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Testing the ability of plasmid DNA content in *Rhodotorula mucilaginosa* S6 to degradation hydrocarbon compounds and transfer through conjucation

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Abstract
The current study included isolation of yeasts from the soils of Bay Hassan oil field in Kirkuk Governorate and the K1 oil filling station in Erbil Governorate/ Iraq, and it was diagnosed based on phenotypic, culture and biochemical tests. The results showed that the yeasts belong to the species: *Saccharomyces cerevisiae* and *Rhodotorula mucilaginosa*. Also, *Escherichia. coli* bacteria were obtained from the exit of infants in Ibn Al-Atheer Teaching Hospital in Mosul/Iraq and was diagnosed based on biochemical tests. The resistance of isolates was studied for 16 antibiotics and 8 heavy metal ions. The resistance of *R. mucilaginosa* S6 and *S. cervisiae* S3 isolates to 16 antibiotics and 8 heavy metal ions was studied. The results showed that all of the studied isolates were resistant to Ap, Fc, Clin and Ct, while they were sensitive to the antibiotic Ax, and the results were uneven with the rest of the antibiotics. The yeasts showed absolute resistance to the heavy metal ions $NiSO_4$ and $K_2Cr_2O_7$ and varied with respect to the rest of the heavy metals. The ability of E. coli to resist antibiotics and heavy metal ions has been studied. The results showed that the two tested *E. coli* isolates were resistant to (Ap, Cf, Clin, Ct, Er, Fc, Str, Rif, Nys, Pen, Ax, Itr, Tc and Cot), while the *E. coli* wasolate was water. (2) The isolate from water is sensitive to the antibiotic Van, while the isolate E. coli urine (1) isolated from the urine was sensitive to the antibiotic Gen. While the two isolates were resistant to $NiCl_2$, NiSo4, $CuSO_4$ and ZnSo4 and sensitive to $CdCl_2$ and $COCl_2$. The results of isolation and transfer of plasmid DNA content of yeasts and bacteria using agarose gel electrophoresis technique showed that isolate *R. mucilaginosa* S6, isolate *S. cervisiae* S3, and bacteria *E. coli* (1) contain plasmid DNA. The plasmid DNA content of the *R. mucilaginosa* S6 that the curing colonies did not bear the characteristic of hydrocarbon hydrolysis, and this indicates that the genes responsible for this characteristic are located on the plasmid DNA. The curing colonies showed sensitivity towards the studied antibiotics and heavy metals with a percentage of 1-92% except for NiSO4 and $COCl_2$. Also, the results of the neutralization of the isolate *S. cervisiae* S3 showed a loss of resistance to antibiotics with a range of 3-93%. The results of neutralizing the isolate E. coli showed a loss of antibiotic resistance with a range of 7-97%, except for Ax, which was confirmed by gel electrophoresis. The conjugation process was successful between the donor isolate *R. mucilaginosa* S6 and the neutral *S. cervisiae* S3 isolate as a receiver with a conjugation frequency of 2.1 × 10-8. This study proved that the plasmid DNA transferred from the isolate *R. mucilaginosa* S6 carries the genes for resistance to the Er antibiotic and carries the genes responsible for the degradation of hydrocarbon compounds. In addition, an attempt was made to conjugate across the kingdoms between yeast *R. mucilaginosa* S6 as a host and *E. coli* as a acceptor. The results showed that the plasmid DNA carrying Rif resistance genes has the ability to move from yeast to bacteria with a conjugation frequency of 5.8 × 10-8. In addition, the results showed that the neutralizing bacteria have the ability to receive and localize the plasmid from the yeast through the conjugation process, and the colonies resulting from the conjugation showed the characteristic of hydrocarbon degradation, and this confirms that the genes encoding the characteristic of hydrocarbon degradation are located on the plasmid DNA.

Keywords: plasmid DNA, Hassan oil, *Rhodotorula mucilaginosa*

Introduction
The term plasmid was coined for the first time by the scientist Lederberg in 1952 (Helinki, 2022) [16]. They are characterized as small double-stranded extrachromosomal DNA molecules, which can be linear or in most cases circular in shape. It has the ability to self-replicate independently of chromosomal DNA (Hohnhelz, 2018) [17]. Due to their presence in large numbers and with the continuous discoveries of new plasmids, and in order to facilitate their study, many systems have been developed to divide them, depending on the number of copies of plasmids in the host, into two types: Stringent plasmids, which are present in the cell in small numbers of 1-2 copies, and their replication is linked to the replication of the DNA of the cell. Relaxed plasmids, which are characterized by large numbers ranging from 10-100 copies/cell, in which DNA replication is not associated with host cell replication (Nancy and Janine, 2004) [29]. Plasmids are also classified into conjugated group, which is a group of plasmids that are similar in the region of origin of replication (ori) and cannot remain in the host cell for a long time. An unconjugated group comprising different plasmids in the region of origin of replication, has the ability to remain in the host cell stably. Also, there is a simple category between the two types called Mobilized Plasmid that contains some genes required for the process of transfer to the other cell (Banu and Prasad, 2017; Thomas and Summers, 2008) [3, 35]. In addition, the possibility of dividing plasmids into five types depending on their function is known as Fertility Plasmids. Also known as F plasmids that contain transfer genes that

allow the transfer of genes during the conjugation process, resistance plasmids that contain genes that enable the cell to resist toxins or antibiotics, virulence plasmids that increase the pathogenicity of the microbes they harbor, and lysate plasmids Degradative plasmids that contribute to the digestion of complex compounds such as salicylic acid, camphor, xylene, and toluene because they contain genes for enzymes related to the analysis of complex compounds, and col plasmids, which possess genes responsible for manufacturing bacteriocin proteins capable of defending their host and killing various attacking strains(Al-Shuailyah, 2020) [1].

Plasmids mediate the transfer of genetic material between different species and genera of microorganisms through conjugation (Ozdemir, 2018) [30]. The transfer of genetic material is unidirectional, i.e. from the donor cell to the recipient cell, with the help of Fertility Plasmid. Conjugating plasmids possess genes that stimulate the transmission of plasmids called transduction genest transfer genes (tra genes) (Virolle *et al*., 2020) [39]. For the transmission process to occur, several requirements must be met, in addition to the genes of the transmission, as they need a specialized site on the plasmid called the transfer site (orit) and a protein called Relaxase, which is the recognition protein (orit) to produce the DNA strand that will be transmitted (Gama, 2017) [11]. The occurrence of the process of horizontal gene transfer is not limited to bacteria only, it can occur in phytoplankton, parasites and fungi, including yeasts. For example, the association between yeasts and bacteria, the genes BIO3 and BIO4 were transferred from the intestinal bacterium *E. coli* to yeast. *S. cerevisiae*. The association could also occur between the same filamentous fungi, as the genes for toxic secondary metabolism were transferred from *A. nidulans* to the fungus *Podospora anserina*, and the genes for secondary metabolism ACE1 from the fungus Magnapor the grisea were transferred to the mushroom *A. clavatus* and fuminosin genes from *Sordariomycete* spp. to *A. niger* (Morogovsky et al., 2022). [28] In another study by Gonçalves and Gonçalves (2019) [13] it was found that the yeast *Wiekerhamiella stramerella* acquired the characteristic of producing vitamin B1 (thiamine) through the transmission of the gene responsible for the formation of this vitamin from *E. coli*. The research aims to reveal the location of genes responsible for the degradation of hydrocarbon compounds on chromosomes or plasmid DNA and the possibility of their transmission.

Materials and Methods

Source of Isolates

Yeast *Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae* were isolated from the soils of Bay Hassan oil field in Kirkuk Governorate and the K1 oil filling station in Erbil Governorate/ Iraq according to the isolation practice of (Pan *et al*., 2009) [32] Yeasts were diagnosed based on th diagnostic characteristics of yeast Kurtzman and Fell,1998; Pitt and Hocking, 2009) [24, 33]. In addition to *Escherichia coli* was obtained from the strain bank of the Department of Life Sciences/College of Science/University of Mosul. The accuracy of the diagnosis was confirmed using Analytic Profile Index (API20E) and Eosin Methylene Blue (EMB) differential medium.

Antibiotic Resistance Test

The antibiotic resistance test was conducted according to Ernst and Chan [27]. The antibiotics used in this study were: Amoxicillin (Ax), Ampicillin (Ap), Candizol (Cd), Clotrimazol(Ct), Clindamycin (Clin), Erythromycin (Er), Fluconazole (Fc), Getamicin (Gm), Kenazol (Ken), Itraconazole (Itr), Co-trimazole (Cot), Nystatin (Nys), Penicillin (Pen), Rifampin (Rif), Streptomycin (Str), Tetracycline (Tc), Vancomycin (Van) at 100 microg / ml, and Cephalexin monohydratefuel(Cf) and Clotrimazol (Ct) were used at a concentration of 30 µg/ m (Ernst and Chan, 1985) [8].

Heavy Metal Resistance Test

This test was conducted according to (Dar, 2004) [6]. The heavy metals used in this test were as follows: Zinc Sulfate ($ZnSO_4$), Copper Sulfate ($CuSO_4$),Potassium Dichromate ($K2Cr_2O7$), Nickel Chloride ($NiSO_4$), Mercury Chloride ($HgCl_2$), Cadmium Chloride ($CdCl_2$), Nickel Chloride ($NiCl_2$) and Cobalt Chloride ($CoCl_2$) at a concentration of 100µg/ ml.

Curing of Plasmid DNA Content of Yeast *S. cerevisiae* S3

(Toh-e and Wickner's, 1980) [37] method was followed in curing a yeast isolate plasmid *S. cerevisiae* use of ethidium bromide as curing. Inoculate 5 ml of liquid YPG with a single colony of yeast colonies, incubate the medium at 28°C for 48 hours, then take 0.1 ml of yeast cell cultures and add 5 ml of YPG medium containing ethidium bromide at concentrations 0, 100, 150, 200, 250 and 300 µg/ml, media were incubated at 28°C for 48 hours until colonies appeared. 0.1 ml was taken and spread on plates. YPG Agar was then incubated at 28°C for 48 hours, after which the growth of the colonies was observed and the lethal concentration of ethidium bromide was determined. 0.1 ml of growing yeast cell cultures on YPG medium was added to 5 ml of YPG medium containing half the lethal concentration of ethidium bromide. The media were incubated at 28°C for one night. Then a decimal dilution series was prepared down to 8-10 dilution. 0.1 ml of the last three dilutions were taken and spread on plates containing YPG agar medium. The plates were incubated at 28°C for 48 hours. Then he prepared the main plate, the master plate, by randomly transferring 100 colonies of yeast to petri dishes containing YPG agar medium. The plates were incubated at 28°C for 48hours, then he tested the growth of these developing colonies on the main plate Master Plate by transferring the colonies to the added YPG agar medium. Mechanism of antibiotics and heavy metals in concentrations that the yeast can resist. The dishes were incubated at 28°C for 48 hours. The number of neutral colonies lost, resistant to antibiotics and heavy metals, was counted.

Curing of Plasmid DNA Content from *E. coli*

The *E. coli* was cured according to (Tomoeda et al., 1968) [38] method. A single colony of *E. coli* was inoculated with 5 ml of Nutrient broth, incubated at 37 C for 24 hours, 0.1 ml of microbial culture was taken and inoculated with 5 ml of nutrient broth containing ethidium bromide at concentrations 0, 100, 150 200, 250, and 300 µg/ ml, incubated at 37˚C for 24 hours and then spreading 0.1 ml on

Nutrient agar plates with a sterile glass diffuser (L-Shape) The plates were incubated at 37°C for 24 hours. Colon growth is observed, lethal concentration was determined. Then 0.1 ml of the growing bacterial culture was taken on a Nutrient broth and added to 5 ml of the nutrient broth containing half the lethal concentration of ethidium bromide, incubated at 37°C for 24 hours. Decimal dilutions were prepared from this culture and 0.1 ml of the last three dilutions (10-6, 10-7 and 10-8) were spread on plates containing Nutrient agar medium. The plates were incubated at 37˚C for 24 hours. The main dish was prepared by transferring master plate. From 100 colonies growing on nutrient media, the developing colonies were transferred on the main plate to the medium of antibiotics and heavy metals that were able to resist them. The number of colonies that failed to grow in these media was counted as neutral isolation.

Conjugation of *S. cerevisiae* S3 and *R. mucilaginosa* S6 Isolates

The yeast isolate *S. cerevisiae* S3 was used as a recipient to study the transformation of plasmids from *R. mucilaginosa* S6 by conjugation between two heterosexual strains, as the two isolates were different in two genetics (resistance and sensitivity) to antibiotics. Heinemann and Sprague (1989, 1991) method was followed in the conjugation process by inoculating two samples of 5 mL liquid YPG medium with a single colony from each of the donor and recipient cells separately and incubating for 24 hours at 28°C. On the next day, 0.1 ml was taken from each culture of the donor and recipient yeast cells and replanted with 5 ml of YPG medium separately. The incubator was incubated in a 150 cycles / minute vibrating incubator at 28°C for 5-8 hours to reach the logarithmic phase. The optical density was (0.5-0.6) at the wavelength of 580nm.Then the cells were deposited by means of a cooled centrifuge 4°C for 10 minutes at 8000 rpm. Wash the precipitate by adding 5 ml of TNB buffer, centrifuging cooled at 8000 rpm for 10 minutes and discarding the liquid. The precipitate was suspended by adding 5 ml of TNB buffer solution and mixing 0.5 ml of donor cell culture with 0.5 ml of recipient cell culture, i.e. in a 1: 1
ratio. Several decimal dilutions were prepared from the conjugation mixture down to the 10-8dilution and 0.1 ml of the last three dilutions were spread on YPG media containing two antibiotics at the final concentrations used which were used as genetic markers, as well as preparing control plates by spreading 0.1 ml. From each culture of the donor and recipient cells separately on those plates containing the two genotypes, all plates were incubated at 28°C for 5 days. The results of the conjugation were observed, then the coupled colonies were transferred to the YPG food medium containing the two antibiotics. The plates were incubated for 5 days at 28˚C to ensure the stability of the characteristic. The following equation was used to calculate the Conjugation Frequency.

Conjugation frequency= (paired mixture of ml (1) in paired cells count) / (cultivated from ml (1) in recipient cells in total count).

Conjugation between *R. mucilaginosa* S6 Yeast and *E. coli*

Conjugation was carried out according to Heinemann and Sprague(1989,1991) method by inoculating two samples of YPG medium and Nutrient broth in a volume of 5 ml with a single colony of donor cells represented by yeast. *R. mucilaginosa* S6 and recipient cells represented by *E. coli* separately, which differ by two genetic parameters (resistance and sensitivity) to antibiotics. Incubated at 28°C and 37°C for 24 hours. On the next day, 0.1 ml was taken from each culture of the donor and recipient cells and
re-inoculated with 5 ml of the same media mentioned above separately, and the incubation was done in a vibratory incubator at 150 cycles/ 54 minute for 24 hours at 28°C and 37°C respectively until Reaching the logarithmic phase, with the Optical Density (0.5-0.6) at the wavelength of 580 nm. The cells were deposited with a cooled centrifuge at 8000 rpm for 10 minutes at 4°C. Wash the precipitate by adding 5 mL of TNB buffer and rapidly cooled central centrifugation 8000 cycles/minute duration of 10 minutes and put out the liquid. Suspend the precipitate with 5 mL of the same buffer solution. Mixing 0.5 ml of each of the donor and recipient cell cultures and prepared a series of decimal dilutions until dilution 10-8, then taking 0.1 ml of the last three dilutions and spreading to the nutrient media containing the antibiotics used as genetic markers, in addition, control plates were used to spread 0.1 ml. From the donor and recipient bacterial culture separately on nutrient plates containing the same antibiotics. All dishes were incubated for 5 days at 37°C. After the incubation period ended, the number of paired colonies was counted and these colonies were purified on the same nutrient medium to ensure that the trait was confirmed, then the coupling frequency was calculated according to the following equation:

Conjugation frequency= (paired mixture of ml (1) in paired cells count) / (cultivated from ml (1) in recipient cells in total count).

Results and Discussion

Resistance of Isolates under Study to Antibiotics

It is evident from the observation of Table (1) that *S. cerevisiae* isolate showed resistance to antibiotics (Ap, Cf, Str, Fc, Clin, Ct, Nys, Pen, Tc and Van) while it showed sensitivity to the antibiotic Ax, Cot, Gm, Er, Rif and Itr. As for the yeast *R. mucilaginosa*, it was resistant to all antibiotics used except for Ax, Cot, Van, Nys and Tc. Based on Delgado and Remers (1998) [7], antibiotic resistance is due to the presence of genes responsible for this resistance and these genes are carried on a chromosome of cell or on the plasmid, expresses the enzymes responsible for breaking down antibiotics and converting them into the inactive form. Resistance to penicillins results from the possession of the enzyme Penicillinase, which converts penicillin to Penicilloic acid, and the antibody becomes ineffective in destroying the cell wall (Forbes *et al*., 2007) [10]. Plasmids and mutant elements also contribute to giving bacteria resistance to anti-Streptomycin and Kanamycin by inhibiting protein building and stopping the elongation of

the peptide chain and thus cell death (Wassef *et al*., 2010) [40]. Thus, it prevents the entry of the antibody through the membrane (Daini *et al*., 2011) [5] and resists Trimethoprim by modifying the enzyme Dihydrofolade reductase by encoding dfr genes that are located either on the plasmid or the chromosome (Kumar *et al*., 2012). As for resistance to chloramphenicol, it results from the presence of genes encoding the enzyme Chloramphenicol acetyl transferase, which works to stop the action of the antibiotic as an inhibitor of the cell protein and thus imparting resistance to the bacteria (Alton and Vapnek, 1979) [2]. In a study by Malla obaia, 2020 it was found that *Candida guilliermondii*, *C. krusei*, *C. norvegensis* and *C. utilis* isolates showed a difference in their resistance to the antibiotics used. *C. utilis* showed resistance to all antibiotics except for Nystatin, while C. krusei was sensitive to all antibiotics and resistant to Candizole and Nystatin. The rest of the isolates varied in the antibiotic resistance used. As for the isolate of *E. coli* bacteria, it was sensitive only to the antibiotics Ax, Cf, Cm, Gm, Nm, Nys and Str, and resistant to the rest of the antibiotics used. Kawane, 2012 [21] observed when *E. coli* isolates isolated from drinking water were tested for 13 antibiotics that they were more resistant to Penicillin, Clindamycin, Cephoxithin, and Metronidazole.

The Resistance of the Isolates under Study to Heavy Metals

The two yeast isolates showed resistance to heavy metal $NiCl_2$ and $COCl_2$, $K_2Cr_2O_7$, $CuSO_4$ and varied for the rest of the heavy metals as shown inTable (2). It has been observed that there is a close relationship between antibiotic resistance and heavy metals due to the possibility that theresistance genes for both are located on the same R-plasmid (Kawane and Tambekar, 2004) [20]. The E. coli bacteria were resistant to all heavy metal except $COCl_2$, $HgCl_2$ and $CdCl_2$ Table (2). Ilyas and Rehman, (2018) found that isolates of yeasts Trichosporon asahii and Pichia kudriavzevii from industrial effluents removed heavy metals Cd, As, Cu, and Pb in varying proportions. As for the researchers, García-Béjar *et al*., (2020) [12], they noted that 33

R. mucilaginosa isolates out of 213 eliminated 50% of the zinc, and the yeast *Diutina rugosa*, showed the best results in removing the same element. In another study, isolated yeast strains *Geotricum* sp. The contaminated coastal soils were resistant to Zn, Cu, and Ni. Sütterlin *et al*., (2018) noted in his study 88 isolates of *E. coli* bacteria isolated from urine samples in Sweden, Spain and Germany that all of them showed high resistance to Ag and Cu + 2, while they were sensitive to As and Na. Essa *et al*., (2018) [9] found also showed that all isolated *E. coli* isolates were resistant to all heavy metals Hg, Cd, Cu, Ag, Zn and Pb used.

Curing the Plasmid DNA Content

After treated *S. cerevisiae* and *E. coli* with several concentrations of ethidium bromide (100, 150,200, 250, and 300 microgramsμg/ ml) to determine the lethal concentration of these isolates. It was found that the concentration of 300 μg/ ml was effective in killing and on this basis the concentration of 150 g/ ml was adopted as half the lethal concentration in the next part of the experiment for the purpose of Curing the plasmid DNA content of *S. cerevisiae* and *E. coli*, respectively. The ethidium bromide proved to be highly efficient in removing the plasmid DNA content, taking into account the good proportions of the developing colonies. These results are in line with the findings of (Pahwa *et al*., 2012) [31].

Curing of Plasmid DNA Content from *S.cerevisiae* S3

The curing results of the plasmid DNA content of *S. cerevisiae* showed that the percentages of curing colonies for the antibiotics Ap, Str, Clo, Clin, Tc, Van, Fc, Nys, Pen, Cf, and Van were as follows: 6, 40, 15, 49, 93, 34, 100, 100,100,100, and 75% respectively, as for heavy metals $ZnSO_4$, $CuSO_4$, $NiCl_2$ and $COCl_2$ the percentages of curing colonies were 3, 100, 86,100 and 25%, respectively. This confirms the occurrence of the genes responsible for showing resistance to antibiotics and curing heavy metals on the plasmid DNA, while the antibiotics in which the yeast retained its resistance status are located on the chromosomal DNA, indicating the inability to curing the resistance to these antibiotics. In general, ethidium bromide is an effective substance in curing plasmid DNA, as it works to stop and inhibit plasmid replication (Jamuna *et al*., 2010) [19]. These results were similar for many studies when curing plasmid DNA of yeasts such (Malla-Obaid, 2018) [5].

Table 1: The antibiotic resistance and sensitivity of the yeast and bacterial isolates under study

	Antibiotics															
Yeast	**Ct**	**Clin**	**Gm**	**Nys**	**Pen**	**Cf**	**Van**	**Tc**	**Fc**	**Str**	**Am**	**Itra**	**Er**	**Cot**	**Rif**	**Ax**
R. mucilaginosa	R	R	R	S	R	R	S	S	R	R	R	R	R	S	R	S
S. cerevisae	R	R	S	R	R	R	R	R	R	R	R	S	S	S	S	S
E.coli	R	R	S	R	R	R	R	R	R	R	R	R	R	r	S	R

Table 2: The resistance of yeast and bacterial isolates under study to heavy metal

Yeast	**Heavy metal**							
	$CoCl_2$	**$NiCl_2$**	**$CdCl_2$**	**$HgCl_2$**	**NiSO4**	**$K_2Cr_2O_7$**	**$CuSO_4$**	**$ZnSO_4$**
R. mucilagenosa	R	R	R	R	R	R	R	S
S. cervisiae	R	S	S	S	R	R	R	R
E. coli	S	R	S	S	R	R	R	R

Curing of Plasmid DNA Content from *E. coli*

The results indicate that E. coli bacteria recorded curing of antibiotics Ap, Cd, Cm, Clin, Ct, Er, Fcz, Kc, Ls, Pen, Rif, Tc, Tm, and Van with the following percentages 64, 100, 17, 35, 100. 100, 0,0, 20, 46, 19, 16, 26, and 50%. While the

curing of heavy metals $CuSO_4$, $K_2Cr_2O_7$, $PbCl_2$, $NiCl_2$ and$COCl_2$ was shown in percentages 0, 18,100, 40, and 15%, respectively. This result shows that the curing process removes the plasmid DNA that carries the genes for antibiotic resistance, while the genes that retained the resistance status are located on the chromosomal DNA. This is indicated by studies (Chigor *et al*., 2010; Daini *et al*., 2011; Malla-Obaida, 2018) [5].

Conjugation between *R. mucilaginosa* S6 and *S. cerevisiae* S3

The conjugation process was performed to test the ability of *R. mucilaginosa* S6 to transfer its genetic material located on the plasmid to both *S. cerevisiae* S3 and *E. coli*, whose plasmids were cured to clarify the role that the transported plasmids play in transmitting antibiotic resistance and to ensure that these genes fall within the sequences. The nitrogenous bases of chromosomal DNA, not plasmid.

Conjugation between *R. mucilaginosa* S6 and *S. cerevisiae* S3 isolates for the pairing process between two isolates of *R. mucilaginosa* S6 and *S. cerevisiae* S3, there must be at least two different genotypes between *R. mucilaginosa* S (as Donor strain) and *S. cerevisiae* S3(as Recipient Strain). Two signs of each isolate were identified, namely, that the yeast possessed resistance to antibiotic Er and was sensitive to the antibiotic Tc, while *S. cerevisiae* S3 was the exact opposite of any resistance to the antibiotic Tc and sensitive to antibiotic Er. It was observed that the conjugation process led to the transmission of antibiotic resistance Ct trait from the yeast *R. mucilaginosa* S6 to *S. cerevisiae* S3 and coupling frequency (2.1 × 10-8) as a function of the colonygrowth resulting from conjugation on the YPG nutrient medium containing the antibiotic Er and Tc. Control models were developed in this test represented by the donor strain and the receiving strain, separately, on the medium containing the antagonists used as a genetic marker in conjugation, so no growth was observed for both strains on this medium. This indicates the ability of the plasmid DNA in this isolate to move and transfer to another isolate of yeast isolates. This indicates the ability of the plasmid DNA in this isolate to move and transfer to another yeast isolate. It was also noted that the transmission of the decomposition of hydrocarbon compounds in the colonies resulting from the conjugation. This obtained result indicates that the genes responsible for the degradation of hydrocarbon compounds in the yeast *R. mucilaginosa* S6 are located on the plasmid DNA. Researcher(Kelly et al., 2012) [22] succeeded in transferring mercury resistance genes on the plasmid DNA from *S. cerevisiae* to another species of the same strain by conjugation with a coupling frequency of 1.3 × 10-2.(Soofy, 2013) [34] managed three conjugation attempts between *S. cerevisiae* isolates during which the antibiotic resistance donor genes were transmitted: Chloramphenicol, Ampicillin, Getamicin, Tetracycline, Penicillin as well as the heavy metals $CuSO_4$ and $NiCl_2$.In another study Malla Obaida, (2017) the coupling between *R. mucilaginosa* BA61 and *S. cervisiae* BA179 curing isolates was successful as a receptor with a coupling frequency of 0.65 x 10-8. This study demonstrated that the plasmid DNA was transduced from the isolate *R. mucilaginosa* BA61 carries genes for resistance to the Gm antibiotic.

Conjugation between R. mucilaginosa S6 and E. coli Bacteria

As previously mentioned, two isolates carrying two different genetics were chosen, represented by the possession of the yeast that conveys resistance to the antibiotic Rif and the sensitivity to the antibiotic Nys and vice versa for the receiving bacteria. The results showed that the resistance of the antibiotic Nys was transferred from the donor yeast to the recipient bacteria, with a coupling frequency (5.8 × 10-8) in terms of the growth of the paired colonies when they were re-cultivated on the food medium containing the antibiotic Rif and the antibiotic Nys. The control models used in this experiment did not observe the growth of any associated colony on the food media containing the antibiotics used. This indicates that the plasmid DNA in *R. mucilaginosa* S6 could be transferred to *E. coli*. It was also noted that the transmission of the decomposition of hydrocarbon compounds in the colonies resulting from the conjugation. (Haslett, 2006) transferred the plasmid DNA carrying the antibiotic resistance genes Tetracycline, Trimethoprim and Ampicillin from E. coli JB139 to *S. cerevisiae* SY1229. (Soofy, 2013) [34] made two attempts to conjugate yeast and bacteria, the first between yeast as a donor and *E. coli* DH5α as a receptor and observed the transfer of the plasmid DNA carrying the genes providing resistance to the antibiotics Chloramphenicol, Tetracycline, Ampicillin, Getamicin, Penicillin and $CdCl_2$ and the heavy metal $ZnSO_4$, and the second attempt. The pathogenic bacteria E. coli as a receptor and isolate of the neutral yeast YET28 as a receptor. It was found that the isolate of the neutral yeast had the ability to receive and localize the plasmid DNA from the bacteria through the conjugation process. The results showed that the plasmid DNA carrying Rif resistance genes has the ability to move from yeast to bacteria at a conjugation frequency of 3.05×10-8. The results also showed that neutralizing bacteria have the ability to receive and localize the plasmid from yeast through the conjugation process (Malla-Obaida *et al*., 2018) [27]. Another study succeeded in conducting the conjugation process to test the ability of the yeast *D. hansenii* to transfer its genetic material located on the plasmid to each of the yeast *S. cerevisiae* and *E. coli* whose plasmids were curing to demonstrate the role of transduced plasmids in transmission of antibiotic resistance (Malla Obaida, 2020) [25].

Conclusion

The results showed that the plasmid DNA carrying the Rifampicin resistance genes has the ability to move and move from yeast to bacteria with a conjugation frequency of 5.8 × 10-8. The results also showed that the cured bacteria have the ability to receive and localize the plasmid from the yeast through the conjugation process.

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