

# 40 Hz sound exposure alters dissolved oxygen levels, gene expression, and colony formation in *Saccharomyces cerevisiae*

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## Abstract

The influence of acoustic stimulation on the physiology of microorganisms has gained increasing attention. However, the effects of sound exposure on dissolved oxygen levels, gene expression, and biofilm formation in yeast remain poorly understood. In this study, we investigated the effects of 40 Hz sound exposure on dissolved oxygen levels in yeast culture medium and purified water, as well as global gene expression and colony formation in *Saccharomyces cerevisiae* BY4741. We found that 40 Hz exposure significantly increased dissolved oxygen levels in yeast culture medium, but not in purified water. RNA-seq and DNA microarray analyses revealed that 40 Hz exposure significantly altered the expression of genes involved in cell adhesion, cell wall organization, and stress response. Notably, the expression of FLO11 and several PAU genes, which are important for yeast biofilm formation, was upregulated by 40 Hz exposure. Consistent with these findings, 40 Hz-exposed yeast exhibited significantly higher colony-forming ability compared to the unexposed control. Our results suggest that 40 Hz sound exposure can enhance dissolved oxygen levels and biofilm formation in *S. cerevisiae*, potentially through the upregulation of adhesion-related genes. These findings provide novel insights into the molecular basis of acoustic effects on yeast physiology and have implications for the control of yeast behavior in biotechnological processes.

*Keywords:* Yeast, *Saccharomyces cerevisiae*, Low-frequency sound

## 1. Introduction

Yeast is a vital eukaryotic microorganism used in the production of various fermented foods and beverages, such as bread, beer, wine, and soy sauce [1]. The physiology of yeast is influenced by environmental factors, including temperature, pH, osmotic pressure, and oxygen availability, which affect its growth, metabolism, and stress responses [2]. Understanding the effects of environmental factors on yeast physiology is crucial for optimizing and controlling fermentation processes.

Recently, the impact of acoustic stimulation on microorganisms has attracted increasing interest. Sound is a mechanical wave characterized by its frequency and amplitude. Previous studies have reported that ultrasound and audible sound can affect the growth and metabolism of bacteria and yeast [3, 4]. For example, Aggio et al. [5] demonstrated that acoustic vibration influences the metabolite profile of *S. cerevisiae*. However, the molecular mechanisms underlying the effects of sound on yeast physiology remain elusive, particularly regarding dissolved oxygen levels and gene expression responses. Dissolved oxygen plays a critical role in yeast physiology, affecting growth, metabolism, and fermentation performance [6]. Oxygen is essential for the synthesis of

unsaturated fatty acids and sterols, which are important components of cell membranes [7].

Moreover, dissolved oxygen levels can influence the expression of genes involved in respiration, fermentation, and stress responses [8]. Therefore, investigating the effects of sound exposure on dissolved oxygen levels in yeast culture medium may provide valuable insights into the mechanisms of acoustic stimulation on yeast physiology.

Furthermore, the impact of sound exposure on yeast biofilm formation has not been explored. Biofilms are multicellular communities of microorganisms attached to solid surfaces and encased in an extracellular matrix [9]. Yeast biofilm formation is a significant concern in food industry and medical settings. In sake brewing, for instance, the formation of yeast biofilms can lead to contamination and deterioration of product quality [10].

Therefore, investigating the effects of sound exposure on yeast biofilm formation may provide valuable insights into controlling yeast behavior in various applications. In this study, we aimed to elucidate the effects of 40 Hz sound exposure on dissolved oxygen levels, global gene expression, and colony formation in *S. cerevisiae* BY4741. We measured dissolved oxygen levels in yeast culture medium and purified water with and without 40 Hz exposure. We employed RNA-seq and DNA microarray analyses to assess the transcriptomic response of yeast to 40 Hz exposure. Colony-forming ability was

evaluated as an indicator of biofilm formation. Our findings offer new insights into the molecular basis of yeast responses to acoustic stimulation and have implications for the modulation of yeast physiology in biotechnological processes.

## 2. Methodology

**Yeast strain and culture conditions.** *S. cerevisiae* strain BY4741 (MATa his3 $\Delta$ 1 leu2 $\Delta$ 0 met15 $\Delta$ 0 ura3 $\Delta$ 0) was used in this study. Yeast cells were pre-cultured in YPD medium (1% yeast extract, 2% peptone, and 2% glucose) at 30°C and 180 rpm for 24 h. For main cultures, 40 mL of YPD medium in 300 mL Erlenmeyer flasks were inoculated with the pre-culture to an initial OD<sub>600</sub> of 0.1 and incubated at 30°C and 120 rpm for 48 h.

**Sound exposure conditions.** For sound exposure experiments, a subwoofer speaker (Yamaha NS-SW050, 20 cm diameter, 50 W output) was used to generate a 40 Hz sinusoidal wave at a sound pressure level of 100 dB (measured at 25 cm from the sound source). Culture flasks were placed in a sound-insulated incubator, and the sound was continuously applied for 48 h. Control experiments were conducted in an identical incubator without sound exposure. All experiments were performed in triplicate.

**Dissolved oxygen measurement.** Dissolved oxygen levels in yeast culture medium and purified water were measured using a portable dissolved oxygen meter (HORIBA OM-71). Measurements were taken at 0, 24, and 48 h of incubation with or without 40 Hz sound exposure. Four experimental conditions were tested: yeast culture medium with 40 Hz exposure, yeast culture medium without exposure, purified water with 40 Hz exposure, and purified water without exposure. Each condition was measured in quadruplicate.

**RNA extraction and RNA-seq analysis.** Total RNA was extracted from yeast cells using the hot phenol method (11) and treated with DNase I. RNA-seq libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina) and sequenced on an Illumina NovaSeq 6000 platform (2 × 150 bp). Raw reads were quality-filtered using Trimmomatic (12) and mapped to the *S. cerevisiae* S288C reference genome (R64-1-1) using HISAT2 (13). Read counts were obtained using feature Counts (14) and differentially expressed genes (DEGs) were identified using DESeq2 (15) with a false discovery rate (FDR) < 0.05 and |log<sub>2</sub> fold change > 1. Gene Ontology (GO) enrichment analysis of DEGs was performed using cluster Profiler (16) with an FDR < 0.05. DNA microarray analysis. DNA microarray analysis was performed using the Affymetrix Gene Chip

**Yeast Genome 2.0 Array.** Total RNA was reverse-transcribed and labeled using the GeneChip 3' IVT Express Kit (Affymetrix). Labeled cRNA was hybridized to the microarray, washed, and stained using the

GeneChip Hybridization, Wash, and Stain Kit (Affymetrix). Microarray data were analyzed using the Transcriptome Analysis Console (TAC) software (Affymetrix) to identify DEGs (FDR < 0.05 and |log<sub>2</sub> fold change| > 1) and perform GO enrichment analysis (FDR < 0.05).

**Colony formation assay.** Yeast cells from 48 h cultures with or without 40 Hz exposure were diluted and spread onto YPD agar plates. After incubation at 30°C for 72 h, the number of colonies was counted. Statistical significance was assessed using Student's t-test with a p-value < 0.05.

## 3. Preliminary Results

Effects of 40 Hz sound exposure on dissolved oxygen levels. Dissolved oxygen levels in yeast culture medium and purified water were measured under four conditions: yeast culture medium with 40 Hz exposure, yeast culture medium without exposure, purified water with 40 Hz exposure, and purified water without exposure (Table 1). In yeast culture medium, 40 Hz exposure significantly increased dissolved oxygen levels compared to the unexposed control (p < 0.01). In contrast, 40 Hz exposure did not affect dissolved oxygen levels in purified water.

Effects of 40 Hz sound exposure on gene expression in *S. cerevisiae*. RNA-seq analysis identified 883 DEGs (429 upregulated and 454 downregulated) in response to 40 Hz sound exposure. DNA microarray analysis revealed 667 DEGs (325 upregulated and 342 downregulated). The overlap between the two methods was 55.2% for upregulated genes and 58.7% for downregulated genes.

Table 1. Dissolved oxygen levels (mg/L) in yeast culture medium and purified water with or without 40 Hz sound exposure.

Condition	0h	24h	48h
Sterile YPD + 40 Hz	6.78 ± 0.05	6.83 ± 0.04	6.89 ± 0.06
Sterile YPD (control)	5.30 ± 0.06	5.06 ± 0.29	4.93 ± 0.27
Purified water + 40 Hz	7.31 ± 0.06	7.29 ± 0.07	7.28 ± 0.05
Purified water(control)	7.35 ± 0.03	7.33 ± 0.04	7.32 ± 0.05

GO enrichment analysis of the common DEGs showed that the upregulated genes were enriched in "cell adhesion," "fungal-type cell wall organization," and "response to stimulus," while the downregulated genes were enriched in "ribosome biogenesis," "rRNA processing," and "translation"

Effects of 40 Hz sound exposure on colony formation in *S. cerevisiae*. Yeast cells exposed to 40 Hz sound exhibited significantly higher colony-forming ability compared to the unexposed control ( $p = 0.003$ ). The mean  $\pm$  SD of colony-forming units (CFU) per mL was  $(3.2 \pm 1.1) \times 10^2$  for the control and  $(7.6 \pm 2.4) \times 10^2$  for the 40 Hz-exposed group.

#### 4. Discussion

This study demonstrates that 40 Hz sound exposure significantly increases dissolved oxygen levels in yeast culture medium and alters the gene expression profile of *S. cerevisiae* BY4741. The increased dissolved oxygen levels in culture medium, but not in purified water, suggest that the effects of 40 Hz exposure on dissolved oxygen are mediated by the presence of yeast cells. One possible explanation is that 40 Hz exposure may enhance the mixing and mass transfer of oxygen in the culture medium by inducing vibrations and microbubble formation (17). Alternatively, 40 Hz exposure may stimulate the metabolic activity of yeast cells, leading to increased oxygen consumption and subsequent dissolution of atmospheric oxygen into the medium.

The upregulation of genes involved in cell adhesion and cell wall organization, such as FLO11 and PAU genes, suggests that 40 Hz exposure may enhance the biofilm-forming ability of yeast. FLO11 encodes a GPI-anchored cell wall protein that promotes cell-cell and cell-surface adhesion, while PAU genes encode cell wall-associated proteins that are important for maintaining cell wall integrity (18, 19). The coordinated upregulation of these genes in response to 40 Hz exposure may contribute to the enhanced colony-forming ability observed in this study.

The higher colony-forming ability of 40 Hz-exposed yeast supports the hypothesis that acoustic stimulation can promote biofilm formation in *S. cerevisiae*. Colony-forming ability is a widely used indicator of biofilm formation in yeast (20). However, further investigations are needed to directly assess the effects of sound exposure on biofilm structure and development.

Yeast biofilm formation is a critical concern in various industrial and clinical settings. In the food industry, biofilm formation by spoilage yeast can lead to contamination and product deterioration (10). In contrast, the biofilm-forming ability of yeast is exploited in bioethanol production to enhance the attachment of cells to cellulosic substrates and improve fermentation efficiency (21). The findings of this study suggest that acoustic stimulation could be a potential tool for modulating yeast biofilm formation in these contexts.

The increased dissolved oxygen levels in yeast culture medium exposed to 40 Hz sound may have implications for fermentation processes. Dissolved oxygen is a critical factor in yeast physiology, affecting growth, metabolism, and product formation (6). In beer fermentation, for

example, high dissolved oxygen levels at the beginning of fermentation are essential for yeast growth and flavor development, while low oxygen levels are desired during the later stages to maintain beer stability (22). The ability to modulate dissolved oxygen levels using acoustic stimulation could potentially be used to optimize fermentation processes and improve product quality.

However, this study has several limitations. First, only a single yeast strain (BY4741) was examined, and the generalizability of the findings to other strains or industrial yeasts remains to be determined. Second, the effects of sound frequencies and intensities other than 40 Hz and 100 dB were not investigated. Third, gene expression and dissolved oxygen levels were analyzed at a limited number of time points, and the temporal dynamics of these responses to sound exposure remain unknown. Future studies should address these limitations to gain a more comprehensive understanding of the acoustic effects on yeast physiology.

#### 5. Conclusion

This study reveals that 40 Hz sound exposure increases dissolved oxygen levels in yeast culture medium, alters gene expression, and enhances colony formation in *S. cerevisiae* BY4741. The upregulation of adhesion-related genes, such as FLO11 and PAU genes, suggests that acoustic stimulation may promote biofilm formation in yeast.

These findings provide new insights into the molecular basis of yeast responses to sound and have implications for the control of yeast behavior and optimization of fermentation processes in biotechnological applications. Further research is warranted to explore the potential of acoustic stimulation as a tool for modulating yeast physiology and biofilm formation in various industrial settings.

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### Authors Introduction

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