

Quantitative analysis of biofuel MET6 expression level in a treated *Saccharomyces cerevisiae* with Broccoli sprouts (*Brassica Oleracea* L.) extract

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Abstract

Methionine and cysteine are essential sulfur-containing amino acids that play critical roles in cellular metabolism. Methionine is the amino acid initiating eukaryotic protein biosynthesis and is activated by S-adenosylmethionine. In fungi, methionine biosynthesis is catalysed by the cobalamin-independent methionine synthase (*MET6*). Although chemical synthesis provides methionine for most applications, sulfur is commonly supplied as inorganic sulfate and must be reduced before incorporation into amino acids. This study evaluated whether natural sources rich in organosulfur compounds, such as broccoli sprouts, could influence methionine biosynthesis. *MET6* gene expression was quantified by qRT-PCR in *Saccharomyces cerevisiae* wild-type (BY4742) and *met15Δ* mutant strains treated with or without broccoli sprout extract. According to the Two-way ANOVA analysis, *MET6* expression was significantly downregulated after two hours of broccoli treatment. A concurrent reduction in cell growth was observed in both strains compared to untreated controls.

Introduction

Methionine is a vital sulfur-containing amino acid that plays an essential role in metabolic processes of various organisms, both animal and plant. Despite most organisms rely on external sources for methionine, plants can produce it internally. Beyond its role as a protein building block, methionine serves additional functions. It acts as a precursor to S-adenosylmethionine (AdoMet), which plays key roles in methyl-group transfer reactions, polyamine synthesis, and the production of the plant hormone ethylene (Ravanel et al. 1998). Furthermore, a study of (Walvekar et al. 2018) showed that the presence of methionine (even when other amino acids are limited) causes the production of all other amino acids and nucleotides needed to maintain anabolism where the generation of metabolites and co-factors is necessary for reductive biosynthesis forms and the basis of this anabolic program. Many plants, fungi, and bacteria can synthesize methionine from various sources, including carbohydrates, as well as inorganic and organic sources of nitrogen with sulfur. In contrast, all animals, inclusive humans, are reliant on dietary intake to obtain methionine. Currently, the industrial production of methionine primarily relies on chemosynthesis encompassing acrolein, methyl mercaptan, and hydrogen cyanide. This chemo-synthesized methionine is extensively utilized across various industries (Ravanel et al. 1998; Willke, T. 2014). Yet, a key challenge in this process is the requirement for a sulfur origin, which is typically supplied as inorganic sulfate, and needs to be reduced to create methionine. Therefore, there has been rising delight in alternative and more sustainable processes that utilize natural resources (Becker, and Wittmann. 2012; Stipanuk and Ueki. 2011). Biofuels have been featured as a promising alternative to conventional fossil fuels, presenting potential solutions to environmental concerns and energy sustainability. Among various biofuel sources, *Saccharomyces cerevisiae*, a yeast species widely utilized in bioethanol production, stands out for its efficiency in fermenting sugars into ethanol. However, enhancing yeast performance through genetic modifications and bioactive compound treatments has garnered significant attention to improving biofuel production efficiency (Sharma et al. 2022). Broccoli seedlings (*Brassica oleracea* L.) have garnered significant attention in recent years due to their high content of health-enhancing phytochemicals. These include nitrogen-sulfur derivatives such as

glucosinolates and isothiocyanates, as well as polyphenols, minerals, and vitamins. Notably, the concentrations of these bioactive compounds are considerably higher in seedlings compared to mature broccoli inflorescences (Moreira-Rodríguez et al.2017). Glucosinolates (GLSs) are nitrogen-sulfur compounds (β -D-thioglucoside-n- hydroxysulfates) and important plant secondary metabolites, almost exclusively found in the genus Brassica, specific to kale, cabbage, and broccoli (Ishida et al.2014). GLSs are classified into three types: glucoraphanin, glucoerucin, and glucobrassicin, which are enzymatically converted into sulforaphane (SFN), erucin (ERN), and iberin, respectively (Clarke et al. 2011). Sulforaphane (SFN) is a sulfur-rich compound known for its potential to lower the risk of various cancers. It is formed when glucoraphanin meets myrosinase enzymes, which are only released and activated when the plant is damaged. Therefore, cruciferous vegetables must be cut, chopped, or chewed to trigger sulforaphane production, releasing myrosinase. (Franklin et al.2014; Asif et al. 2023). Importantly, betaine (trimethyl glycine) is a natural constituent of broccoli, the use of betaine is usually followed by an increase in plasma methionine and a fall in plasma total homocysteine, where betaine acts as a methyl donor and thereby converts homocysteine to methionine (Feng et al.2011; Mcrae et al. 2013). Additionally, broccoli contains betaine (trimethyl glycine), a compound with significant physiological roles. Betaine acts as a methyl donor, converting homocysteine into methionine, which is associated with increased plasma methionine levels and decreased plasma total homocysteine levels (Feng et al.2011; Mcrae et al. 2013). The current study aimed to stimulate methionine biosynthesis at a low cost from natural biological sources such as Broccoli (*Brassica oleracea L.*) extract, which is rich in organosulfur-containing compounds.

Methodology

Strains

S. cerevisiae (W-T- BY4742) with genotype (*MATa; his3 Δ 1; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0*) and the deletion-mutant (*met15 Δ - BY4741*) (*MATa; his3 Δ 1; leu2 Δ 0; met15 Δ 0; ura3 Δ 0*). It was procured from Euroscarf (Frankfurt, Germany) (Bachmann et al. 1998).

Yeast extract peptone dextrose (YEPD) agar medium

Yeast extract peptone dextrose (YEPD) agar (3%yeast extract (w/v), peptic 5% (w/v), agar 15% (w/v) (Microgen-NED) was prepared according to the manufacturing instructions: 23.0 g from YEPD was dissolved in 1 Liter of distilled water; the solution's pH was adjusted to 5.5.

Synthetic Complete (SC) medium

Based upon yeast nitrogen base without amino acids (YNB) (sodium chloride, magnesium sulphate, potassium sulphate, calcium chloride, ammonium sulphate) (FORMEDIUM-UK), and a complete supplement mixture (CSM) containing the appropriate amino acids, but lacking methionine

(FORMEDIUM- UK), synthetic complete (SC) medium was prepared according to the manufacturing. instructions: 6,9 g from YNB, and 770 mg of (CSM) complete supplement mixture were dissolved in 1 liter of distilled water, then pH was adjusted to 5.5.

Broccoli extract preparation

The broccoli used in this study was purchase from a local market. Broccoli extract was prepared through washing broccoli heads, then single florets were cut 5 cm in length and 0.7–0.9 cm in width (stem), A capped glass bottle was used to put 50 g of cutter florets and immersed in a thermostatic bath at 40 oC for 13 min to convert GFN into SFN. After blanching, broccoli florets were withdrawn from the thermostatic bath and cooled at room temperature. The broccoli pieces were then crushed in a screw mill and compressed to obtain broccoli juice. Broccoli juice was mixed with 200 ml of distilled water, then filtered via sterile gauze to remove any solids. At the end stage of preparation, the broccoli extract was precipitated via centrifuge at 4500 rpm for 30 min at 20°C for further purification (Gonzalez et al.2021).

Yeast culture

growth conditions of yeast strains

The *Saccharomyces cerevisiae* strains BY4742 wild type (WT) and the deletion-mutant (met15Δ-BY4741) were routinely maintained and grown either in yeast extract peptone dextrose (YEPD) broth (3%yeast extract (w/v), peptic 5%(w/v), 2% (w/v) glucose), or YEPD agar (same but containing 2% (w/v) agar), or in yeast nitrogen base (YNB) medium (sodium chloride, magnesium sulphate, potassium sulphate, calcium chloride, ammonium sulphate (FORMEDIUM-UK) supplemented with a complete supplement mixture (CSM) which containing the appropriate amino acids, but lacking methionine (FORMEDIUM- UK). Cultures were grown with shaking at 120 rev/min and 30°C overnight to stationary phase in standard YEPD or selective YNB broth, before dilution into fresh medium and further growth for 4–5 h before experimental use (Holland and Avery, 2009). Short-term storage of all yeast strains took place at 4°C on the appropriate agar plates. However, for long-term storage, glycerol stocks were produced with 20% (w/v) glycerol and kept at – 80°C.

Growth curve assay in broth media

The SC medium which consisted of yeast nitrogen base (YNB) without all amino acids (YNB) and supplemented with a complete supplement mixture that containing the appropriate amino acids, but lacking methionine (CSM-MET), was used as described by (Sherman 2002). For cells growth assay, individual colonies were transferred from YEPD agar plate into 50 mL of SC medium using a 250 ml shake flask. Cultures were grown and incubated at 28°C with shaking (120 rev/min) overnight until they reached the stationary phase. Subsequently, samples were diluted to OD600 ~ 0.02 and tenfold diluted

aliquots transferred to fresh SC medium and allowed to grow for further 4 hours to exponential phase at the same conditions before experimental use. For growth curve assays, cultures were divided equally into two groups:

Group I: BY4742 (WT) and the deletion-mutant (*met15Δ*- BY4741) were left without treatments as a control.

Group II: BY4742 (WT) and the deletion-mutant (*met15Δ*- BY4741) were treated with Broccoli extract alone at a final concentration of 10%.

All these cultures groups were incubated with shaking and the optical density (OD) at a wavelength of 600 nm (OD₆₀₀) recorded continuously after (0, 2 and 4) hours using a BioTek 16 Powerwave XS microplate spectrophotometer (BIO TEK SYNERGY 2 MICROPLATE READER, Thermo Scientific™, USA), as described (Sherman 2002). Three independent biological replicates for each group were performed for statistical analysis.

RNA extraction

The RNeasy mini kit (Cat. No.74104-Qiagen-DE) was used to extract RNA from *S. cerevisiae* of all groups. Samples containing 1×10^7 cells were moved to sterile microfuge tubes and centrifuged for 10 minutes at 6000 rpm at 4°C. Cell pellets were immediately placed and ground in liquid nitrogen with glass beads using a cooled pestle and mortar. After that, steps were followed as the manufacturer's instructions for the RNeasy mini kit.

RNA Purification

The RNA template was treated with DNase I to remove contaminating DNA using kit RNase-Free DNase (Cat. No.79254- Qiagen-DE). Briefly, the RNA was incubated with 10 µL of 1x DNase I Reaction Buffer, 1 µL of DNase I enzyme, and nuclease-free water to a final volume of 50 µL. The reaction was carried out at 37°C for 15 minutes. After DNase I treatment, RNA was purified using a phenol-chloroform extraction and ethanol precipitation method as described by Vomelova (Vomelova et al. 2009). An equal volume (100 µL) of phenol-chloroform-isoamyl alcohol (25:24:1, pH 4.5-5) (Invitrogen™-USA) was added to the reaction mixture. The solution was mixed thoroughly by shaking and then centrifuged at 16,000 x g for 5 minutes at 4°C. The upper aqueous layer containing the RNA was carefully transferred to a clean tube and 10 µL of 2M sodium acetate (pH 5.2) (Invitrogen™-USA) and 400 µL of ice-cold 100% ethanol (Invitrogen™-USA) were added to the aqueous layer to precipitate the RNA. The solution was mixed gently by inversion and incubated at -80°C for 1 hour to allow for RNA precipitation. The mixture was centrifuged at 16,000 x g for 15 minutes at 4°C. The supernatant was carefully removed and discarded. The RNA pellet was washed once with 1 mL of 70% ethanol by centrifugation at 16,000 x g for 15 minutes at 4°C. The supernatant was discarded, and the remaining pellet was air-dried for 5–10 minutes. Finally, the purified RNA was resuspended in 25 µL of sterile, nuclease-free water for downstream

applications. Then, RNA concentration and purity were measured by using (NanoDrop Thermo scientific, USA) and gel electrophoresis.

RNA Integrity Assessment Using Agarose Gel Electrophoresis

Dissolved agarose gels were used to separate nucleic acids based on size. These gels were prepared by heating agarose in 1x TAE buffer (RBI® Research Products International) in 1x TAE buffer (242g Tris-base, 200 ml 0.5M EDTA pH 8.0, 57.1ml CH₃COOH, made up to the volume required with H₂O) and heating it in a microwave for 1 minute and 40 seconds, and heating it in a microwave. After cooling, ethidium bromide was added to visualize the separated RNA. The gels were then placed in a specialized tray, electrophoresed in a tank, and loaded onto the gel. The gels were run for 40–60 minutes at 100V, and the separated RNA was visualized using BioSpectrum Imaging Systems.

First-strand cDNA synthesis method

RNA, being a single-stranded molecule, cannot be directly amplified. To overcome this limitation, reverse transcription was performed to convert RNA into complementary DNA (cDNA), which is suitable for amplification. cDNA was synthesized from total RNA using the GoScript® Reverse Transcription System (Cat. No. A5001, Promega, USA), following the manufacturer's instructions. All steps were performed in a biosafety cabinet (BSC) to minimise contamination, and reagents were kept on ice during preparation.

Quantitative RT-PCR (qRT-PCR)

Real-time PCR was performed using an Applied Biosystems™ StepOne™ Real-Time PCR System machine was performed in triplicate reactions, each comprising 100ng purified cDNA, 100nM MET6 and ACT1 - specific primers shown in Table (1), a 20 µL reaction containing 2 µL of first-strand cDNA, 10 µL of master mix qPCR Kit (EverGreen, HavenSci, KSA), 1 µL of 10M F primer, 1 µL of 10M R primer, and 6 of nuclease-free ddH₂O. Reactions were performed in MicroAMP 96-well optical plates sealed with optical adhesive film and monitored with a 7500 Fast Real-Time PCR System (Applied Biosystems) using 7500 software v2.0.4 (Applied Biosystems). MET6 gene-specific primers and ACT1 housekeeping gene primers were selected as a reference for measuring the amount of total RNA in the sample. The optimal annealing temperature was chosen based on Taq DNA polymerase's gradient PCR reactions. The samples were denatured for 5 minutes at 95°C, followed by 40 cycles of 10 seconds at 95°C, 30 seconds at 60°C, and 15 seconds at 95°C, followed by 1 minute at 60°C finally 15 seconds at 95°C, on the (Applied Biosystems). The expression of the housekeeping control gene ACT1 and the data were analyzed by the 2⁻(Delta Delta C(T)) method (Livak and Schmittgen. 2001), and in all cases, a melt curve was included after the amplification stage, where agarose gel electrophoresis and melting-curve analysis were used to confirm a single PCR product. Amplification was quantified from a standard curve constructed from reactions with defined copy numbers of the expected PCR product.

Table 1
Q RT-PCR Primers (*MET6* and *ACT1*)

Primer	Sequence	Expected size Of DNA product (bp)
<i>MET6</i>	FWD 5'-GAAGCTGCCGGTATCAAGGT-3'	105
	REV 5'-GGCAGCCCAGGTGTAGTAAG-3'	
<i>ACT1</i>	FWD 5'-GGAAATCACCGCTTTGGCTC-3'	86
	REV 5'-AACCACCAATCCAGACGGAG-3'	

Statistical Analysis

Statistical analysis of the data was conducted using two-way ANOVA with Prism 10 software to determine whether differences were statistically significant. Statistical significance was considered when the p-value was below 0.05 ($p < 0.05$), while p-values greater than or equal to 0.05 ($p \geq 0.05$) indicated no significant difference.

Results

Morphological characteristics of yeast *S. cerevisiae* cells on YEPD agar medium

For morphological study, the wild-type strain of *S. cerevisiae* (BY4742-WT), and the mutant strain *met15Δ*(BY4741) were prepared and tested for growth in YEPD medium firstly. Morphological description of yeast colonies showed that the shape of colonies of both yeast cells of *S. cerevisiae* (BY4742-WT), and the mutant strain *met15Δ*(BY4741) as shown in Figure (1), were round, creamy in color, and had smooth surfaces. However, the sizes of both cells types vary considerably. Table (2)

Table 2
Morphological characteristics description of untreated wild-type (WT) and mutant *met15Δ* cells

Morphological description	BY4742-WT	<i>met15Δ</i>
shape	Round	Round- Oval
Color	creamy	creamy
Surface	smooth surfaces	smooth surfaces
Size of colony	2–3 μm in diameter	0.5-1 μm in diameter

Absence of methionine decreases the growth rates of the deletion mutant cells (*met15Δ*) compared to the wild type cells

To compare the growth level of the (BY4742-WT)cells and *met15Δ*(BY4741) cells, spectrophotometer at OD600 was used to measure the growth level of the WT (BY4742) and the mutant cells (*met15Δ*) in the broth synthetic complete (SC) media, which is YNB medium supplemented with all amino acids except

methionine (YNB-MET), for 2hr. Results indicated that there was a remarkable increase in wild-type cells growth compared to the mutant strain *met15Δ*, and a statistically significant difference was discovered ($p < 0.0072$). according to the two-way ANOVA statistical analysis test as shown in figure (2). This made these cells suitable and might be used to evaluate if the expression level of *met 15* influences the growth rate of yeast cells.

Broccoli extract affects the level of cells growth of both wild-type and mutant cells

Glucosinolates (GLSs) and sulforaphane (SFN) are among the most studied bioactive compounds, which found abundantly in broccoli sprouts that belongs to Brassica genus (Ishida et al. 2014). In addition, Glucosinolates (GLSs) are nitrogen- and sulfur-containing compounds that undergo enzymatic conversion into sulforaphane (SFN), erucin (ERN), and iberin (Clarke et al.2011). SFN is a sulfur-rich compound and activated when glucoraphanin meets myrosinase enzymes (Coutinho et al. 2023). This sulfur is an essential source for growth and used primarily to synthesis methionine, for this reason, sulforaphane molecules are the leading candidates for investigating the broccoli ingredient (Li et al. 2023). Therefore, the same assay that was mentioned above but with adding a 100 µl/ml broccoli extract in the SC (YNB-MET) media lacking methionine was used for both BY4742 wild-type cells and the deletion-mutant cells (*met15Δ*) to test whether broccoli extract may raise cells growth of both wild-type and mutant *met15Δ* in media lacking methionine. After exposure to 100 µl/ml of broccoli extract, a remarkable growth rate decreases of both types of cells wild-type BY4742 and the deletion mutant *met15Δ* was observed compared to the untreated strains (control), and a significant decrease of a treated *met15Δ* cells growth was seen compared to a treated wild-type cells figure (3).

Furthermore, this reduction in the cells growth level of a treated wild-type and *met15Δ* cells was significant compared to the untreated cells (control) according to the two-way ANOVA statistical analysis test where ($p < 0.0001$) figure (3).

These data suggest that the sulfur-rich compounds that found abundantly in broccoli sprouts may not be effective in compensating the lack of methionine or may act as antagonism for methionine biosynthesis, and thereby growth reduction was observed in both cell types. Therefore, more investigation was needed, thus, quantification of *MET6* gene expression levels in both treated - untreated cells is necessary to determine whether the broccoli extract is indeed affecting *MET6* gene expression levels or may act as antagonistic factor against methionine biosynthesis which may elucidate the cause of observed growth level drop of both cell types.

Quantification of purified RNA concentrations using a Nano-Drop spectrophotometer and gel electrophoresis.

The extraction of a high-quality RNA from both wild-type and the deletion mutant *met15Δ* cells is a critical step in further gene expression analysis. The consistent RNA yield and concentration across all

samples indicate the reliability of the extraction method used. All extracted and purified RNA was quantified using a Nano-Drop spectrophotometer (Nano-Drop Technologies), results showed that the average of RNA concentration in both BY4742 wild-type (WT) and the deletion mutant (*met15Δ*) cells untreated (C) and treated with broccoli (B) extracts was approximately range between 100 to 300 ng/μl, with a minimum variation between the biological replicates. Furthermore, agarose gel electrophoresis was used to confirm these extracted and purified RNA concentrations that were determined by Nano-drop. All samples untreated and treated with Broccoli extract were analyzed by agarose gel electrophoresis and results showed the integrity of the extracted RNA in all samples, as evidenced by the presence of clear RNA bands and no degradation of RNA was observed, which indicating that a high-quality RNA extraction (Fig. 4) and (Fig. 5). This yielded RNA is qualified now to use as a template to synthesis the first-strand cDNA and subsequently used for quantitative RT-PCR (q RT-PCR).

The Broccoli extract alters the MET6 expression levels of the wild-type and the deletion mutant(*met15Δ*)cells

Quantification analysis of *MET6* gene expression levels in both the untreated and treated wild-type (BY4742) and the deletion-mutant cells (*met15Δ*) using q RT-PCR was performed in triplicate reactions, each comprising 100 ng purified cDNA to verify whether broccoli extract may affect *MET6* expression level, and consequently will elucidate the cause that affected the treated cells growth rates that were showed previously.

Broccoli extract downregulates the expression level of MET6 of both the wild- type and the mutant *met15Δ* cells

As a result of the significant reduction in yeast cells growth levels that had been observed previously (Fig. 3) in a treated wild-type (BY4742) and the deletion mutant (*met15Δ*) cells with a broccoli extract compared to the untreated cells, test whether broccoli extract could affect *MET6* gene expression levels is a crucial, and thereby may elucidate the cause of cells growth reduction. Therefore, quantification analysis of *MET6* gene expression levels in both the untreated and treated wild-type and the deletion-mutant cells (*met15Δ*) was conducted using q RT-PCR in triplicate reactions, each comprising 100 ng purified cDNA. The quantification assay results showed a remarkable down regulation *Met6* expression level in both the wild-type and the mutant *met15Δ* cells that were treated with the broccoli extract for 2 hrs compared to the untreated cells (control), and these observed differences were statistically significant according to the two-way ANOVA statistical analysis test ($p < 0.0001$). Besides, there was a statistically significant decrease in the expression level of *Met6* of the treated wild type compared to the *met15Δ* mutant cells as showed (Fig. 6).

According to these results with the broccoli extract, the reason of the remarkable decrease of cells growth rates in media lacking methionine that were observed previously (Fig. 3) of both treated strains may owing to the insufficient compensation role of the sulfur-rich compounds of the broccoli extract of methionine, which may act as antagonistic factors against methionine biosynthesis, and thus, broccoli

extract became ineffective in compensating methionine deficiency. Therefore, these results may elucidate the cause of the observed growth level drop of both cell types.

Discussion and Conclusions

Methionine is one of the common sulfur-containing amino acids integrated into the biosynthesis of almost all eukaryotic proteins. *Saccharomyces cerevisiae Met6* is described as cobalamin-independent methionine synthase, which involved in methionine regeneration and biosynthesis. In fungi, the ultimate step of methionine biosynthesis is motivated by *Met6* (Sadhu et al. 2014). Chemically produced methionine are used for most applications. However, Sulfur is usually provided in the form of inorganic sulfates, which require significant reduction before being converted to methionine. Therefore, stimulating the production of cobalamin-independent methionine synthase (*Met6*) using natural sources rich with organosulfur-and nitrogen-containing compounds, such as broccoli (*Brassica oleracea L.*), was the aim of this study. Recent study showed that absence of *MET15* gene in BY4741 strain affected specifically sulfur metabolism, as well as sulfur absorption pathways of the mutant (*met15Δ*-BY4741) strain was altered and led to differences in cells growth rates between these strains of wild-type and its isogenic mutant (*met15Δ*-BY4741) (Van Oss et al. 2022). In addition, further study demonstrated that *MET6* gene plays an important role in sulfur metabolism pathways next *MET15*. Its expressed methionine acts as precursor for all proteins biosynthesis where is reported as the first amino acid starts translation process (Stipanuk, 2020; Lauinger and Kaiser, 2021). Additionally, this study highlighted the impact of an aqueous broccoli (*Brassica oleracea*) extract on *S. cerevisiae* cells growth in media lacking methionine, and hence quantification analysis of *MET6* expression levels was conducted. The growth curve assay results showed a remarkable reduction and statistical significant differences in cells growth rate of both types of *S. cerevisiae* strains wild-type and the deletion mutant *met15Δ* compared to the untreated strains (control) which is consistent with the previous studies findings that described broccoli myrosinase as a growth toxic for *S. cerevisiae* cells when *Brassica napus* myrosinase MYR1 was introduced (Chen and Halkier, 1999; Jimenez et al. 2022). Moreover, a remarkable down regulation *Met6* expression level in both the wild-type and the deletion mutant *met15Δ* cells that were treated with the broccoli extract compared to the untreated cells (control) was remarked, and these observed differences were statistically significant, however, this reduction in *Met6* expression level in a treated wild-type was greater than that observed in *met15Δ* mutant cells. This finding is consistent to earlier studies that showed broccoli sulforaphane was involved in suppressing the expression levels of cancer stem cell-related genes *SOX2* and *OCT4* in human HNSCC cells, diminishing their colony and sphere formation capacity (Elkashty and Tran, 2020) but no previous studies about broccoli effect on *S. cerevisiae Met6* expression level have been detected. Additionally, a further investigation (Zhang et al. 2020) highlighted SF's ability to downregulated *AMACR* and *ARLNC1* genes is associated with cancer progression in prostate cancer patients. As well as (Satomi et al. 2022). and Yoshida (2015) studies described the impact of components found in broccoli sprouts extract in gene expression regulation where stimulate genes activity associated with detoxification and the synthesis of glutathione in the liver of human and rats. Broccoli's glucosinolates are hydrolysed by myrosinase to produce bioactive substances such as

sulforaphane, which has been linked to many health advantages including anticancer effects (Elkashty and Tran. 2020).

Therefore, the current study results may elucidate the insufficient compensation of the sulfur-rich compounds of the broccoli extract in methionine biosynthesis and thereby become ineffective in compensating methionine deficiency, and this may elucidate the cause of the distinct drop in growth level of both yeast cells types.

Declarations

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding Declaration

The authors declare that no funding was received to support this research.

Author Contributions

NOUF K. ALQAHTANI performed the experiments, wrote, participated in manuscript editing, and performed the experimental analyses.

Alawiah M. Alhebshi designed the study, performed the experimental analyses, revised, and edited the manuscript and gene expression testing.

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Figures

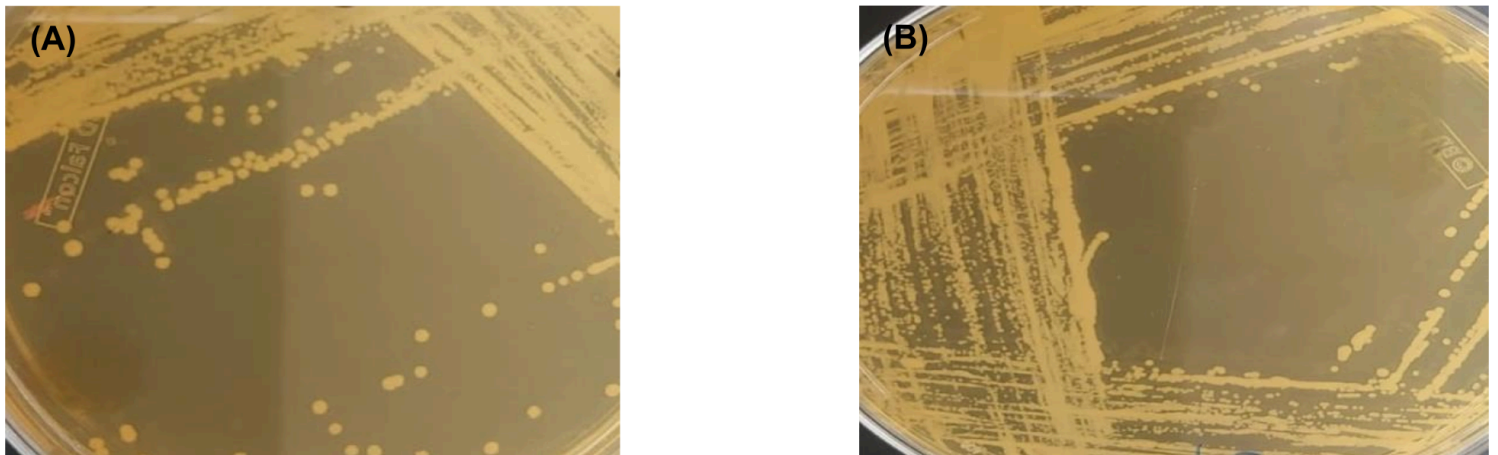


Figure 1

(A) Morphological characteristics of wild-type (WT) and (B) mutant *met15Δ* colonies on complete YEPD agar medium after 24 h incubation at 28 °C

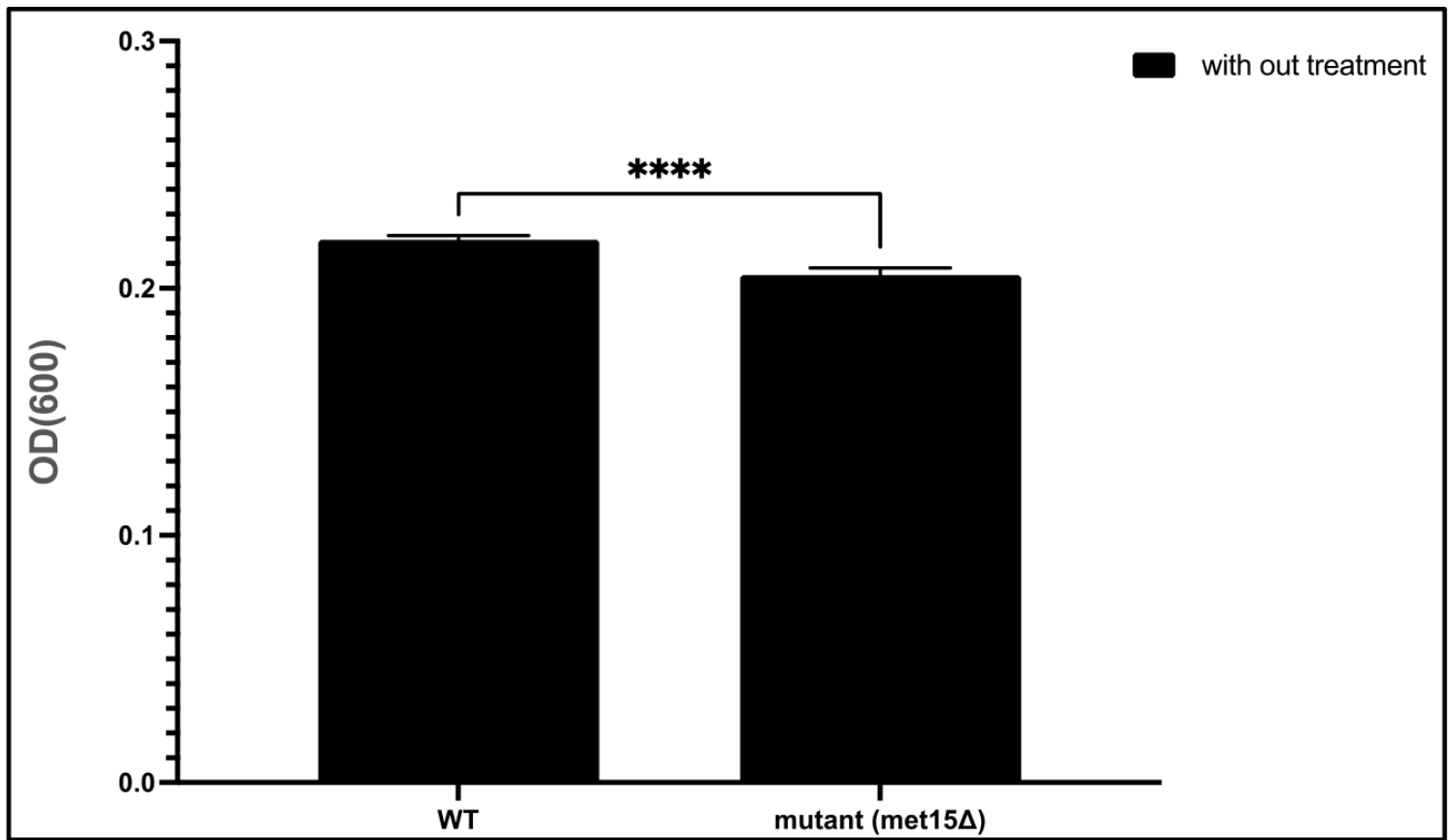


Figure 2

Growth curve assay comparing the cells growth rates of (WT) and mutant (*met15Δ*) cells after incubation at 28C° in broth synthetic complete (SC-MET) media lacking methionine with shaking in a BioTek power wave microplate spectrophotometer (Gen5). A spectrophotometer with OD600 was used to measure the growth levels of both cell types. Mean data are shown. + SEM were calculated from three independent biological samples.

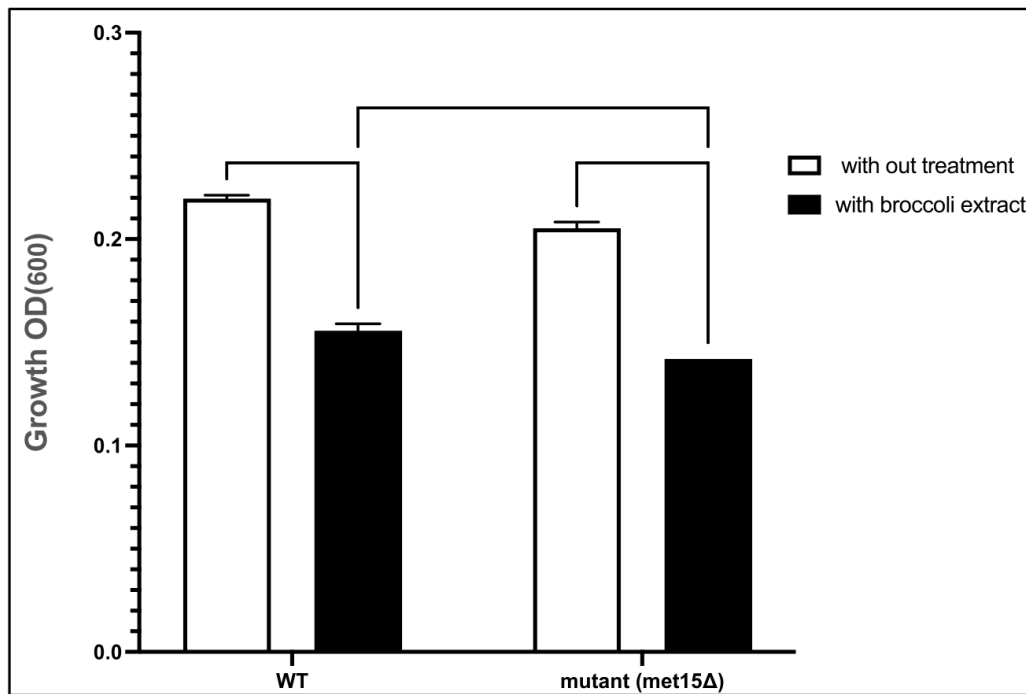


Figure 3: Broccoli extract decrease the growth rates of both wild type and mutant *met15Δ* cells. Growth rates of both wild type and mutant *met15Δ* cells after incubation at 28 C° in the absence (□) or presence (■) of 100 µl/ml broccoli extract in broth synthetic complete (SC-MET) media minus methionine in a BioTek power wave microplate spectrophotometer (Gen5) with shaking. A spectrophotometer with OD600 was used for these growth level measurements of both cell types in media lacking methionine. Mean data are shown. + SEM from three independent biological samples were calculated statically.

Figure 3

See image above for figure legend

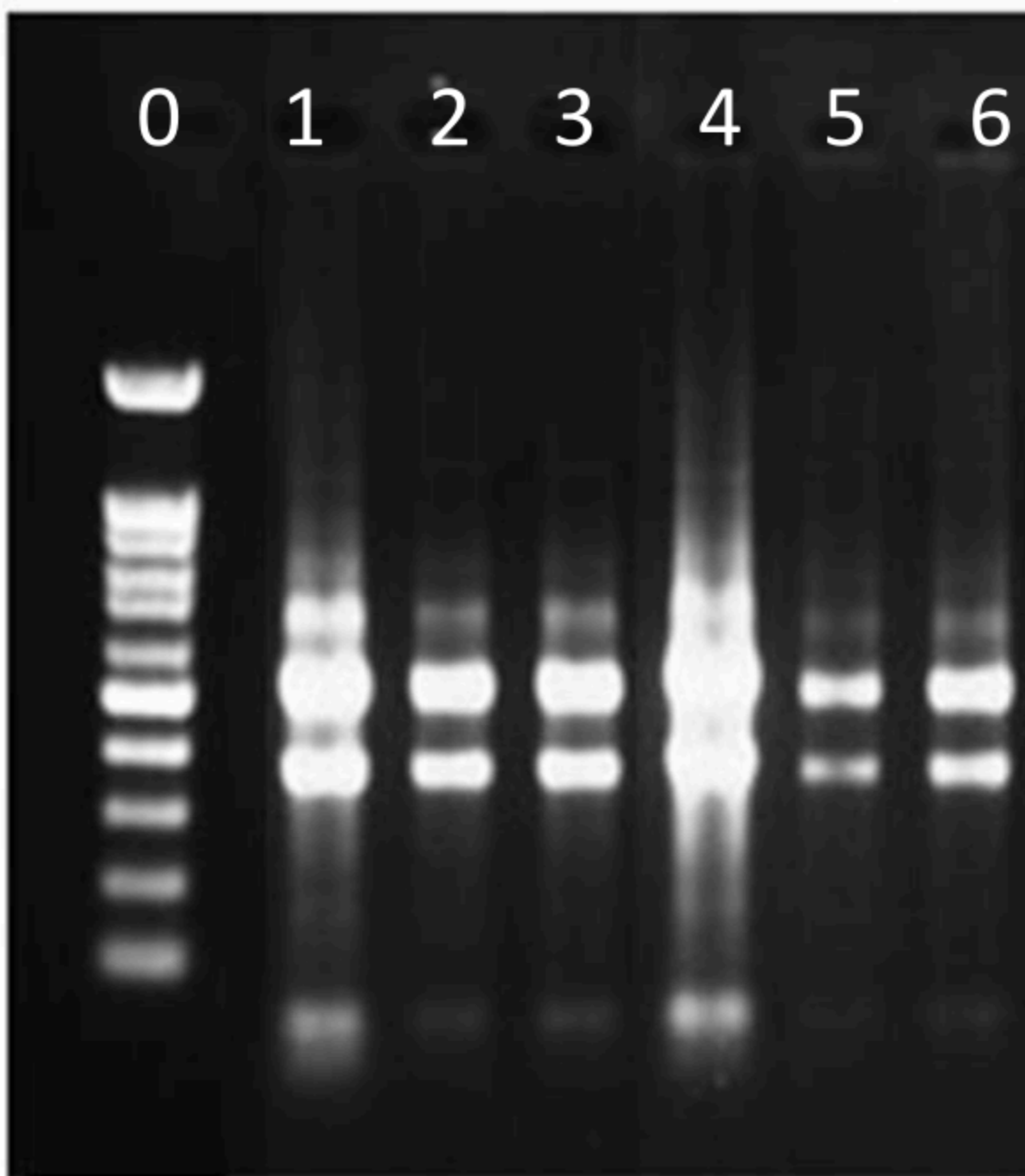


Figure 4

Agarose gel electrophoresis confirmed the integrity of the extracted and purified RNA. RNA extracted from three biological replicates for each sample of the BY4741 wild type strain treated or not with the extract of broccoli, then 1 μ l of each sample was loaded into the gel wells as shown: Lane 0: 100bp DNA ladder; Lanes 1, 2 and 3: RNA extracted from the untreated BY4741 wild type strains after 2 hr; Lane 4, 5 and 6: RNA extracted from the treated BY4741 wild type strains with broccoli extract.

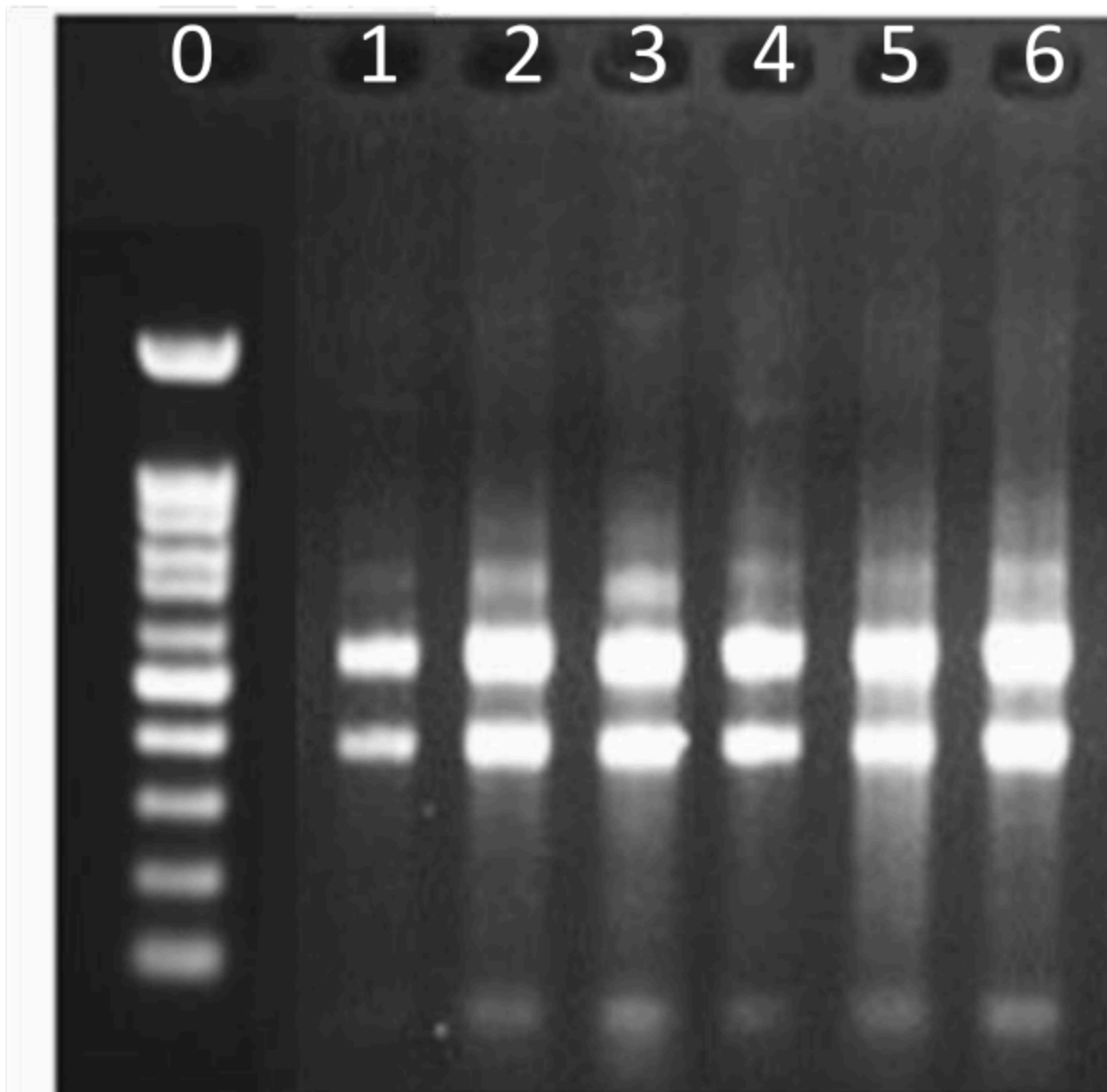


Figure 5

Agarose gel electrophoresis confirmed the integrity of the extracted and purified RNA. RNA extracted from three biological replicates for each sample of the deletion mutant *met15Δ* cells treated or not with the extract of broccoli, then 1 μ l of each sample was loaded into the gel wells as shown: Lane 0: 100bp DNA ladder; Lanes 1, 2 and 3 RNA extracted from the untreated deletion mutant *met15Δ* strains after 2 hrs; Lane 4, 5 and 6 RNA extracted from the treated deletion mutant *met15Δ* strains with broccoli extract after 2 hrs.

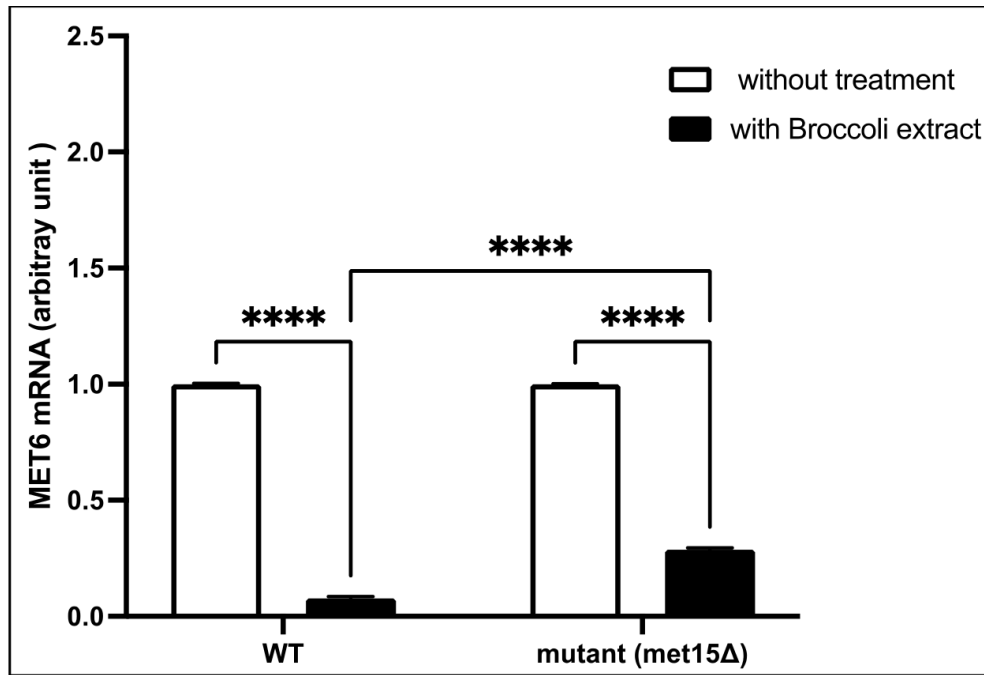


Figure 6: Q RT-PCR analysis of *MET6* expression level. RNA was extracted from wild-type (WT) and *met15Δ* mutant cells which were pre-incubated for 2 h in the absence (□) or presence (■) of 100 μl/ml broccoli in YNB medium supplemented with all amino acids except methionine (CSM-MET). *MET6* mRNA was quantified using q RT-PCR with standardized RNA additions in all reactions, performed in triplicate for each of the three independent experiments. The results are presented as mean ± SEM.

Figure 6

See image above for figure legend