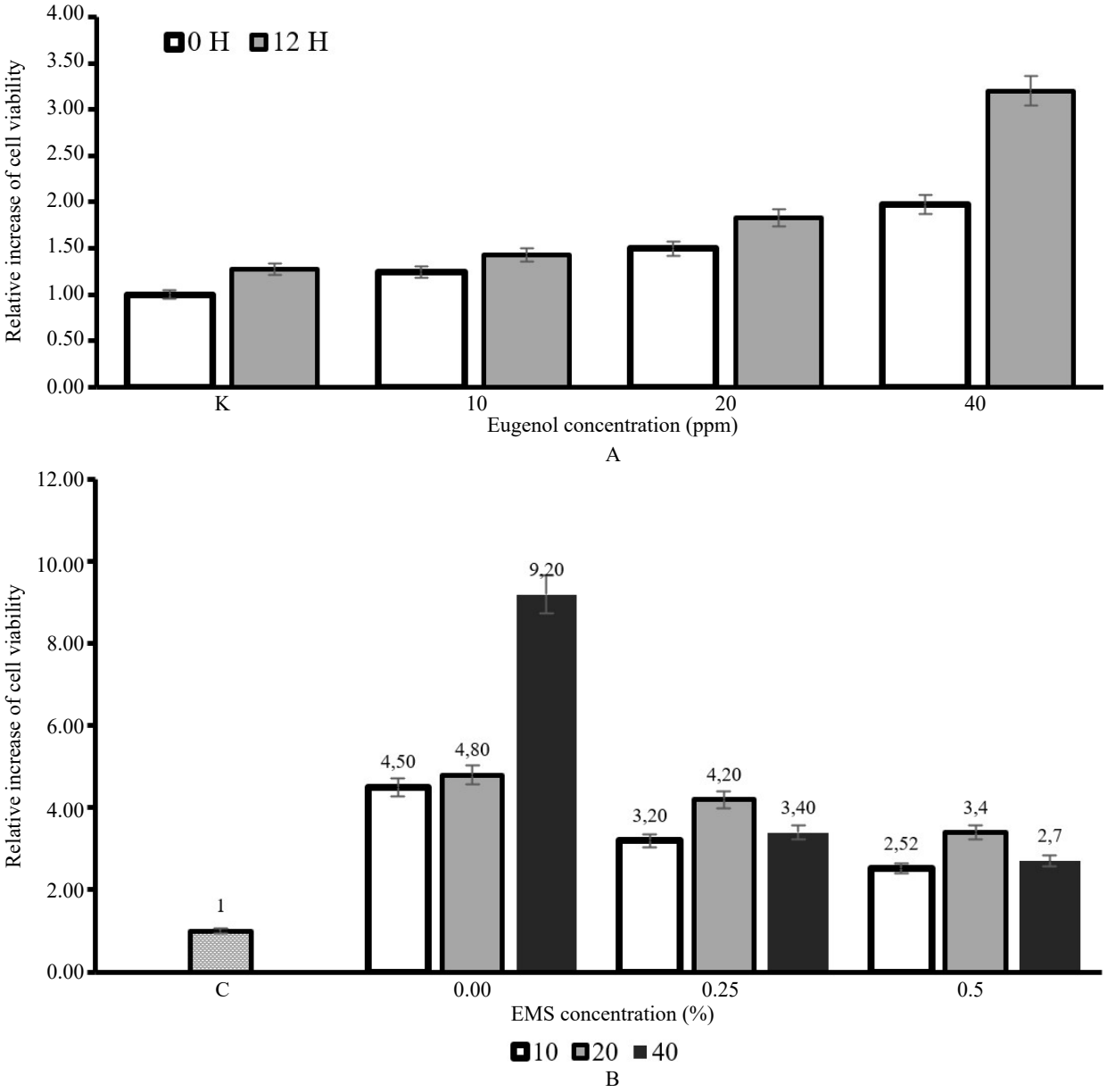
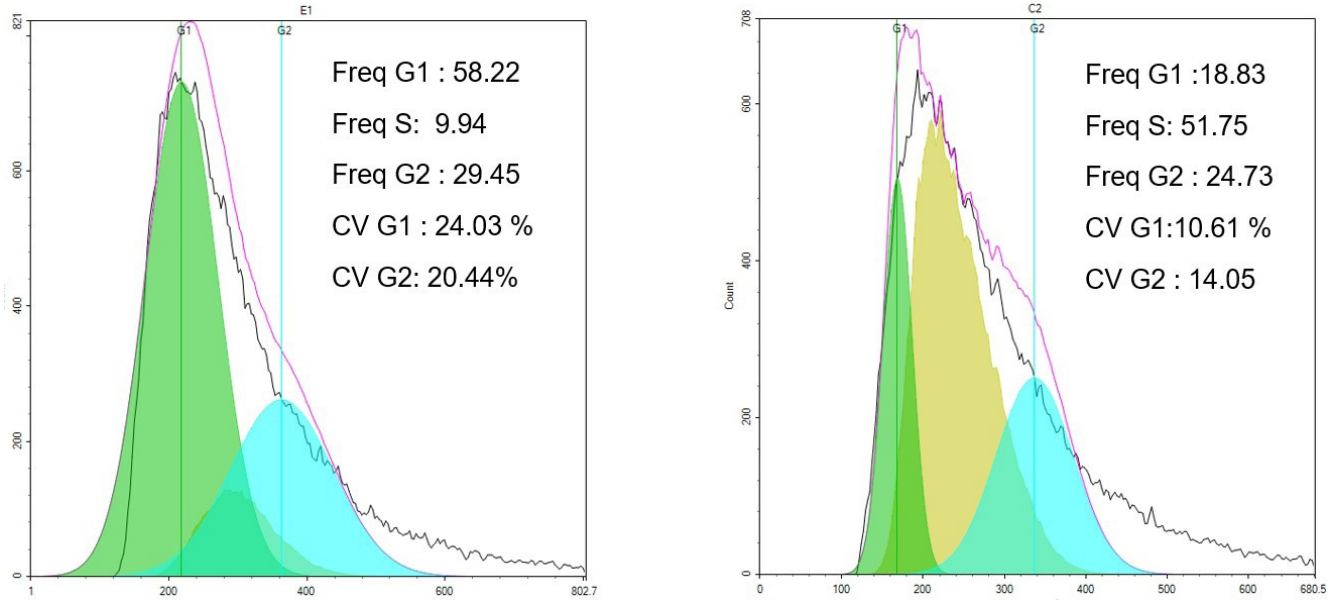


Figure 1. (A) Effect of UV exposure on *S. pombe* treated with eugenol (10, 20, and 40 ppm). Yeast cells cultured without eugenol were used as a control (K) treatment. Cell viability was determined using the plate count assay at 0 hours (before UV treatment) and 12 hours (after UV treatment). (B) The effect of EMS treatment (10, 20, and 40 ppm) on the viability of *S. pombe*, which was cultured in YES medium supplemented with different concentrations of eugenol (10, 20, and 40 ppm)



A B

Figure 2. Effect of the eugenol treatment on the cell cycle of *S. pombe*. Yeast cells were treated with (A) eugenol and (B) without eugenol. The total amount of each yeast cell phase was mentioned in the right corner of each figure. All figures and data represent the means of three independent experiments

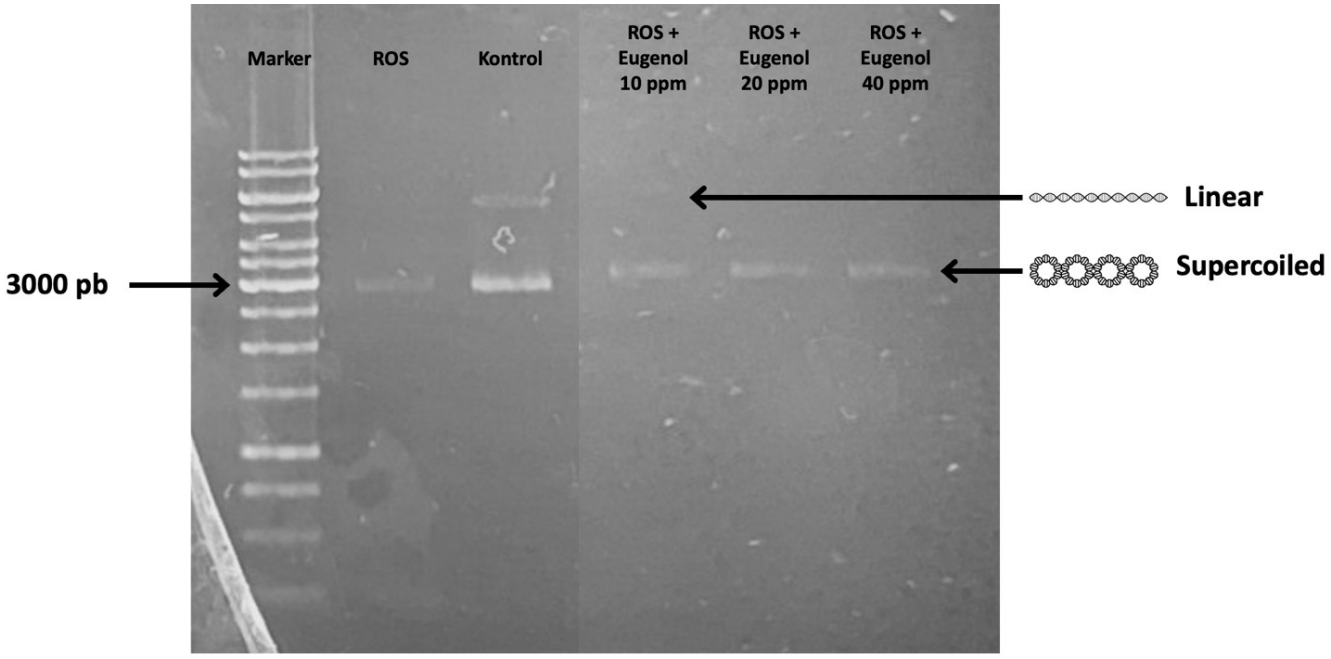


Figure 3. DNA nicking assay to elucidate the effect of eugenol administration on pBR322 plasmid exposed to ROS (Fenton reaction). Eugenol was treated at different concentrations of 10, 20, and 40 ppm. ROS treatment indicates the presence of Fenton reactions only, while control treatment indicates the presence of a DNA plasmid without eugenol Fenton reagents

an intact supercoiled form compared to the control treatment.

3.5. Gene Ontology-based Transcriptomic Analysis

Gene ontology analysis was performed to categorize transcriptome data into three ontology groups, including biological process (BP), cellular component (CC), and molecular function (MF) (Figure 4A, B, and C). The analysis showed that eugenol can regulate the expression of genes involved in various cellular phenomena. A total of 5083, 2463, and 2788 genes in the BP, CC, and MF gene ontology clusters of yeast cells were modulated in expression by eugenol treatment, respectively. The clusters of genes involved in cellular processes (1,219 gene sequences), cell anatomy (999 gene sequences), and binding (1,065 gene sequences) in the BP, CC, and MF gene ontologies were most significantly altered in expression by eugenol treatment.

3.6. Principal Component Analysis (PCA) of Transcriptome

Transcriptome data analysis was performed using the PCA method to categorize genes whose expression increased, decreased, or remained unchanged following eugenol treatment. The results indicated that eugenol can modulate the expression of genes involved in DNA repair mechanisms. The PCA analysis revealed three primary groups of genes: those that were upregulated, down-regulated, and unchanged after eugenol administration (Figure 5). Specifically, 445 genes showed increased expression, 1,610 genes exhibited decreased expression, and 30 genes remained unaffected

In the mismatch repair response group, 2 genes showed an increase in expression, while 21 genes exhibited a decrease following eugenol treatment. In the base excision repair response group, 2 genes exhibited increased expression and 6 genes showed decreased expression in response to eugenol. For the nucleotide excision repair group, 8 genes showed increased expression, and 37 genes showed decreased expression after eugenol treatment. The PCA analysis results for each response category reveal that the graphs consistently divide into two groups: genes with increased expression and genes with decreased expression due to eugenol (Figure 6).

4. Discussion

UV exposure can induce the formation of ROS that disrupt metabolic processes (Kozmin & Jinks-Robertson 2013). Exposure to ROS causes oxidative stress and results in increased production of free radicals in cells. Eugenol has antioxidant activity, which neutralizes the toxicity of UV exposure and supports yeast cell viability. Indeed, the viability of yeast *S. pombe* increased following eugenol treatment in UV-exposure conditions. The antioxidant defensive system of eugenol neutralizing ROS in cells has several mechanisms of action by accelerating the neutralization reaction of free radicals by enzymes, reducing free radicals with electron donors, and binding oxidant metal ions with binding proteins (Gülçin 2011; Bezerra *et al.* 2017; Parween *et al.* 2021). A similar phenomenon has also been reported by the antioxidant agent quercetin, which elicits antimutagenic effects against UV exposure in the yeast Saccharomyces cerevisiae (Asfahani *et al.* 2023). In addition to UV protection, eugenol showed potential as an antimutagen against EMS treatment. EMS can cause DNA changes, such as oxygen alkylation of guanine bases, and alter the arrangement of nitrogenous bases, resulting in transitions, insertions, and deletions. Mutations in yeast cells can be prevented by inhibiting the penetration of the mutagen, EMS, into cells by adding antioxidants that may inactivate free radicals produced by EMS (Ali 2022).

The results of the cell cycle assay showed a delay in the G1 phase of yeast *S. pombe* towards the S and G2 phases due to the administration of eugenol. Such a phenomenon may indicate a cell cycle arrest, suggesting potential antiaging properties of eugenol. The cell cycle arrest that occurs is a delay in yeast cells entering the G2 phase, resulting in cells being maintained longer in the G1 phase (Zhuang & Miskimins 2008). The results of the cell cycle analysis obtained in this study are in accordance with previous research, which states that the addition of clove flower fraction to yeast cells can suppress and slow down the G1 phase, reducing it from 16 to 60% (Lesmana *et al.* 2021). DNA nicking assay also confirmed the potential of eugenol in protecting DNA from free radicals. Observations showed that in the results of electrophoresis, most of the plasmid is in the form of supercoiled rather than in linear or open

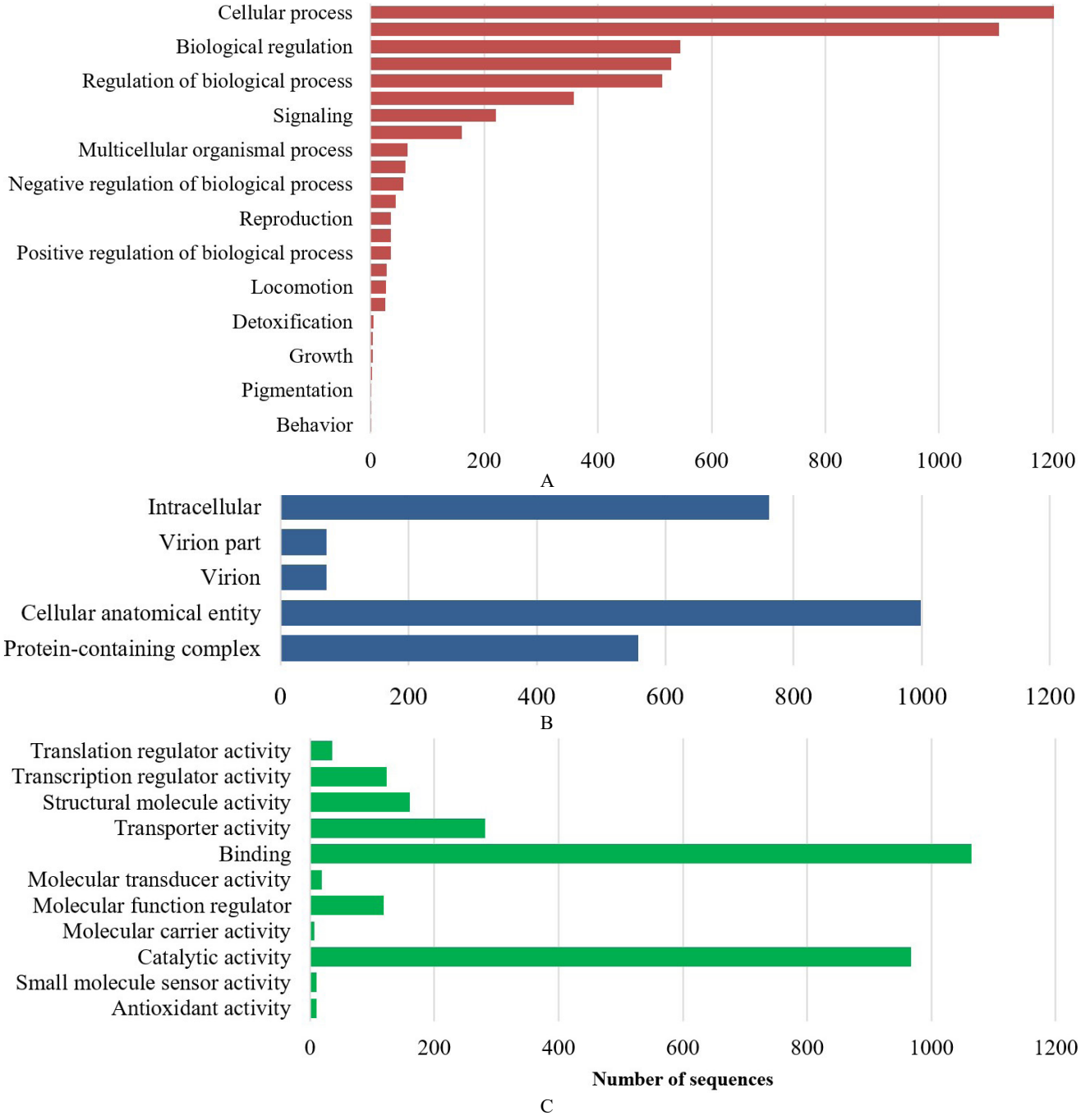
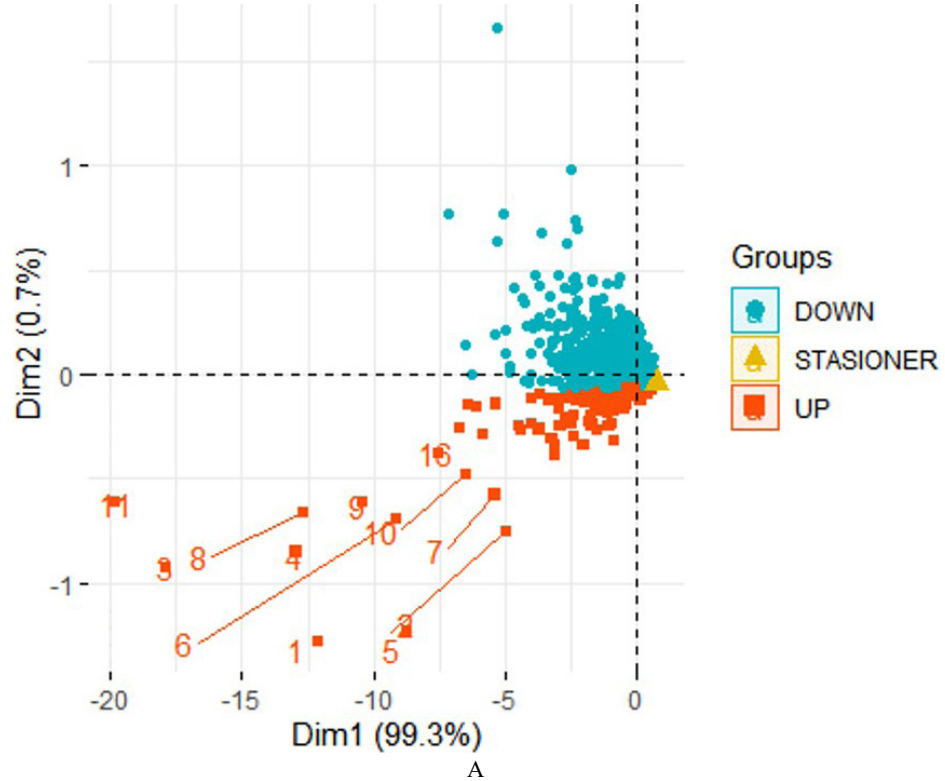
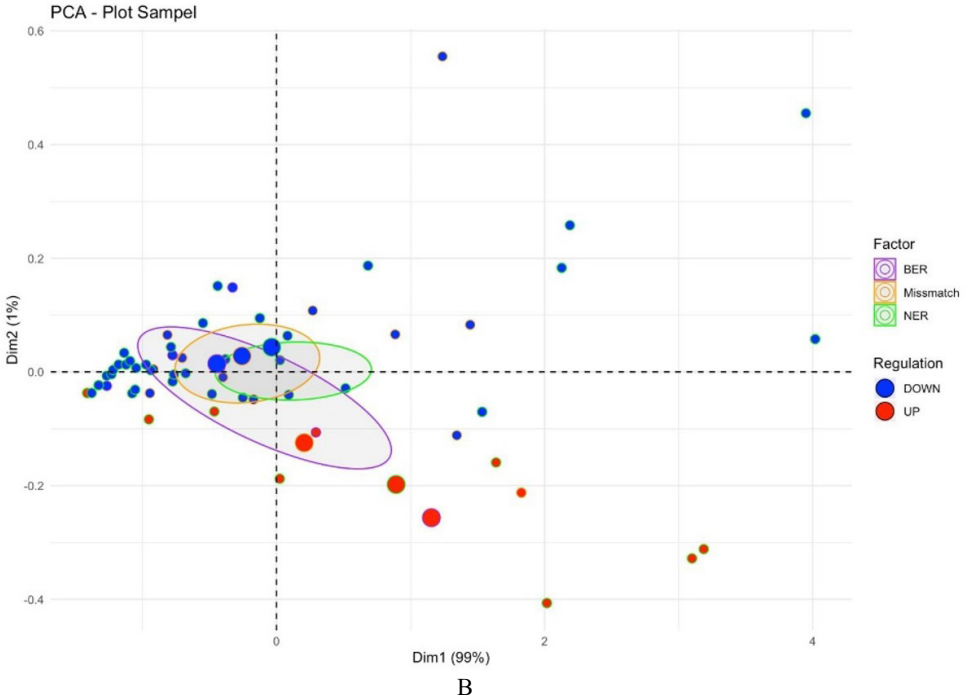


Figure 4. Distribution of (A) biological process, (B) cellular component, and (C) molecular function gene ontology terms in the transcriptome of *Schizosaccharomyces pombe* treated with eugenol (10 ppm)



A



B

Figure 5. (A) A grouping graph of *S. pombe* transcriptomes showing increased (up), decreased (down), or unchanged (stationary) expression following eugenol treatment. (B) A grouping graph depicting gene expression changes in *Schizosaccharomyces pombe* after eugenol treatment across all response categories: Mismatch Repair (mismatch), Base Excision Repair (BER), and Nucleotide Excision Repair (NER)

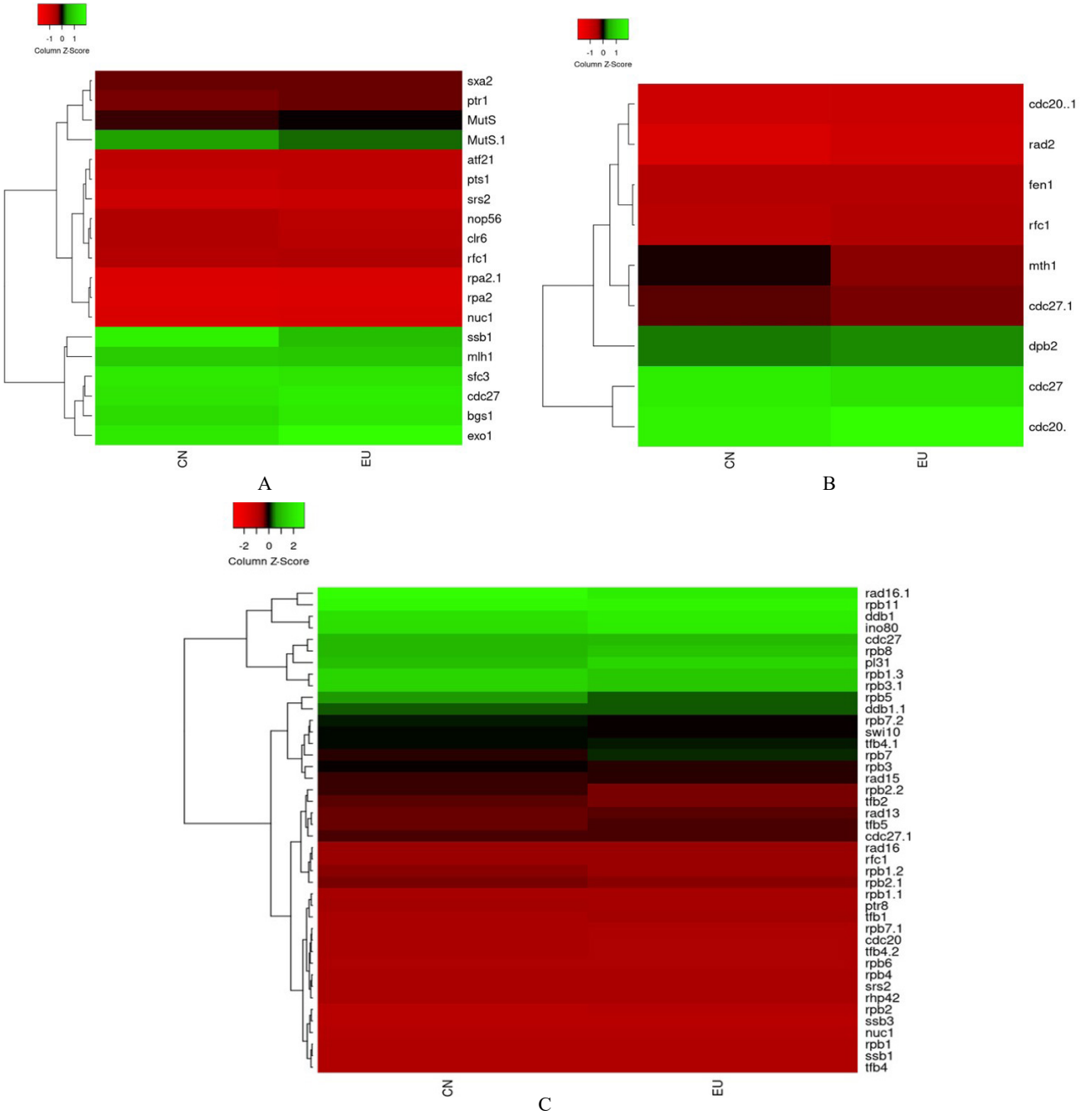


Figure 6. Grouping heatmap illustrating gene expression changes in *Schizosaccharomyces pombe* cells following eugenol administration (EU) compared to without eugenol (CN) treatment. Gene expressions are clustered in three DNA damage response categories: (A) mismatch repair, (B) base excision repair, and (C) nucleotide excision repair

circular. The presence of supercoiled plasmid indicates undamaged, intact plasmid DNA (Leba *et al.* 2014).

The addition of eugenol caused cells to experience a slowdown in the G1 phase, resulting in non-dividing cells due to delayed cell senescence. The slowdown can be expected because there is regulation of genes that play a role in slowing the G1 phase of the cell cycle, as indicated by the results of transcriptomic analysis. The results of cell cycle analysis using gene ontology are in accordance with a previous report, which stated that cells may undergo a slowdown in the G1 phase due to decreased expression of DNA-related genes (Leonov *et al.* 2017). DNA is a crucial component in the progression of the G1 cycle towards *S. pombe* cells,

which experience a slowdown in the cell cycle phase, often caused by a lack of DNA and damage to the DNA structure. Slowing down is necessary for cells to reproduce or repair their genetic material (Junqueira & Carneiro 2003). One of the cellular phenomena related to cell aging is cell cycle regulation.

The PCA analysis revealed significant changes in the expression of cell cycle-related genes following eugenol treatment. Specifically, the analysis identified alterations in the expression of genes involved in cell cycle regulation, including *cdc18, orc6, res1, res2*, and *hsk1*. The *cdc18* gene is crucial for initiating DNA replication and works with the orc1 gene to regulate the start of the S phase (Vas *et al.* 2001). A reduction in *cdc18* expression indicates cell cycle arrest, as downregulation of this gene aligns with findings from a previous study, which suggests that cdc18 contributes to slowing the G1 phase (Peng *et al.* 2005). The protein produced by cdc18 is Mini Chromosome Maintenance (MCM), which is essential for DNA replication in eukaryotic cells (Lei 2005). Similarly, the *orc6* gene plays a role in DNA replication and regulates the transition from the G1 phase to the S phase. The orc6 gene encodes a subunit of the origin recognition complex. A decrease in orc6 expression suggests that eugenol may prolong the lifespan of *S. pombe* (Chuang *et al.* 2002).

The *res1* and *res2* gene clusters, upregulated by eugenol, are reported to function as regulators of DNA synthesis in cells and activate transcription of genes required for the G1/S phase transition (Whitehall *et al.* 1999). Previous studies have reported that the *res1* and *res2* genes encode products that activate DNA transcription factors in the G1 phase of the S-phase cyclin genes (Costanzo *et al.* 2003). The decrease in the expression of the *res1* and *res2* genes resulting from eugenol treatment in *S. pombe* is thought to cause a slowdown in the G1 phase of the cell cycle. This is characterized by the inactivity of genes required for the G1 phase transition towards *S. pombe*. Eugenol also regulated hsk1 gene expression. The increase in HSK1 gene expression suggests that the cell may initiate the DNA replication process with the assistance of the *READ3* and *CDS1* genes (Snaith *et al.* 2000). This condition indicates that the cell is poised to enter the S phase. The cause of increased gene expression may be due to the chronological age of *S. pombe* used, which is relatively old (Chen & Runge 2009). The product of the hsk1 gene may stimulate cells to enter the S phase due to the phosphorylation of *cdc19* (MCM2) (Brown & Kelly 1998). Although there is an increase in expression, hsk1 protein activity does not necessarily increase because it can be regulated post-translationally. Therefore, further analysis of the effect of eugenol on HSK1 protein activity is needed.

In conclusion, transcriptome data analysis categorized genes related to DNA damage influenced by eugenol treatment in yeast *S. pombe*. The analysis demonstrated that eugenol could modulate the expression of genes involved in DNA damage response mechanisms induced by oxidative stress, including those in Mismatch Repair, Base Excision Repair, and Nucleotide Excision Repair. Indeed, treatment of eugenol could increase yeast viability following exposure to mutagens (UV and EMS). In addition, eugenol was confirmed to prolong yeast lifespan by modulating the cell cycle. These data indicate the potential pharmacological effect of eugenol as an antimutagen at the cellular level, which therefore supports its further study in multicellular organisms.

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