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# Assessment of the Developmental Profiles of ZHY3 and W303 Yeast Strains in Zinc-Modified Media

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## Abstract

All organisms require zinc to sustain their vital functions. In the case of Zn deficiency, plant production, as well as animal and human health, are adversely affected. Baker's yeast (*Saccharomyces cerevisiae*) is a model organism for scientific research and is commonly used in metal deficiency/toxicity studies. This study aims to identify the first stage in the identification of Zn uptake genes, which determines the conditions of Zn-deficient environments that inhibit yeast development. The YNB medium, the basic nutrient medium for yeast cultivation, contains enough Zn conducive to yeast growth. Chelating agents (EGTA or EDTA) have been added to the YNB medium to create conditions of insufficient Zn. The yeast strains ZHY3 (Zn uptake gene mutant) and W303 (wild type – WT) were tested in these environments. The growth profiles of the yeasts were compared in nutrient media containing different concentrations of the chelating agents. The concentrations of EGTA and EDTA that inhibited the growth of the W303 strain were determined to be 25 mM and 1.6 mM, respectively, while the concentrations that inhibited the growth of the ZHY3 strain were determined to be 12.5 mM and 0.01 mM, respectively. These findings are

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significant for understanding the effects of Zn deficiency on different yeast strains and highlighting the importance of Zn uptake genes. It is believed that the identified concentrations and the results obtained will contribute to studies related to Zn uptake and Zn uptake genes.

***Keywords:*** Yeast; Zinc; ZHY3; W303; EDTA; EGTA.

## ZHY3 ve W303 Maya Irklarının Çinko Zenginleştirilmiş Ortamdaki Gelişim Profillerinin Değerlendirilmesi

### Öz

Tüm organizmalar hayati fonksiyonlarını sürdürebilmek için çinkoya ihtiyaç duymaktadır. Çinko eksikliğinde bitkisel üretim, hayvan ve insan sağlığı olumsuz etkilenmektedir. Ekmek mayası (*Saccharomyces cerevisiae*) bilimsel araştırmalar için model bir organizmadır ve metal eksikliği/toksisitesi çalışmalarında yaygın olarak kullanılmaktadır. Bu çalışmada çinko alım genlerinin tanımlanması çalışmalarında ilk aşama olan, mayanın gelişmesini önleyen yetersiz çinkolu ortam koşullarının belirlenmesi hedeflenmiştir. Mayanın yetiştirildiği temel besin ortamı olan YNB, mayanın büyümesine elverişli yeterli miktarda çinko içermektedir. Yetersiz çinko koşulları oluşturması için şelat ajanları (EGTA ya da EDTA) YNB besin ortamına eklenmiştir. ZHY3 (çinko alım genleri mutant) ve W303 (Yabanıl – WT) maya ırkları bu ortamlarda test edilmiştir. Mayaların büyüme profilleri farklı konsantrasyonlarda şelat ajanı içeren besin ortamlarında karşılaştırılmıştır. W303 ırkının büyümesini engelleyen EGTA ve EDTA konsantrasyonları sırasıyla 25 mM ve 1.6 mM olarak belirlenirken ZHY3 ırkının büyümesini engelleyen EGTA ve EDTA konsantrasyonların sırasıyla 12.5 mM ve 0.01 mM olarak belirlenmiştir. Bu bulgular, çinko eksikliğinin farklı maya ırkları üzerindeki etkilerini anlamak ve çinko alım genlerinin önemini vurgulamak açısından önemlidir. Belirlenen konsantrasyonların ve elde edilen sonuçların çinko alımı ve çinko alım genleri ile ilgili çalışmalara katkıda bulunacağı düşünülmektedir.

***Anahtar Kelimeler:*** Maya; Çinko; ZHY3; W303; EDTA; EGTA.

### 1. Introduction

Zinc (Zn) is a trace element essential for the survival of living organisms and is the second most abundant trace element in the human body after iron [1]. Zn acts as a cofactor in approximately 300 enzymes and plays roles in various biological processes, including

indoleacetic acid production, photosynthesis, pollen formation, and maintaining cell membrane integrity [2-3]. A healthy adult body contains 1-2.5 grams (g) of Zn, and humans require an average daily intake of 10 milligrams (mg) of Zn. Individuals who receive less than this amount may experience Zn deficiency, leading to conditions such as anemia, vision problems, loss of appetite, immune system issues, delayed wound healing, and severe autoimmune diseases [1]. It is estimated that 17% of the world's population suffers from Zn deficiency [4]. Today, many people use Zn-supplemented medications and commercial products to increase their Zn levels. In plants, Zn deficiency can cause issues such as chlorosis, stunted growth, an increase in reactive oxygen species (ROS), and losses in yield and quality. To prevent these problems, Zn-fortified fertilizers are used, but they do not provide a permanent solution and can result in significant economic losses. Nearly half of the world's soil is deficient in Zn [5]. Moreover, even when sufficient Zn is available in the soil, many plant species cannot efficiently absorb it [2].

Baker's yeast (*Saccharomyces cerevisiae*) is one of the most well-known yeast species. Its simple structure, ease of genetic manipulation, rapid growth, and low cost make it a model organism in biotechnological studies [6]. The W303 yeast strain is a derivative of a yeast strain, S288C. It carries mutations in leu2, *trp1*, *ura3*, *ade2*, and *his3* genes and is known for its high transformation efficiency. It has been used in various studies involving gene expression, protein localization, cell cycle regulation, and DNA repair. YPD (Yeast Extract Peptone Dextrose) is a commonly used non-selective medium for yeast cell growth, while YNB (Yeast Nitrogen Base) is a selective medium for uracil auxotrophic yeast. CSM-ura, which contains amino acids except for uracil, is used in the selection of yeast transformants [7].

ZRT1 and ZRT2 were first identified as Zn transporters in yeast [8]. The *ZRT1* gene encodes a high-affinity Zn transporter, while the *ZRT2* gene encodes a low-affinity transporter. Both genes have eight transmembrane domains and share 67% similarity [8]. The ZRT1 protein has been reported to localize to the plasma membrane [9]. At the onset of Zn deficiency, the expression of the *ZRT2* transporter increases, while during critical levels of Zn deficiency, the expression of the *ZRT1* transporter is upregulated. The ZHY3 yeast strain is derived from the W303 strain. In addition to the mutations carried by W303, ZHY3 also possesses mutations in *ZRT1* and *ZRT2*. Therefore, it cannot take enough Zn from the external environment for growth. ZHY3 is frequently used in complementation tests aimed at determining the function of genes related to Zn uptake/export pathways.

Chelation refers to binding ions and molecules to metal ions [10]. Chelating agents have a reducing effect on the amount of metal ions in the environment. There are two main chelating

agents for Zn: EDTA (ethylenediaminetetraacetic acid) and EGTA (ethylene glycol-bis(β-aminoethyl ether)). EDTA and EGTA are frequently used in growth media to understand the effects of Zn deficiency. EDTA forms a complex by binding divalent free metal ions, and EGTA functions similarly but has a lower affinity for magnesium ($Mg^{2+}$) ions and a higher affinity for calcium ($Ca^{2+}$) ions.

In the present study, the pAG426GPD-ccdB yeast expression vector was transformed into two different yeast strains: ZHY3, which has mutations in the Zn uptake genes (*ZRT1* and *ZRT2*), and W303, which does not have these mutations. EGTA and EDTA chelating agents were then added in varying concentrations to the YNB selective medium containing a standard 25 μM $ZnSO_4$ to chelate the Zn. By creating Zn-deficient conditions in the selective medium, the growth profiles of the W303 and ZHY3 strains were determined.

## 2. Materials and Methods

### 2.1. Transformation of the pAG426GPD-ccdB Vector into W303 and ZHY3 Yeast Strains

In this study, the pAG426GPD-ccdB yeast expression vector (Addgene, plasmid #14156) was used. Thanks to the *URA3* gene presence in the vector, transformant colonies can survive in a medium lacking uracil by producing the URA3 protein.

The transformation of the pAG426GPD-ccdB vector into the W303 and ZHY3 yeast strains was performed using the transformation protocol reported by Benatuil et al. [11]. The transformed W303 and ZHY3 yeast cells were spread onto a YPD agar medium and incubated at 30°C for approximately three days, after which colony formation was observed. A single colony was selected from the resulting colonies, transferred to a liquid YPD medium, and shaken overnight at 30°C and 225 rpm. The next day, the cell concentration was measured as OD600 = 1.2. The cells were diluted in 50 ml of liquid YPD medium to a concentration of OD600 = 0.2. The cells were then incubated in a shaker at 30°C and 225 rpm until the OD600 reached 1.2. After approximately 5 hours, the cells were centrifuged at 3000 rpm for 3 minutes, and the pellet was obtained. The pellet was washed twice with 50 ml of cold distilled water and once with 50 ml of cold electroporation buffer. The cells were then centrifuged again at 3000 rpm for 3 minutes. The pellet was resuspended in 20 ml of 0.1 M LiAc/10 mM DTT and shaken at 30°C and 225 rpm for 30 minutes. After incubation, the cells were collected again by centrifugation at 3000 rpm, and the supernatant was removed. The pellet was resuspended in 100-200 μL of electroporation buffer, and the final volume was adjusted to 1 ml. The cells were added to electroporation cuvettes

(previously kept on ice), with 400 µL of cells in each cuvette. Then, 1 µg of the pAG426-GPD-ccdB plasmid was added to 400 µL of cells. The electroporation was performed under the conditions of 2.5kV, 25µF, and 3.0 milliseconds. After electroporation, the cells were transferred to tubes containing 8 ml of a 1:1 mixture of 1M sorbitol and YPD medium. The cells were spread (100 µL per plate) onto prepared YPD and YNB-URA selective agar media. The Petri dishes were incubated at 30°C for about three days until colonies were observed.

### 2.2. Determination of EGTA and EDTA Concentrations Inhibiting the Growth of W303 and ZHY3 Yeast Strains

The growth of ZHY3-pAG426GPD-ccdB and W303-pAG426GPD-ccdB yeast cells was observed in a YNB medium containing various concentrations of EGTA (0 – 25 mM) or EDTA (0 – 3.2 mM) to determine the concentrations that inhibit their growth. For this purpose, the cells were grown overnight in 5 ml of liquid YNB at 30°C and 225 rpm. The next day, the OD600 values of the cells were measured using a spectrophotometer and diluted to 1/10 serial dilutions starting from an OD600 of 0.2. Each cell sample (5 µL) was spotted onto plates containing YNB medium with the predetermined concentrations of EGTA or EDTA. The spotted cells were incubated at 30°C for about three days.

## 3. Results and Discussion

### 3.1. Transformation of the pAG426GPD-ccdB Vector into W303 and ZHY3 Yeast Strains

The transformation of the pAG426GPD-ccdB vector into the W303 and ZHY3 yeast strains was successfully performed (Fig.1 and Fig. 2). Colony formation was observed in the transformed cells within approximately three days. Since the W303 and ZHY3 cells without the vector did not produce the URA3 protein, no colony formation was observed on the URA-deficient YNB medium (-URA) for these cells (C, D).

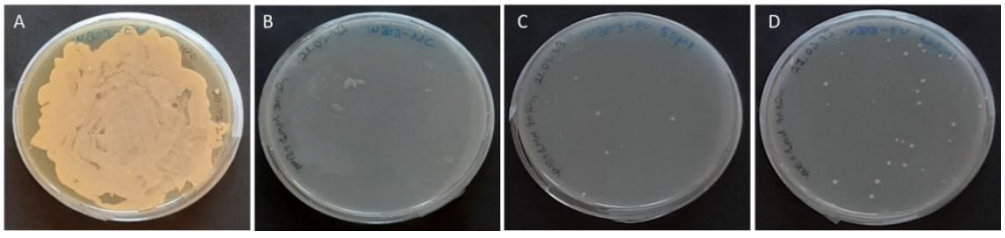


**Figure 1:** Transformation of the pAG426GPD-ccdB vector into W303 yeast cells. (A) W303 cells with the vector in YPD medium (positive control), (B) W303 cells without the vector in YNB medium (negative control), (C-D) W303 cells with the vector in YNB medium.

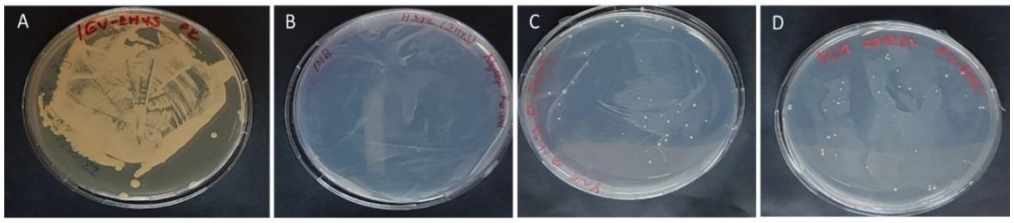


**Figure 2:** Transformation of the pAG426GPD-ccdB vector into ZHY3 yeast cells. (A) ZHY3 cells with the vector in YPD medium (positive control), (B) ZHY3 cells without the vector in YNB medium (negative control), (C-D) ZHY3 cells with the vector in YNB medium.

The W303 and ZHY3 cells containing the vector were spotted in serial dilutions onto YNB media containing 0, 0.5, 1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, and 25 mM EGTA (Fig. 3) or YNB media containing 0, 0.0005, 0.001, 0.002, 0.01, 0.025, 0.05, 0.075, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mM EDTA (Fig. 4).

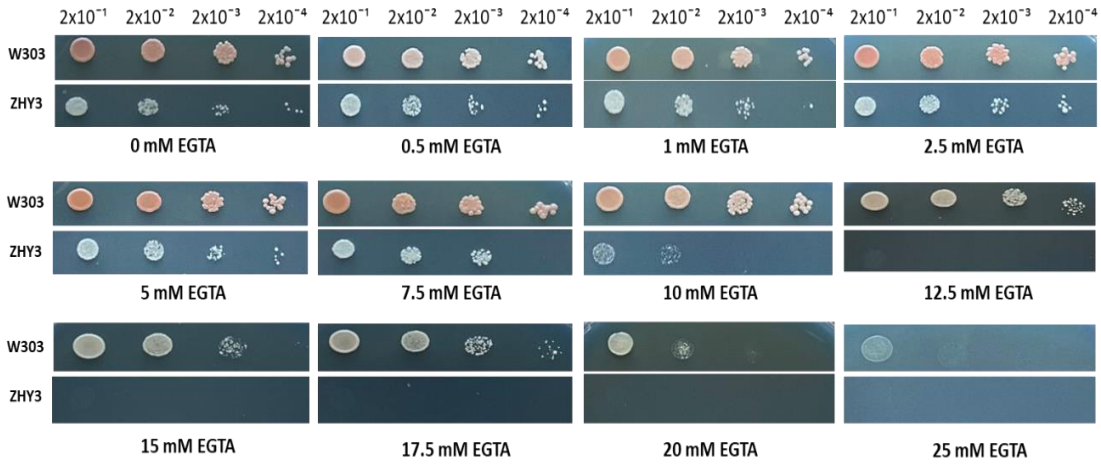


**Figure 3:** Growth profiles of W303 and ZHY3 yeast strains containing the pAG426GPD-ccdB vector in YNB medium with varying concentrations of EGTA.

The cells of the transformed W303 strain were not critically affected by Zn deficiency, but they did not grow on YNB medium containing 25 mM EGTA. In contrast, the cells of the transformed ZHY3 strain were critically affected by Zn deficiency, and their growth significantly decreased starting from the medium containing 12.5 mM EGTA. In all media, the W303 strain showed more growth than the ZHY3 strain.

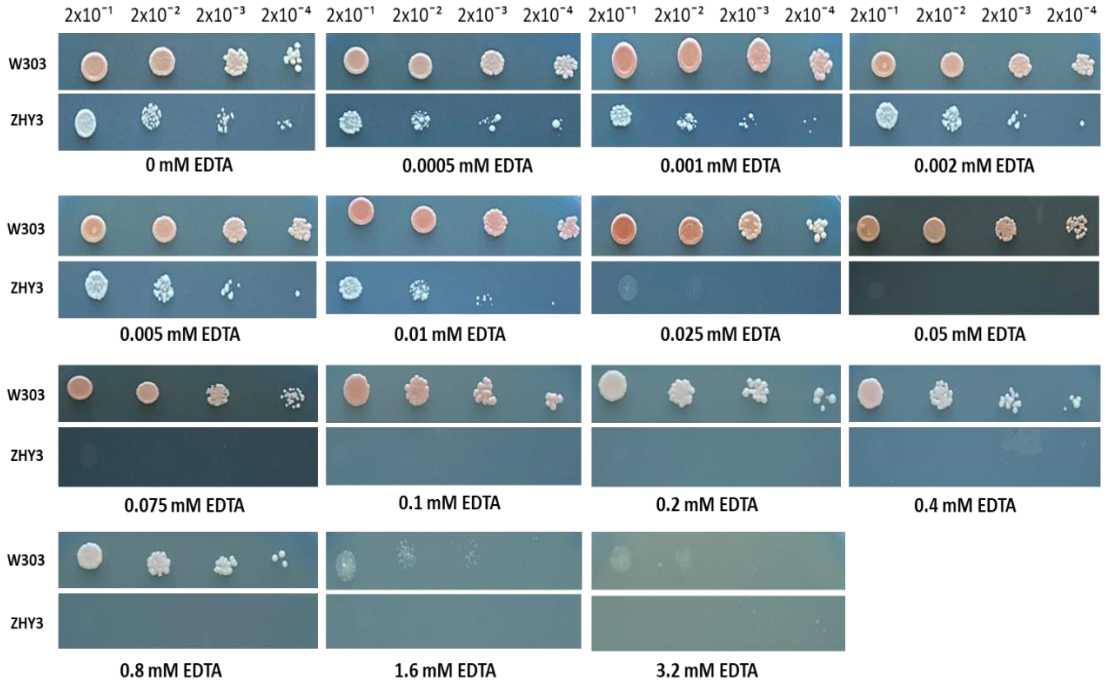


**Figure 4:** Growth profiles of W303 and ZHY3 yeast strains containing the pAG426GPD-ccdB vector in YNB medium with varying concentrations of EDTA.

The transformed W303 cells continued to grow in the YNB medium containing 0-0.8 mM EDTA, but growth slowed down in the medium containing 1.6 mM EDTA, and it was completely halted in the medium with 3.2 mM EDTA. On the other hand, the transformed ZHY3 cells were critically affected by Zn deficiency and were unable to grow in the medium containing as little as 0.01 mM EDTA. Across all EDTA and EGTA concentrations, the W303 strain exhibited more growth than the ZHY3 strain.

## 4. Discussion and Conclusion

Zinc deficiency is a widespread issue affecting agricultural productivity and human health. Insufficient zinc levels result in notable reductions in plant yield and quality while also contributing to various health problems in humans. To prevent the negative effects of Zn deficiency in plants, it is crucial to fully understand the mechanisms of plant Zn uptake and to identify the genes associated with Zn absorption. Studies addressing Zinc deficiency have been used the ZHY3 yeast strain, which has mutations in intracellular Zn uptake genes, alongside other wild-type strains. However, the concentration of Zn deficiency that restricts ZHY3's growth varies across studies. For instance, Kozak et al. [12] used the wild-type DY1457 strain and the derived ZHY3 strain in their study. They observed that DY1457 cells could grow in a YNB medium containing 7.5 mM EGTA, whereas ZHY3 cells were unable to grow at both 5 and 7.5 mM EGTA concentrations. Similarly, Li et al. [13] reported that DY1451 cells could grow at 0.4 mM EDTA,

while ZHY3 cells did not show any growth at that concentration. Yang et al. [14] found that wild-type cells could grow in a medium with 5 mM EGTA, but ZHY3 cells were unable to grow in EGTA-containing environments.

In the present study, the growth profiles of the wild-type yeast strain W303 and the ZHY3 strain, derived from W303 but with mutations in Zn uptake genes, were compared in YNB media containing different concentrations of the chelating agents EDTA and EGTA. The impact of the Zn uptake genes *ZRT1* and *ZRT2* was clearly observed in these strains. Zn chelation occurred in YNB media with varying concentrations of EDTA and EGTA, creating progressively increasing Zn-deficient conditions. In YNB media containing EGTA, W303 cells were able to grow up to 25 mM, while ZHY3 cells, due to mutations in Zn uptake genes, were unable to grow starting from 12.5 mM EGTA. Similarly, in YNB media containing EDTA, W303 cells grew up to 0.8 mM, while ZHY3 cells showed no growth starting from as low as 0.01 mM EDTA.

These findings, along with previous studies, demonstrate that slight differences in chelating agent concentrations can affect the growth inhibition of yeast strains depending on the specific strain and the technical conditions of the study. The chelating agent concentrations identified in this study will contribute to gene function analysis and characterization studies related to Zn deficiency.

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