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# Investigation of genes responsible for antibiotics resistance in yeast *Zygosaccharomyces rouxii*

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## *Abstract*

**Objective**

The aim of the present study was to identify the genetic location of antibiotic and heavy metal resistance in the yeasts *Zygosaccharomyces rouxii* and *Candida tropicalis*. These yeasts were isolated from mandarin tree leaves collected in Mosul. The study focused on determining whether resistance genes were located on plasmid DNA or chromosomal DNA.

**Materials and methods**

Yeast isolates of *Z. rouxii* and *C. tropicalis* were examined for the presence of plasmid DNA. Two methods were used to locate resistance genes. The first method involved plasmid curing using ethidium bromide at a concentration of 150 $\mu g\ mL^{-1}$. This treatment was applied to both yeast species to remove plasmid DNA. After curing, the sensitivity of yeast colonies to different antibiotics and heavy metals was tested. Gel electrophoresis was used to confirm the loss or retention of plasmid DNA. The second method involved conjugation experiments to study the transfer of plasmid DNA between yeast isolates. In this method, *Z. rouxii* was used as the donor strain and cured *C. tropicalis* was used as the recipient strain.

**Results**

Plasmid curing was successful in both yeast species. Because many cured colonies became sensitive to the tested antibiotics and heavy metals. In *Z. rouxii*, the curing percentage ranged from 12% to 94%, except for cephalexin monohydrate and zinc sulfate ($ZnSO_4$), where resistance was not lost. In *C. tropicalis*, loss of resistance ranged from 17% to 80% for antibiotics and from 15% to 85% for heavy metals, except for ketoconazole and $ZnSO_4$. These results suggest that resistance to these agents is encoded on chromosomal DNA. Gel electrophoresis supported these findings. Conjugation experiments showed successful plasmid transfer from *Z. rouxii* to cured *C. tropicalis*, with a conjugation frequency of $0.84 \times 10^{-8}$.

**Conclusions**
The study demonstrated that resistance genes in *Z. rouxii* and *C. tropicalis* can be located on either plasmid or chromosomal DNA. The transferred plasmid from *Z. rouxii* was found to carry resistance genes for nystatin. It can confirm the role of plasmid DNA in antibiotic resistance.
**Keywords:** Antibiotic, *Candida tropicalis*, conjugation, plasmids, resistance genes

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**Introduction**

Some types of yeast are characterized by having extrachromosomal genetic elements called plasmids. They are double-looped segments of DNA and can replicate independently of the host cell's chromosome (Møller, 2015). Their types in yeast vary according to their structure and function, including Yeast Episomal Plasmid (YEp), Yeast Centromeric Plasmid (YCp), Yeast Integrative Plasmid (YIp), Yeast Replicating Plasmid (YRp), and Yeast Linear Plasmid (YlP), which are the least common (Hohnholz et al., 2017). Although plasmids are not essential for the life of their host, their presence may give the host important additional traits that enable it to live under exceptional conditions (Miljkovic-Selimovic et al., 2008). These characteristics are resistance to antibiotics (Ramesh et al., 2010), enzymes and toxins (Talaro and Chess, 2014), resistance to heavy metals and disinfectants (Szczepanowski et al., 2005), autotrophicity, and other features (Hanson et al., 2003), replication start site (ARS) or artificial chromosome centers (CEN), and selectable markers, such as HIS3, LEU2, URA3, used They also can bear regulatory genes, like LACZ or GFP, which allows investigating gene regulation and cellular regulation (Hohnholz et al., 2023). The past few years have seen the dramatic improvements in this area, with autotrophic yeast strains being developed that could take in a great number of plasmids at once. This has made it possible to express several genes simultaneously, allowing tremendous

opportunities in complex protein expression and artificial genetic modification (Frost et al., 2018). This has made plasmids very relevant as they can be used to express a desirable foreign gene and also in gene editing hence, they provide a perfect platform in biotechnology, pharmaceutical, and genetic engineering (Chan et al., 2013; Frost et al., 2018). It is possible to isolate plasmids in the form of partially circular DNA (Franklin and Snow, 2005). They may also be lost spontaneously out of cells with low frequencies. This is referred to as spontaneous curing. The rate of loss is more common in cells that are exposed to compounds that insert between bases of DNA. The most efficient ones are acridines including proflavine, acridine orange, ethidium bromide, and sodium dodecyl sulfate (Mickelsen et al., 1985). One of the strongest and most relevantly active curing agents is ethidium bromide which attaches itself to the DNA or RNA and suppresses the activity of the DNA polymerase and RNA polymerase (Crameri et al., 1986). Cells might also be deleted of plasmids when they are cultivated in the presence of urea as well as high temperatures (Mickelsen et al., 1985). The transferability of plasmids between cells, inter-, and intra-species of bacteria of the same type or between different species (Yah et al., 2007), and between different genera define plasmids. This process is referred to as conjugation (Sher et al, 2023). Contact between two cells, one of them is referred to as the donor cell and the other as recipient cell is involved in conjugation process (Dale and Park, 2004). It is through this that a copy of the plasmid is copied to the receiving cell (Rees and Wilkins, 1989). The conjugation process can also help in transferring a lot of characteristics of one species to another including antibiotic and heavy metal resistance. Resistance traits can be transmitted in a single unit to other cells through conjugation since it is done by resistance R-plasmid (Sher et al., 2023). The study was intended to explore the plasmid present in the *Z. rouxii* yeast and determine where the genes present in the plasmid or chromosomal DNA give the resistance to antibiotics.

## Materials and methods

**Source of isolates**: *Z. rouxii* and *C. tropicalis* isolates which had been identified previously in the laboratories of the 17 department of life sciences college of science in the university of Mosul were utilized.

**Antibiotic resistance testing**: Antibiotic resistance testing was performed on neutralized and non-neutralized isolates according to Ernst and Chan (1985). The antibiotics used in this study were: Amoxicillin (Ax), Ampicillin (Ap), Candizol (Cd), Chloramphenicol (Cm), Clindamycin (Clin), Erythromycin (Er), Fluconazole (Fc), Getamicin (Gm), Ketoconazole (Kc), Lamisil (Ls), Neomycin (Nm), Nystatin (Nys), Penicillin (Pen), Rifampin (Rif), Streptomycin (Str), Tetracycline (Tc), Trimethoprim (Tm), and Vancomycin (Van) at a concentration of 100 µg/mL.

Cephalexin monohydrate (Cf) and Clotrimazole (Ct) were used at a concentration of 30 µg/mL. micrograms/mL.

**Heavy metal resistance test:** This test was performed on neutral and non-neutral isolates as described by Edet et al. (2023). The heavy metals used in this test were: zinc sulfate, copper sulfate, potassium sulfate, lead chloride, mercuric chloride, cadmium chloride, nickel chloride and cobalt chloride at a concentration of 100 µg/mL.

**Isolation of plasmid DNA content from *Z. rouxii* and *C. tropicalis* yeast isolates:** Plasmid DNA was isolated from *Z. rouxii* and *C. tropicalis* yeasts using the Yeast Plasmid DNA Mini-Preps Kit supplied by BIO BASIC INC., Canada, and the manufacturer's recommended operating steps were followed.

**Quality control test of plasmid DNA extraction**: This test was conducted to determine the suitability of the DNA for use in molecular experiments during the current study, which included as below:

**1- Determination of plasmid DNA integrity:** Electrophoresis was performed on plasmid DNA extracted from *Z. rouxii* and *C. tropicalis* yeast isolates using a 1% agarose gel. The agarose gel was prepared by dissolving 1 g of agarose powder in 100 mL of 10% TBE solution. The mixture was heated and stirred continuously using a magnetic stirrer until the turbidity disappeared and the mixture became clear during boiling. It was then allowed to cool to 60°C. The mixture was poured into the electrophoresis tray after attaching the well-forming comb to one end of the gel. The pouring was carried out slowly so as not to form bubbles which are removed with the help of a pipette in case they appeared. The mixture was then left to solidify and then the comb was removed carefully to not scratch the gel in the formed wells. The gel was then loaded into the electrophoresis tank filled with 200 mL of 10% TBE solution making sure the gel surface was soaked. 7-10 µL of the DNA of the sample was then injected into the wells created by the comb. The electrophoresis tray was linked to the power supply and ran by passing 80 V/cm current on it once the electrodes were connected with reversed polarity (negative to positive). The duration of the electrophoresis was 180 minutes. 5. Once the migration process was done, the gel was checked by exposing it to ultraviolet light through an ultraviolet generator lamp at the wavelength of 240 nanometers to allow the visualization of the DNA bands. The procedure of imaging and recording the migration outcomes in terms of the agarose gel was done using the Gel Documentary System technique.

**2- Plasmid DNA concentration and purification evaluation:** A Nano Drop Micro Volume Spectrophotometers was used to check the concentration and purity of plasmid DNA, which was extracted out of the chosen yeast isolates. The instrument calibration was carried out by placing a 3-5 mL drop of solution (Elution Buffer) on the lower lens with a pipette. The upper lever,

which holds the upper lens, was then repositioned so that the added drop was positioned between the upper and lower lenses. After calibration, the drop was removed using a pipette, and a fresh drop of plasmid DNA solution was added to the same Elution Buffer. The reading was then displayed on the instrument's screen.

**Curing of plasmid DNA content in yeasts *Z. rouxii* and *C. tropicalis:*** The method of Toh-e and Wickner (1980) was followed in curing the plasmid of *Z. rouxii* and *C. tropicalis* yeast isolates using ethidium bromide as a Curing agent. Five millilitres of YPG liquid culture medium were inoculated with a single yeast colony and incubated at 28°C for 48 hours. Then, 0.1 millilitres of the yeast cell cultures were added to 5 millilitres of YPG medium containing ethidium bromide at concentrations of 0, 100, 150, 200, 250, and 300 µg/mL. The media were incubated at 28°C for 48 hours until colony formation. Another 0.1 millilitres were spread onto YPG agar plates and incubated at 28°C for 48 hours. Colony growth was then observed, and the lethal concentration of ethidium bromide was determined. Finally, 0.1 millilitres of the yeast cell cultures growing on YPG medium were added to 5 millilitres of YPG medium containing half the lethal concentration of ethidium bromide. The media were incubated at 28°C overnight. Then, a series of decimal dilutions were prepared up to a $10^{-8}$ dilution. 0.1 mL of the last three dilutions were taken and spread onto plates containing YPG agar. The plates were incubated at 28°C for 48 hours. Next, a master plate was prepared by randomly transferring 100 yeast colonies onto Petri dishes containing YPG agar. These plates were incubated at 28°C for 48 hours. The growth of these colonies was then tested by transferring them onto YPG agar supplemented with antibiotics and heavy metals at concentrations to which the yeasts could resist. These plates were incubated at 28°C for 48 hours. The number of cured colonies lacking resistance to the antibiotics and heavy metals was counted.

**Conjugation between yeast isolates:** The yeast isolate *C. tropicalis* was used as the recipient isolate to study the auto transformation of plasmids from the yeast isolate *Z. rouxii* by conjugating two strains of different genera. The two isolates differed in two genetic markers (resistance and susceptibility) to antibiotics and heavy metals. The conjugation process followed the method of Heinemann and Sprague (1989 and 1991), by inoculating two 5 mL samples of liquid YPG medium with a single colony of both donor and recipient cells separately and incubating them for 24 hours at 28°C. The following day, 0.1 mL of each donor and recipient yeast cell culture was taken and re-cultured on 5 mL of YPG medium separately. The cells were incubated in a shaker at 150 rpm at 28°C for 5–8 hours to achieve the logarithmic phase, with an optical density of 0.5–0.6 at 580 nm. They were then precipitated by cryo-centrifugation at 4°C for 10 minutes at 8000 rpm. The pellet was washed with 5 mL of TNB buffer and centrifuged again at 8000 rpm for 10 minutes, and the liquid was discarded. The precipitate was resuspended

by adding 5 mL of TNB buffer and mixing 0.5 mL of donor cell culture with 0.5 mL of recipient cell culture (1:1 ratio). Several decimal dilutions of the conjugation mixture were prepared up to a $10^{-8}$ dilution. 0.1 mL of the last three dilutions was spread onto YPG medium containing the two antibiotics at the final concentrations used (Table 2), which served as genetic markers. Control plates were also prepared by spreading 0.1 mL of both the donor and recipient cell cultures separately onto the plates containing the two genetic markers. All plates were incubated at 28°C for 5 days. Conjugation results were observed, and the conjugated colonies were then transferred onto YPG medium containing the two antibiotics and incubated for 5 days at 28°C to confirm the stability of the trait. The following equation was used to calculate the conjugation frequency.

$$\text{Conjugation frequency} = \frac{The\ number\ of\ paired\ cells\ in\ (1)\ ml\ of\ the\ conjugation\ mixture}{The\ total\ number\ of\ cells\ received\ in\ (1)\ ml\ of\ culture}$$

## Results and discussion

**Antibiotic resistance of the studied isolates:** Table 1 shows that the two tested yeast isolates exhibited resistance to the antibiotics Ax, Ap, Cd, Clin, Nm, Rif, Str, and Tm, while they showed sensitivity to the antibiotics Ct, Er, Gm, and Kc. The results varied with the remaining antibiotics. A study by Fakruddin et al. (2017) demonstrated that Saccharomyces yeast isolates varied in their resistance to different antibiotics. Most isolates showed clear resistance to erythromycin, chloramphenicol, rifampin, gentamicin, streptomycin, tetracycline, penicillin, ampicillin, and amoxicillin, while all were sensitive only to nystatin. Obaida and Ramadan (2020) demonstrated that *Candida albicans* yeast isolates from patients with oral candidiasis exhibited pervasive resistance to most of the studied antifungals, namely fluconazole, itraconazole, ketoconazole, and terbinafine, with the exception of one isolate that showed sensitivity to fluconazole and itraconazole. Regarding nystatin, all isolates showed sensitivity, with the exception of one. Antibiotic resistance is attributed to the presence of genes responsible for this resistance. These genes are located on the cell's chromosome or on a plasmid and express enzymes responsible for breaking down antibiotics and converting them into their inactive form (Wilson, 1998; Castañeda-Barba et al., 2024).

**Table 1. The antibiotic resistance and sensitivity of the yeast isolates under study**

| Yeast | Antibiotics | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AX | AP | Cd | Cf | Cm | Clin | Ct | Er | Fcz | Gm | Kc | Ls | Nm | Nys | Pen | Rif | Str | Tc | Tm | Van |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Z. rouxii* | R | R | R | S | R | R | S | S | R | S | S | S | R | R | R | R | R | S | R | S |
| *C. tropicalis* | R | R | R | R | S | R | S | S | S | R | S | R | R | S | S | R | R | R | R | R |

R: Refer to the resistance status; S: Refer to the sensitivity status

**Resistance of the isolates under study to heavy metals:** The two yeast isolates showed resistance to the heavy metals $ZnSO_4$, $HgCl_2$ and $CoCl_2$, and sensitivity to $CuSO_4$ and $PbCl_2$ to varying degrees with respect to the rest of the heavy metals, as shown in Table 2.

**Table 2. The resistance of yeast isolates under study to heavy metals**

| Yeast | Antibiotics | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **ZnSO4** | **CuSO4** | **K2Cr2O7** | **PbCl2** | **HgCl2** | **CdCl2** | **NiCl2** | **CoCl2** |
| *Z. rouxii* | S | S | R | R | S | S | R | R |
| *C. tropicalis* | S | R | R | R | S | S | R | R |

R: Refer to the resistance status; S: Refer to the sensitivity status

A close relationship has been observed between antibiotic resistance and heavy metal resistance due to the possibility that the resistance genes for both are located on the same R-plasmid. Fakruddin et al. (2017) noted that Saccharomyces yeast isolates from the fruits and leaves of some plants showed resistance to the heavy metals $CdCl_2$, $PbCH_2COOH$, $CuSO_4$, $ZnSO_4$, $NiCl_2$, and $K_2Cr_2O_7$, with the exception of $HgCl_2$.

**Evaluation of plasmid DNA concentration and purity:** The results of evaluating the concentration and purity of plasmid DNA in the yeasts *Z. rouxii* and *C. tropicalis* were within acceptable parameters, with concentrations of 130 µg/mL and 127 µg/mL, respectively. Purity levels were 1.8 and 1.77, respectively. The ideal purity range for plasmid DNA is between 1.7 and 1.9. An acceptable concentration is greater than or equal to 100 µg/mL. The quantity of the plasmid DNA isolated by the method can be different depending on the type of the plasmid, isolation method and the growth efficiency. The isolation of plasmids in yeast is not as easy as the isolation of the plasmids in bacteria, and this may be because of difficulties in breaking the cell wall, which is made of peptidoglycan, mannan and mannoproteins, and must undergo enzymatic and mechanical proteolytic steps (Bzducha-Wróbel et al., 2014).

**Curing of plasmid DNA content:** Having tested isolates of *Z. rouxii* and *C. tropicalis* yeast in the presence of a few concentrations of ethidium bromide (100, 150, 200, 250, and 300 µg/mL) to ascertain lethal concentration with the two isolates, it was revealed that the 300 µg/mL concentration was effective. Thus, the 150 ug/ mL concentration was taken as the half lethal

concentration to use in the subsequent section of the experiment to Curing the plasmid DNA content of *Z. rouxii* and *C. tropicalis*, respectively. The efficiency of ethidium bromide was high to eliminate the plasmid DNA content as long as the ratios of the colony growth were good. These results are consistent with those of Hayes and Malla Obaida (2023).

**Curing and gel migration of plasmid DNA content for *Z. rouxii* and *C. tropicalis* isolates:** The results of plasmid DNA Curing for the Z. rouxii yeast isolate showed that the highest Curing rate (94%) was for the antibiotic Ls, the lowest (12%) for the antibiotic Tc, and the remaining antibiotics varied between 18% and 72%. For heavy metals, the highest Curing rate (88%) was for HgCl2 and the lowest (33%) for CoCl2 (Table 3). For the C. tropicalis yeast isolate, the highest Curing rate (80%) was for the antibiotic Fc and the lowest (17%) for the antibiotic Pen, with the remaining antibiotics showing varying Curing rates. Regarding heavy metals, K2Cr2O7 exhibited the highest Curing rate of 85%, while NiCl2 had the lowest at 15% (Table 4).

**Table 3. Curing of resistance *Z. rouxii* yeast colonies to antibiotics and heavy metal ions**.

| isolation | Number of colonies growing on a master plate medium | %cured colonies | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Antibiotics | | | | | | | | Heavy metal ions | | | |
| | | Cf (30) | Ct (30) | Er (100) | Gm (100) | Kc (100) | Ls (100) | Tc (100) | Van (100) | $ZnSO_4$ (100) | $HgCl_2$ (100) | $CdCl_2$ (100) | $CoCl_2$ (100) |
| *Z. rouxii* | 100 | R | 72 | S | S | 55 | 94 | 12 | 18 | R | 88 | S | 33 |

R: Refer to the resistance status; S: Refer to the sensitivity status (100% Curing)

**Table 4. Curing resistance *of C. tropicalis* yeast colonies to antibiotics and heavy metal ions**.

| Isolation | Number of colonies growing on a master plate medium | %cured Colonies | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Antibiotics | | | | | | | Heavy metal ions | | | | |
| | | Cm (100) | Ct (30) | Er (100) | Fc (100) | Kc (100) | Nys (100) | Pen. (100) | $ZnSO_4$ (100) | $K_2Cr_2O_7$ (100) | $HgCl_2$ (100) | $NiCl_2$ (100) | $CoCl_2$ (100) |
| *C. tropicalis* | 100 | 42 | 28 | S | 80 | R | 63 | 17 | R | 85 | 48 | 15 | S |

R: Refer to the resistance status; S: Refer to the sensitivity status (100% Curing)

This confirms that the genes responsible for antibiotic resistance and Curried heavy metals are located on plasmid DNA, whereas the antibiotics to which the yeast retained resistance are located on chromosomal DNA, indicating the inability to Curing resistance to these antibiotics. In general, ethidium bromide is an effective plasmid DNA Curing, as it inhibits plasmid replication. These results are consistent with those obtained by Obaida (2020a) in Curing the *Debaromyces hansenii* yeast plasmid. Plasmid DNA was extracted from the cured isolates and electrophoresed on agarose gel to observe their plasmid DNA content and compare it to the non-cured isolates (Figure 1-A and C). It was found that the cured isolates did not show any plasmid DNA bands compared to the non- Curing isolates.

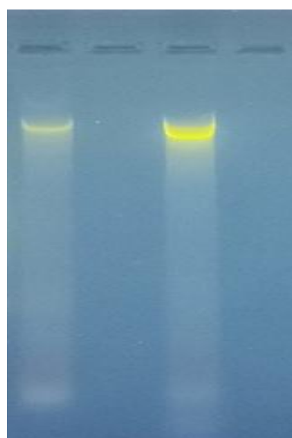

**Figure 1. Curing Neutralization of plasmid DNA content in the yeast isolates under study. *(A)* Non- cured *Z. rouxii, (B)* cured *Z. rouxii, (C)* Non- cured *C. tropicalis, (D)* cured *C. tropicalis***

**Conjugation:** The conjugation process was carried out to test the ability of the yeast *Z. rouxii* to transfer its genetic material located on the plasmid to the yeast *C. tropicalis* whose plasmid had been cured, in order to clarify the role that transferred plasmids play in transferring the trait of resistance to antibiotics and to confirm that the genes responsible for this are located within the sequences of nitrogenous bases of chromosomal DNA and not the plasmid.

**Conjugation between two yeast isolates of *Z. rouxii* and *C. tropicalis:*** For conjugation to occur between two isolates, there must be at least two different genetic markers between them *Z. rouxii* (as the donor strain) and *C. tropicalis* (as the recipient strain). Two markers were determined for each isolate: the donor yeast possessed resistance to the antibiotic Nys and sensitivity to the antibiotic Ls, while *C. tropicalis* exhibited the opposite, being resistant to the antibiotic Ls and sensitive to Nys. It was observed that the conjugation process resulted in the

transfer of resistance to the antibiotic Nys from *Z. rouxii* to *C. tropicalis*, with a conjugation frequency of $0.84 \times 10^{-8}$, as indicated by the growth of the resulting colonies on YPG medium containing the antibiotics Nys and Ls. Control models, consisting of the donor and recipient strains, were grown separately on medium containing the antibodies used as a genetic marker for conjugation. No growth was observed in either strain on this medium. This demonstrates the mobility of the plasmid DNA in this isolate and its ability to transfer to other yeast isolates. Kelly et al. (2012) successfully transferred mercury resistance genes located on plasmid DNA from *S. cerevisiae* to another strain of the same yeast via conjugation at a conjugation frequency of $1.3\times10^{-2}$. Another study by Obaida (2020b) successfully transferred antibiotic and heavy metal resistance genes between *Debaromyces hansanii* and *S. cerevisiae*. Hayes and Malla Obaida (2023) successfully performed a coupling attempt between *Rhodotorula mucilaginosa* yeast isolate and *S. cerevisiae*, during which the genes responsible for the hydrocarbon degradation trait of *S. cerevisiae* were transferred at a coupling frequency of $2.1 \times 10^{-8}$.

**Conclusion:** These results confirm the different locations of genes responsible for antibiotic resistance in the yeast *Z. rouxii*. Some genes are located on chromosomal DNA, while others are located on plasmid DNA. Neutralization of the plasmid with ethidium bromide successfully eliminated resistance to some antibiotics, while resistance to others persisted, indicating the presence of these genes on chromosomal DNA. Furthermore, the conjugation experiment demonstrated the transmissibility of the plasmid carrying the Nystatin resistance gene between yeast species, suggesting the possibility of horizontal transmission of resistance traits between species. The importance of studying the mechanisms of resistance gene transfer between different yeast species lies in understanding the spread of resistance and enhancing future control strategies.

### Author contributions

All activities were performed by B. A. R. M. O.

### Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Ethical approval**

Not applicable.

**Conflict of Interest**

The author declare that they have no competing interests or financial conflicts of interest related to this study.

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# بررسی ژن‌های مسئول مقاومت آنتی‌بیوتیکی در مخمر *Zygosaccharomyces rouxii*

**بدیعه عبدالرزاق ملا عبیده**

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## چکیده

**هدف:** هدف از این مطالعه شناسایی محل ژنتیکی ژن‌های مقاومت به آنتی‌بیوتیک‌ها و فلزات سنگین در مخمرهای *Zygosaccharomyces rouxii* و *Candida tropicalis* بود. این مخمرها از برگ‌های درخت نارنگی جمع‌آوری‌شده از شهر موصل جداسازی شدند. تمرکز اصلی پژوهش بر تعیین این موضوع بود که آیا ژن‌های مقاومت بر روی DNA پلاسمیدی قرار دارند یا بر روی DNA کروموزومی.

**مواد و روش‌ها:** ایزوله‌های مخمری *Z. rouxii* و *C. tropicalis* از نظر وجود DNA پلاسمیدی مورد بررسی قرار گرفتند. برای تعیین محل ژن‌های مقاومت، از دو روش استفاده شد. روش نخست شامل حذف پلاسمید (plasmid curing) با استفاده از اتیدیوم بروماید با غلظت ۱۵۰ میکروگرم بر میلی‌لیتر بود. این تیمار برای هر دو گونه مخمری به‌منظور حذف DNA پلاسمیدی اعمال شد. پس از حذف پلاسمید، حساسیت کلنی‌های مخمری نسبت به آنتی‌بیوتیک‌ها و فلزات سنگین مختلف بررسی گردید. الکتروفورز ژل برای تأیید حذف یا باقی‌ماندن DNA پلاسمیدی به کار رفت. روش دوم شامل آزمایش‌های کونژوگاسیون برای بررسی انتقال DNA پلاسمیدی بین ایزوله‌های مخمری بود. در این روش، *Z. rouxii* به‌عنوان سویه دهنده و *C. tropicalis* حذف‌پلاسمیدشده به‌عنوان سویه گیرنده مورد استفاده قرار گرفت.

**نتایج:** حذف پلاسمید در هر دو گونه مخمری با موفقیت انجام شد، به‌طوری که بسیاری از کلنی‌های حذف‌پلاسمیدشده نسبت به آنتی‌بیوتیک‌ها و فلزات سنگین مورد آزمایش حساس شدند. در *Z. rouxii*، درصد حذف پلاسمید بین ۱۲٪ تا ۹۴٪ متغیر بود، به‌جز در مورد سفالکسین مونوهیدرات و سولفات روی ($ZnSO_4$) که مقاومت از بین نرفت. در *C. tropicalis*، میزان از دست رفتن مقاومت برای آنتی‌بیوتیک‌ها بین ۱۷٪ تا ۸۰٪ و برای فلزات سنگین بین ۱۵٪ تا ۸۵٪ بود، به‌جز در مورد کتوکونازول و $ZnSO_4$. این نتایج نشان می‌دهد که مقاومت به این عوامل احتمالاً توسط DNA کروموزومی کدگذاری می‌شود. نتایج ژل الکتروفورز نیز این

یافته‌ها را تأیید کرد. آزمایش‌های کونژوگاسیون انتقال موفق پلاسمید از *Z. rouxii* به *C. tropicalis* حذف‌پلاسمیدشده را نشان داد، به‌طوری که فراوانی کونژوگاسیون برابر با $0.84 \times 10^{-8}$ بود.

**نتیجه‌گیری:** این مطالعه نشان داد که ژن‌های مقاومت در *Z. rouxii* و *C. tropicalis* می‌توانند بر روی DNA پلاسمیدی یا کروموزومی قرار داشته باشند. مشخص شد که پلاسمید منتقل‌شده از *Z. rouxii* حامل ژن‌های مقاومت به نیستاتین است. این یافته می‌تواند نقش مهم DNA پلاسمیدی را در بروز مقاومت آنتی‌بیوتیکی تأیید کند.

**کلمات کلیدی:** آنتی‌بیوتیک، پلاسمیدها، ژن‌های مقاومت، کونژوگاسیون، *Candida tropicalis*

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