Dose-Dependent Inhibition of *Saccharomyces cerevisiae* Growth by Thapsigargin: Insights into Antifungal Mechanisms

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ABSTRACT

This study explores the inhibitory effects of thapsigargin on the growth of Saccharomyces cerevisiae. Utilizing a dose-response approach, we evaluated the antifungal efficacy of thapsigargin at concentrations ranging from 0.1 mg/mL to 5 mg/mL, incubated over 2 and 4 days. Results demonstrated a significant dose-dependent inhibition of yeast growth, with maximum inhibition observed at 5 mg/mL after 4 days. Specifically, the percentage inhibition was calculated as 63% compared to the control groups, which included ethyl acetate and sterile water. The findings indicate that thapsigargin exerts potent antifungal effects on S. cerevisiae, with potential implications for food safety and fermentation processes. This research fills existing gaps in the literature regarding the effects of plant-derived compounds on beneficial yeast species, contributing valuable insights into natural antifungal strategies. The results underscore the significance of Thapsia garganica as a viable candidate for further exploration in antifungal applications, particularly in the context of food preservation and biotechnological processes.

***Keywords**: Thapsigargin, Thapsia garganica, Saccharomyces cerevisiae, Antifungal efficacy, Dose-response.*

التثبيط المعتمد على الجرعة لنمو *Saccharomyces cerevisiae* بواسطة الثابسجارجين:
رؤى حول الآليات المضادة للفطريات

مفتاح عبدالقادر بطاو
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الملخص

تبحث هذه الدراسة في التأثيرات التثبيطية للثابسجارجين على نمو *Saccharomyces cerevisiae*. باستخدام نهج استجابة الجرعة، قمنا بتقييم الفعالية المضادة للفطريات للثابسجارجين بتركيزات تتراوح بين 0.1 ملغ/مل و5 ملغ/مل، مع الحضانة لمدة 2 و4 أيام. أظهرت النتائج تثبيطاً كبيراً لنمو الخميرة معتمدًا على الجرعة، حيث تم تسجيل أقصى تثبيط عند تركيز 5 ملغ/مل بعد 4 أيام، حيث تم حساب نسبة التثبيط بـ 63% مقارنةً مع مجموعات التحكم التي شملت الأسيتات الإيثيلي والماء المعقم. تشير النتائج إلى أن الثابسجارجين يمتلك تأثيرات قوية مضادة للفطريات على *S. cerevisiae*، مما يحمل دلالات محتملة على سلامة الأغذية وعمليات التخمير. تسد هذه الدراسة فجوات في الأدبيات الحالية فيما يتعلق بتأثير المركبات النباتية على أنواع الخميرة المفيدة،

المفيدة، مما يضيف رؤى قيّمة حول استراتيجيات طبيعية لمكافحة الفطريات. وتبرز النتائج أهمية *Thapsia garganica* كمرشح جدير بالمزيد من البحث في التطبيقات المضادة للفطريات، خاصةً في سياق حفظ الأغذية والعمليات البيوتكنولوجية.

الكلمات المفتاحية: الثابسجارجين، *Thapsia garganica*، *Saccharomyces cerevisiae*، الفعالية المضادة للفطريات، استجابة الجرعة.

INTRODUCTION

Thapsia garganica, commonly referred to as the "deadly carrot" due to its high toxicity to livestock such as sheep and cattle, has been associated with various medicinal applications alongside its toxic effects (Smitt et al., 1995). The toxic compound thapsigargin (Tg) is present in several parts of the T. garganica plant. According to Abderrahim et al. (2013), Tg was first isolated by Christensen and colleagues in 1978 as a skin irritant. The complete structure and absolute configuration of thapsigargin were subsequently established in 1984 (Christensen, Norup, & Rasmussen, 1984).

Historically, the resin derived from the roots and fruits of T. garganica has been utilized in traditional medicine to treat a range of ailments, including pulmonary diseases, female infertility, catarrh, fever, and rheumatism (Andersen et al., 2015). Recent phytochemical investigations have identified sesquiterpene lactones in *Thapsia garganica*, highlighting thapsigargin's potential for therapeutic applications and antifungal studies (Li et al., 2020).

While existing research predominantly addresses the anticancer and antimicrobial properties of thapsigargin (Alilou & Akssira, 2021; Li et al., 2020), there are notable gaps in the literature regarding its effects on non-pathogenic yeast, specifically *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae, commonly known as baker's or brewer's yeast, is a unicellular fungus that has played a crucial role in food production for centuries. This yeast is primarily employed in baking, brewing, and winemaking, where it ferments sugars to produce carbon dioxide and alcohol (Parapouli et al., 2020). As a model organism in molecular and cellular biology, *S. cerevisiae* provides valuable insights into eukaryotic cell functions, genetics, and metabolic pathways, making it a favored choice for a variety of scientific studies (Vanderwaeren et al., 2022).

Despite its recognized benefits, there are instances where inhibiting the growth of *S. cerevisiae* is desirable. Contamination by unwanted strains can lead to spoilage in food products, adversely affecting flavor and quality, particularly in fermented beverages (Bokulich et al., 2013). Additionally, controlling yeast growth is critical in biotechnological applications for optimizing fermentation processes and studying yeast metabolism (Arevalo-Villena et al., 2017).

Moreover, while *S. cerevisiae* is generally recognized as safe, it can pose risks for immunocompromised individuals, potentially leading to opportunistic infections (Goldstein & McCusker, 2001). Inhibiting the growth of *S. cerevisiae* may also serve as a strategic approach in

bioengineering and synthetic biology applications, where precise control over metabolic pathways is essential for producing desired metabolites (Schindler, 2020).

As the food industry seeks to mitigate spoilage and contamination risks, understanding the dose-response relationship of thapsigargin in yeast cultures is particularly relevant. However, this relationship has not been extensively characterized, with limited studies exploring how varying concentrations of thapsigargin influence yeast growth and viability over time. This study aims to address this gap by systematically investigating the inhibitory effects of thapsigargin on the growth of *Saccharomyces cerevisiae*, focusing on dose-response correlations over defined incubation periods. The specific objectives of this study include:

i. **Evaluating the Efficacy:** Assessing the antifungal efficacy of thapsigargin against *S. cerevisiae* by determining growth inhibition at various concentrations and time points.

ii. **Understanding Mechanisms:** Elucidating the mechanisms underlying the antifungal activity of thapsigargin in yeast, contributing to a deeper understanding of its action and potential applications.

iii. **Providing Practical Insights:** Offering insights into the potential applications of thapsigargin in food preservation and antifungal treatments, thereby supporting the industry's need for natural antifungal alternatives.

Table 1. *List of Materials and Equipment*

Material	Supplier	Concentration/Purity
Thapsigargin	Sigma-Aldrich	5 mg/mL (stock)
Ethyl Acetate	Sigma-Aldrich	99.8% purity
Potato Dextrose Agar (PDA)	Thermo Fisher Scientific	-
Potato Dextrose Broth (PDB)	Thermo Fisher Scientific	-
Saccharomyces cerevisiae L.	University of Salford, UK	-
Eppendorf Tubes (1.5 mL)	Eppendorf	-
Sterile Pipette Tips	Thermo Fisher Scientific	-
Pipettes (1–1000 µL)	Eppendorf	-
Incubator	Thermo Scientific	Adjustable to 23°C
Shaker (for liquid cultures)	New Brunswick Scientific	Gentle shaking

METHODOLOGY

Thapsigargin Preparation

To investigate the effect of thapsigargin on Saccharomyces cerevisiae, a stock solution of thapsigargin was prepared at a concentration of 5 mg/mL, dissolved in ethyl acetate. Serial dilutions were performed on the stock to create working solutions at concentrations ranging from 0.1 mg/mL to 5 mg/mL, covering a gradient that allowed for observation of dose-dependent responses in yeast growth. These working solutions were stored in sterile, labeled containers and used within 24 hours to ensure consistency and minimize degradation.

Yeast Culture Preparation

Saccharomyces cerevisiae L. cultures were sourced from the Alun Hughes Laboratory at the University of Salford, UK. Initial cultures were maintained on PDA plates to ensure strain purity and viability. The cultures were incubated at 23°C for 48 hours to allow colonies to grow under optimal conditions, promoting reliable colony formation for subsequent inoculation in a liquid medium. Colonies formed during this phase were used as a basis for liquid culture establishment, providing a consistent and viable inoculum for experimental procedures.

Preparation of Yeast Liquid Cultures

Following the growth of colonies on PDA plates, a single colony of Saccharomyces cerevisiae was carefully selected and transferred into 5 mL of PDB medium in a sterile environment. This inoculated broth was incubated at 23°C with gentle shaking at 120 rpm for 48 hours. The purpose of this incubation was to ensure that yeast cells were evenly distributed and in a growth phase suitable for testing. This preparation allowed a controlled cell concentration, which was essential for ensuring the reproducibility and accuracy of the experimental setup.

Experimental Setup for Thapsigargin Testing

For the testing phase, each experimental setup involved sterile 1.5 mL tubes containing 100 µL of PDB medium. Each tube was inoculated with 1 µL of the S. cerevisiae liquid culture, establishing the baseline growth environment for the yeast. Three conditions were tested:

1) Treatment samples containing 1 µL of thapsigargin at various concentrations
2) Solvent control with 1 µL of ethyl acetate (the solvent for thapsigargin)
3) A negative control with 1 µL of sterile water.

Each condition was prepared in duplicate to confirm consistency across trials and allow for statistical validation of the results.

Incubation and Monitoring

After the addition of the treatment or control solutions, the tubes were incubated at 23°C for periods of 2 and 4 days to assess both short-term and longer-term responses to thapsigargin. Following each incubation period, cultures were subjected to serial dilutions to achieve appropriate colony densities on agar plates. Dilutions were performed by transferring 100 µL of the culture into 900 µL of sterile medium for a 1:10 dilution, which was further diluted to 1:100 and 1:1000 as needed to reach countable colony numbers.

Plating and Colony Counting

From each of the three diluted samples, 100 µL was plated onto fresh Potato Dextrose Agar (PDA) plates in duplicate to ensure reliable measurement. The plates were incubated at 23°C for 4 days to facilitate observable colony formation. After the incubation period, colonies were manually counted on plates that exhibited between 20 and 100 colonies, following established best practices for colony quantification. This approach allowed for the evaluation of thapsigargin's inhibitory effects on Saccharomyces cerevisiae growth across the tested concentrations. Additionally, one photograph of each agar plate was taken at the end of the incubation period to visually document the yeast colony growth and the impact of the different thapsigargin concentrations.

Repetitions and Replicates

The experiment was repeated on three separate occasions, and the average colony count was taken from these replicates. Additionally, each treatment condition (thapsigargin, solvent, and water control) was run in duplicate within each experimental setup to allow for technical consistency. This dual approach of biological and technical replicates ensured the reproducibility and reliability of the observed growth patterns.

Data Analysis: Inhibition Percentage Calculation

The inhibition percentage of S. cerevisiae growth was calculated by comparing colony counts from the thapsigargin treatments to those from the ethyl acetate control. The following formula was used to calculate inhibition:

$$\textbf{\textit{Inhibition percentage}} = \left(1 - \frac{\textbf{Cell Count with Treatment}}{\textbf{Cell Count with Control (Ethyl Acetate)}}\right) \times 100$$

Positive inhibition values indicated a reduction in growth compared to the ethyl acetate control, while negative values, where colony counts exceeded control levels, indicated enhanced growth in response to thapsigargin. This analysis allowed for the quantification of thapsigargin's impact on yeast proliferation across a concentration gradient.

RESULTS

Dose-Dependent Growth Inhibition of *Saccharomyces cerevisiae* by Thapsigargin at 2 and 4 Days

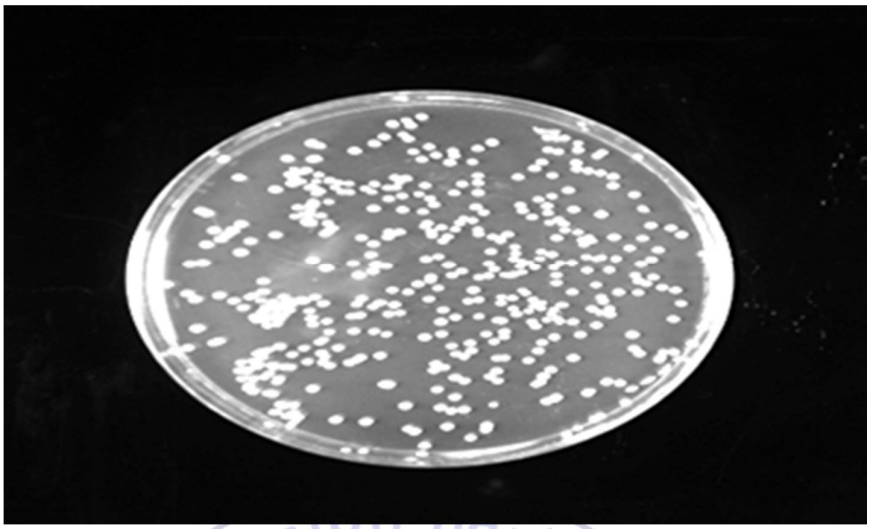
The inhibitory effect of thapsigargin on *Saccharomyces cerevisiae* was assessed at both 2 and 4 days of incubation. Results indicate that thapsigargin's inhibitory effect increases with concentration and time, with higher concentrations leading to more substantial inhibition across both time points. Colony counts and inhibition percentages, compared to the ethyl acetate control, are presented in Table 2.

At 2 days, minimal inhibition was observed at 0.1 mg/mL, with inhibition becoming more pronounced at 1 mg/mL and reaching substantial levels at 5 mg/mL. At 4 days, inhibition percentages increased across all concentrations, highlighting a cumulative effect of thapsigargin over time.

Table 2. Colony Counts and Growth Inhibition of S. cerevisiae by Thapsigargin at 2 and 4 Days

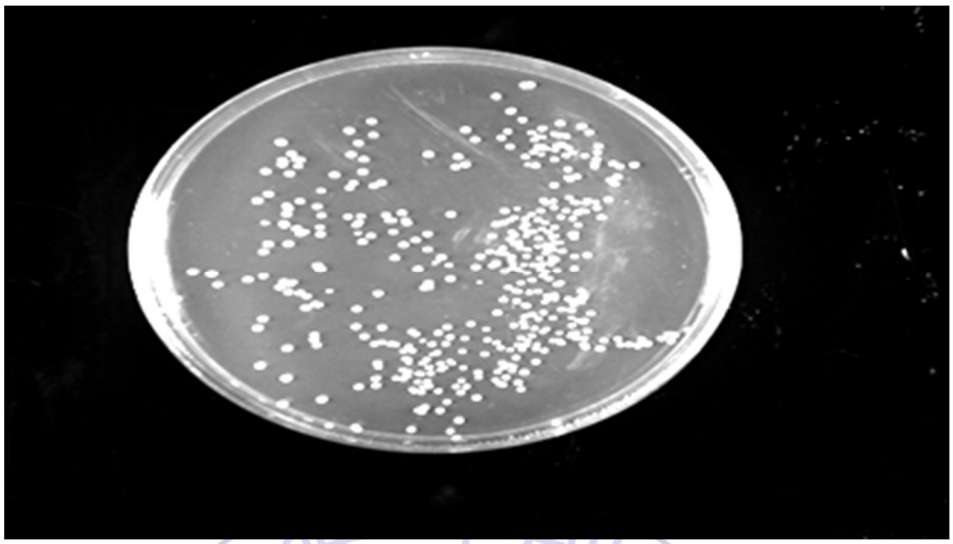
Treatment	Colony Count at 2 Days (Mean ± SD)	Inhibition at 2 Days (%)	Colony Count at 4 Days (Mean ± SD)	Inhibition at 4 Days (%)
Water Control	110 ± 5	-4.8	102 ± 4	-7.4
Ethyl Acetate Control	105 ± 4	0	95 ± 3	0
Thapsigargin 0.1 mg/mL	97 ± 6	7.6	85 ± 5	10.5
Thapsigargin 1 mg/mL	77 ± 7	26.7	70 ± 6	26.3
Thapsigargin 5 mg/mL	39 ± 5	62.9	35 ± 4	63.2

Figure 1. Dose-Dependent Inhibition of Saccharomyces cerevisiae Growth on PDA Plates at 4 Days. Photographs depict the growth of S. cerevisiae colonies treated with three different concentrations of thapsigargin.

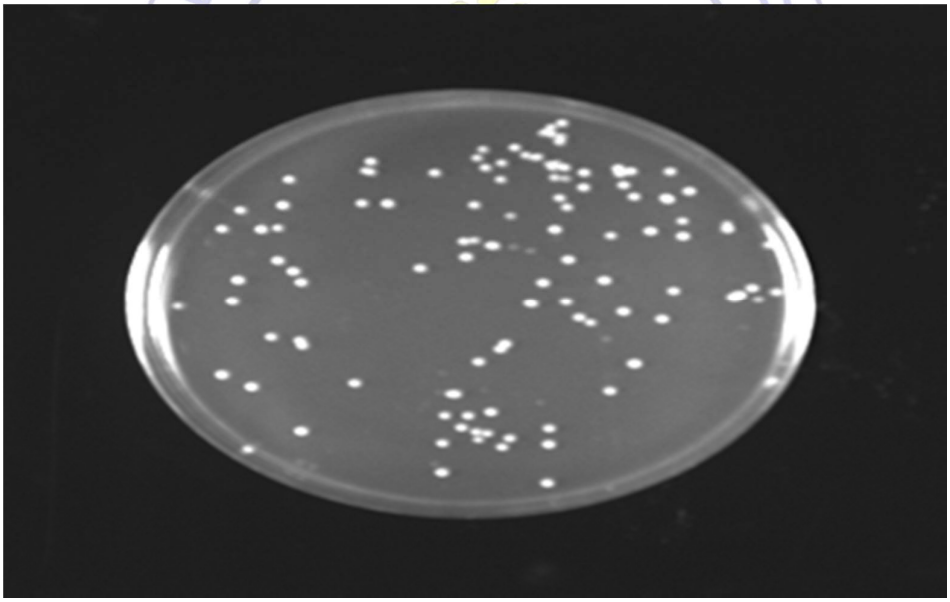


a) Thapsigargin at 0.1 mg/mL, showing minimal inhibition

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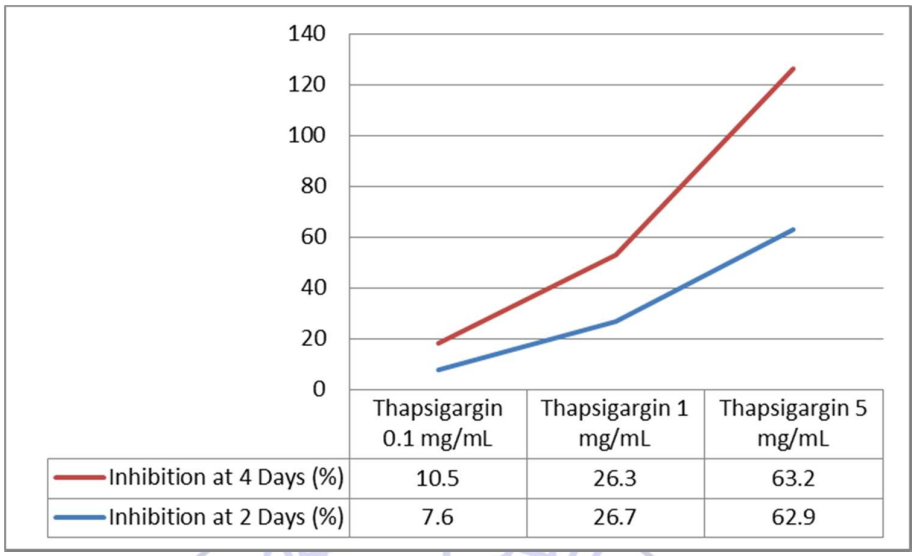


b) Thapsigargin at 1 mg/mL, demonstrating moderate inhibition.



c) Thapsigargin at 5 mg/mL, showing substantial growth inhibition.

Figure 2. Dose-Dependent Inhibition Curve of Saccharomyces cerevisiae Growth in Response to Thapsigargin Treatment at 2 and 4 Days



	Thapsigargin 0.1 mg/mL	Thapsigargin 1 mg/mL	Thapsigargin 5 mg/mL
Inhibition at 4 Days (%)	10.5	26.3	63.2
Inhibition at 2 Days (%)	7.6	26.7	62.9

The graph shows the inhibition percentages of *S. cerevisiae* colony formation at thapsigargin concentrations of 0.1, 1, and 5 mg/mL after both 2 days and 4 days of incubation. Inhibition percentages are calculated relative to the ethyl acetate control, demonstrating increased inhibition with higher concentrations and prolonged incubation.

DISCUSSION

This study explores the inhibitory effects of thapsigargin from *Thapsia garganica* on the growth of *Saccharomyces cerevisiae*. The observed dose-dependent inhibition, particularly the significant growth reduction, aligns with previous findings that demonstrate the antifungal properties of various plant-derived compounds (Alilou & Akssira, 2021). This research adds to this by suggesting a similar potential for growth inhibition in yeast, although the precise mechanisms in *S. cerevisiae* warrant further investigation.

Strengths and Limitations

One of the strengths of this study lies in its systematic approach to assessing the antifungal activity of thapsigargin through various concentrations and incubation times. The use of both ethyl acetate and sterile water as controls enhances the robustness of our findings by providing a clear baseline for comparison. However, a limitation of the study is the lack of exploration into the mechanisms of action behind the observed inhibition. Understanding how thapsigargin interacts with *S. cerevisiae* at the molecular level would provide deeper insights into its antifungal properties and broaden the potential applications in food safety and preservation.

Additionally, while this study contributes valuable data regarding the antifungal potential of *Thapsia garganica*, the sample size and conditions were limited to specific concentrations and incubation periods. Future studies should explore a wider range of concentrations, varying environmental conditions, and include more replicates to strengthen the findings and ensure their applicability in diverse settings.

Future Implications

The implications of this research are significant for both academic and industrial applications. Given the rising concern over antifungal resistance in both clinical and agricultural contexts, *Thapsia garganica* may serve as a novel source of antifungal agents that could complement existing treatments. Future research should focus on elucidating the mechanisms by which thapsigargin affects yeast growth and exploring the synergy between thapsigargin and other antifungal agents.

CONCLUSION

The results indicate a clear, dose-dependent inhibition of *Saccharomyces cerevisiae* growth by thapsigargin, with higher concentrations significantly suppressing colony formation. At a concentration of 5 mg/mL, thapsigargin inhibited growth by approximately 63%, while at 0.1 mg/mL, inhibition was only 10.5%, close to the natural variance observed in untreated samples. This trend suggests that thapsigargin's antifungal activity against *S. cerevisiae* is concentration-dependent, aligning with findings from similar studies where thapsigargin demonstrated potent effects at higher concentrations but minimal impact at lower doses.

The ethyl acetate control inclusion validated that observed inhibition was due to thapsigargin and not its solvent, as yeast growth in the ethyl acetate control closely mirrored the water baseline, with negligible inhibition. This verification underscores the specificity of thapsigargin's inhibitory effects on *S. cerevisiae*.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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