Zagazig J. Agric. Res., Vol. 39 No. (5) 2012 863-869

Zagazig Journal of Agricultural Research
www.zu.edu.eg/agr/journals

THE YEAST *Rhodotorula spp.* and *Candida spp.* AS A RECIPENT EUKARYOTES IN TRANS-KINGDOM CONJUGATION

Amina A. Hassan*

Genetics Dept. Fac. Agric., Zagazig Univ., Egypt

ABSTRACT

Conjugation is often very efficient between members of a given species or genus, it can also occur at a lower rate between phylogenetically distant microorganisms with structurally distinct cell surfaces. This study was attempted to carry out trans-kingdom between *E. coli* as donor and *Rhodotorula spp.*, *Candida spp.* yeasts as recipient. Constructed plasmids (pJan 25 and pGW533) derived from R_4pGWB conjugative plasmid were used. A conjugation mixture consisted of diparental mating between *E. coli* harboring one plasmid and *Rhodotorula* or *Candida*. Plasmid DNA isolation and gel electrophoresis for transconjugants yeast cells indicated the presence of corresponding bands to donor DNA plasmids. The results clearly indicated that, two plasmids were able to transfer to yeast cells. The transfer was started at one h after mating and the frequency was increased after two to 24h., these were established by detection of β- glucuronidase (*gus*) marker gene located on plasmids using specific PCR.This is a good proof that, gene transfer through conjugation may be occur from prokaryotes to eukaryotes.

Keywords: Trans-kingdom, yeast, bacteria, conjugation, plasmid, PCR.

INTRODUCTION

Conjugal gene transfer involves a specific set of transfer (*tra)* functions that mediate the mobilization of DNA containing an origin of transfer (*oriT*) from a donor to a recipient in a process requiring cell-to-cell contact (Lanka and Wilkins, 1995). Conjugation is often very efficient between members of a given specie, *Rhizobium leguminosarum* (Hassan, 2010) or genus, *Candida* and *Saccharomyces* (Mentel *et al.,* 2006) *Rhizobium leguminosarum* and *Bacillus turingiensis* (Hassan, 2010), *Staphylococcus* and *Micrococcus* (Hassan and Mahgoub, 2011). It can also occur at a lower frequency between phylogentically distant microorganisms with structurally distinct cell surfaces (Dodsworth *et al.,* 2010). *E. coli,* for example, mediates conjugal transfer of DNA to such diverse bacterial recipients as cyanobacteria (Wolk *et al.,* 1984). Spirochetes (Picardeau, 2008), a variety of gram-positive bacteria and even mediates conjugal DNA transfer to members of the domain eukaryotes such as *Saccharomyces cerevisiae* (Nishikawa *et al.,* 1990), *Saccharomyces Kluyveri* (Inomata *et al.,* 1994) and mammalian (Waters, 2001) cells. Because of it's broad range of potential recipients, conjugation has proven to be a valuable genetic tool (Losa and Cruz, 2005), and may be an important mechanism of horizontal gene transfer and evolution of eukaryotic genome.

Not only *E. coli* bacteria was able to transfer their DNA to eukaryotes, but also *Agrobacterium tuifaciens* transfered T-DNA to *Saccharomyces cerevisiae* (Bundock *et al.,* 1995 and Piers *et al.,* 1996). Also, there was evidence of horizontal gene transfer between bacteria and *Candida parapsilosis* (Fitzpatrick *et al.,* 2008).

Because of the evolutionary and ecological interest of these studies, it was. Attempted to carry out trans-kingdom conjugation between *E. coli* and *Rhodotorula spp., Candida spp.* yeasts.

* **Corresponding author: Tel.** : +201068468435
E-mail address: kanr2011@yahoo.com

MATERIALS AND METHODS

This study was carried out in Microbial Genetics Lab. Dept. Genetics, Fac. Agric. Zagazig Univ.

Bacterial and Yeast Strains and Plasmids

E. coli strains pGWB533 and pJan25 were used as donors for constructed plasmids (Fig. 1). These plasmids were derived from R4pGWB which contain *rep* (broad host rang replication origin) and *bom*, *cis* (acting elements for conjugational transfer) genes (Nakagawa *et al.*, 2008) and encoding. β-glucuronidase (*gus*) marker gene. These strains were obtained from Matthews Lab., Soybean Genomics Laboratory, Beltsville, USDA, USA. *Rhodotorula spp.* and *Candida spp.* yeast strains, obtained from Microbial Genetics Lab. and Microbiology Lab respectively, Fac. Agric. Zagazig Univ. The yeast colonies were distinguished by shape and color.

Mating Conditions

E. coli Cells were cultured in LB broth (1% tryptone, 0.5% yeast extract and 1% NaCl) at 37°C. Antibiotics tetracycline (Tet) and spctenamycin (Spc) were supplemented at the concentration of 50 and 100 mg/ml, respectively. Yeast was cultured in a rich medium YPD (1% yeast extract, 2% peptone and 2% glucose) at 30°C (Nishikawa *et al.,* 1990). Diparental mating was preformed by mixed bacteria and yeast at the ratio 1:1 and incubated at 30°C. At different interval times (0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 8.0, 12.0, 16.0 and 20.0 h.) the cell mixture was diluted and spreaded on YPD agar plates and incubated at 30°C for 48 h.

Detection of *Gus* Gene in Yeast Cells

Random colonics were picked up and subjected to specific PCR using specific primers for gus gene (*gus* forward, 5`-AGGAAGTGA TGGAGCATCAG-3`, *gus* reverse, 5` - CATCAGCACGTTATCGAATCC- 3`).

PCR Amplification

Individual colonies were selected from plates and grown overnight in 5 ml YPD. Cells from 20 µl solution from overnight cultures were collected by centrifugation and resuspended in 20 µl dd H2O and heated at 100°C for 10 min. The resulted solution containing all cells components were subjected to colony PCR as from each culture were used as a template in a 20 µl PCR reaction to confirm the presence of the *gus* gene in the plasmid using Taq polymerase. Polymerase chain reaction was started with an initial denaturation at 94°C for 5 min. followed by 35 cycles of denaturation at 94°C for 35 sec., annealing at temperature based on the primers used for 35 sec., and extension at 72°C for 2 min per Kb of PCR product. Final extension of the amplification was for 10 min denaturation at 72°C. All PCR reactions were performed using in a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio- Rad, USA) (Hassan and Alzohairy, 2012).

Gel Electrophoresis

Ten µl of PCR products were separated on agarose (1.2%) gel electrophoresis, stained and loaded in 2 µl EZ-VISION™ ONE (Amresco, USA), at 100 Volts in 1X SB (10mM NaOH solution with Boric acid pH is 8.5) and photographed on a UV transilluminator (Pharmacia) by a Canon S5 digital camera with a UV filter adaptor. A negative control which contained all the necessary PCR components except template DNA was included in PCR run.

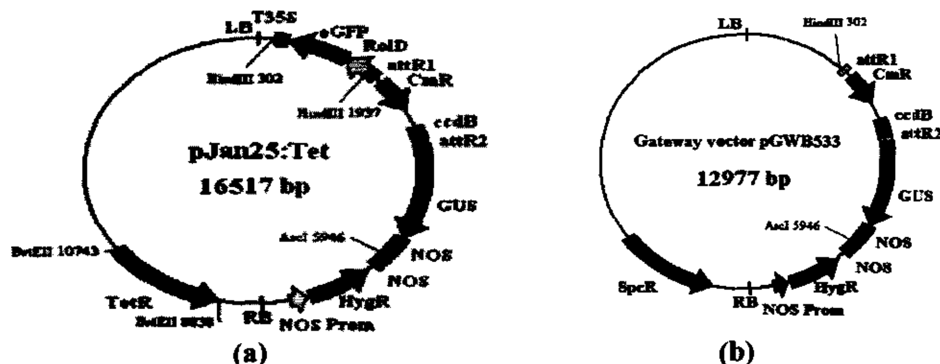

Fig. 1. Maps constructed plasmids used: (a) show the A: pJan25 and (b) show the pGWB533

Plasmid Isolation

Plasmid DNA was isolated from bacterial cells by alkali treatment method described by Kado and Lui (1981) with slight modification.

Yeast cells are different from bacterial cells in that they contain more membranes and are surrounded by a layer of chitin. Therefore, the procedure for plasmid isolation is slightly more complicated. Essentially, one first breaks open the cells with a buffer that contains SDS by vortexing in the presence of glass beads. After the cells burst, an organic solvent (50% phenol/ 50% chloroform) is added to extract cellular proteins and lipids. The solution is then centrifuged and a DNA-binding matrix is added to the aqueous solution (this phase contains the nucleic acids). Once the DNA binds to the matrix, it is washed to remove contaminants and finally unbound from the matrix by adding a buffer with no salt.

Plasmid DNA preparations from yeast cells were carried out as the following: Transfer 1.5 ml of overnight culture to microcentrifuge tube. Centrifuge tube for 1 min, remove supernatant and add 200 microliters of breaking buffer. Add 0.4 gm of glass beads and vortex tube for 1 min. Add 200 microliters of a 50% phenol/ 50% chloroform solution and votex for 1 min (be sure to use gloves at this point), this step will extract proteins/ membranes, centrifuge for 1 min. Transfer aqueous layer (top layer) to new tube and add 600 microliters Binding buffer. Add 15 microliters of DNA-binding matrix (BioRad "Prep-A-Gene"). Be sure matrix is resuspended before adding to your tube. Invert tube manually for 3-5 min. This allows time for the DNA to bind, centrifuge for 1 min. Remove supernatant and add 375 microliters binding buffer to pellet. Resuspend pellet. This step helps remove contaminants that don't bind to matrix. Centrifuge 1 min, remove supernatant, add 375 microliters Wash Buffer and resuspend pellet. This step helps to remove contaminants that bind to matrix. Repeat the previous step. Centrifuge 1 min, remove supernatant. Centrifuge again to remove last traces of Wash buffer. Allow pellet to dry for 10 min so that excess ethanol can evaporate. Resuspend pellet in 30 microliters TE buffer, incubate at 37 degree C for 5 min. Centrifuge 1 min and transfer supernatant to new tube. Label and store tube. It should contain fairly pure plasmid DNA whereby 3 microliters should be sufficient for bacterial transformations.

Buffers

Breaking buffer: 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, 0.1% SDS.

Binding buffer: 6M NaClO4, 50 mM Tris, pH 8.0, 10 mM EDTA.

Wash buffer: 20 mM Tris, pH 7.5, 2 mM EDTA, 400 mM NaCl, 50% ethanol.

TE buffer: 10 mM Tris, pH 8.0, 1 mM EDTA

After electrophoresis on a 0.7% horizontal agarose gel (John and Okpokwasili, 2012), the bands were visualized with a UV transmilluminator and the gel picture was photographed.

RESULTS AND DISCUSSION

Gene Transfer from *E. coli* to Yeast by Conjugation

Yeast cells were mated with *E. coli* cells for 24 h. The mixture was diluted and plated on YPD medium. Random yeast colonies were assayed by PCR to detect *gus* gene which located on donor plasmid. Fig. 2 show gel electrophoresis for *gus* gene. The bands of *gus* gene were presence in 13 from 14 tested colonies (92.9%). Other bacteria were able to transfer their genes to yeast, Bundock *et al.* (1995) and Piers *et al.* (1996) were demonstrated that, *A. tumefaciens* was able to transfer T-DNA into *S. cerevisiae*, Fitzpatrick *et al.* (2008), were indicated that two genes (PR, PhzF) were transferred from bacteria to *Candida parpsilosis*.

Plasmid Transfer from *E. coli* to Yeast

In order to know whether bacteria can transfer their plasmids into recipient yeast cells by conjugation, yeast cells were mated with the *E. coli* cells for 24 h. The mixture was diluted and plated on YPD medium. Twenty random yeast colonies were used for plasmid isolation and gel electrophoresis, and compared with parental yeast cells. Plasmid isolation was carried out to donor and recipient cells. Fig. 3 shows the plasmid profile of donor (*E. coli*), recipient (yeast) and transconjugants.

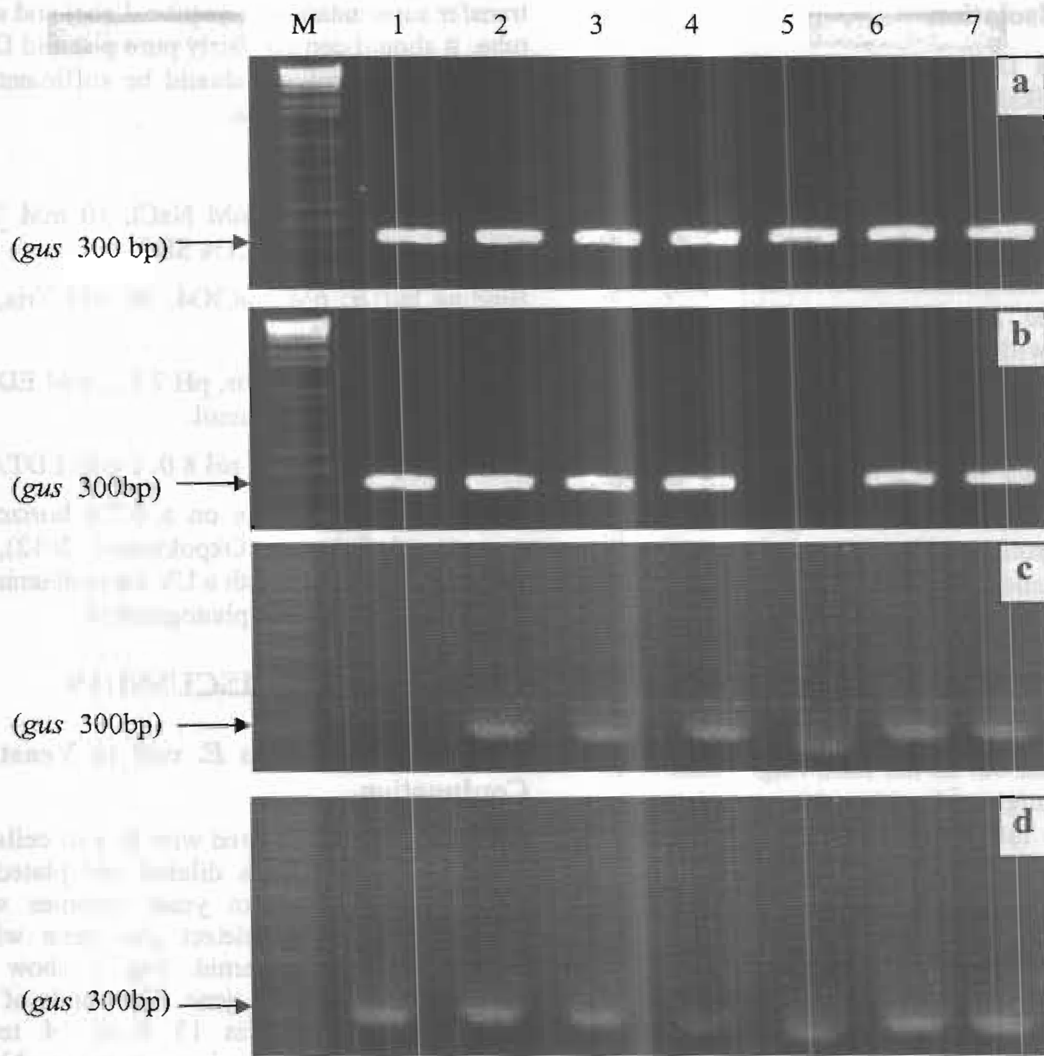

Fig. 2. Detection of *gus* gene in yeast transconjugants: (a,b)transconjugants of *E. coli* (pJan25) x *Rhodotorula sp*, (c,d) transconjugants of *E. coli (pGWB533)* x *Candida sp*

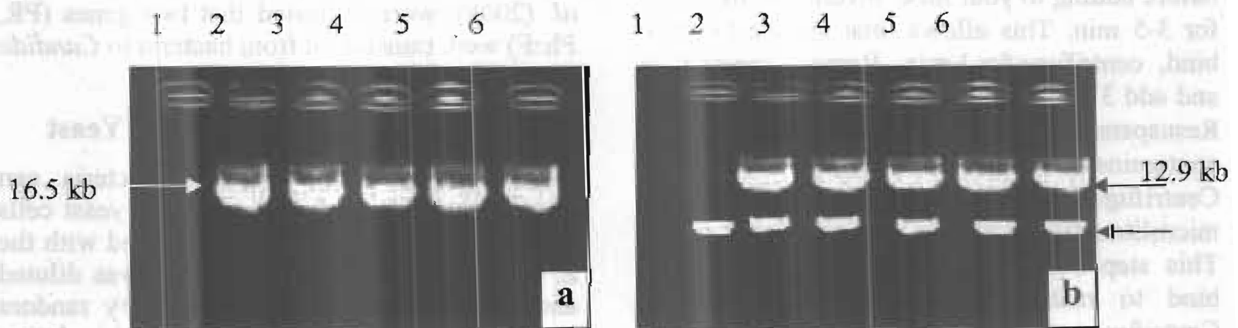

Fig. 3. Plasmids profile of recipient, donor and transconjugants:-(a)lane 1: recipient *Rhodotorula sp*, lane 2: donor pJan25, lanes 3-6: transconjugants of *E. coli* (pJan25) x *Rhodotorula sp*, (b)lane 1: recipient *Candida sp*, lane 2:donor pWGB533, lanes 3-6: transconjugants of *E. coli (pGWB533)* x *Candida sp*.

The results showed the presence of the large constructed plasmids in all *E. coli* donor cells, however, *Rhodotorula spp* did not contain any plasmid. *Candida spp.* Contained a plasmid but smaller than the donor plasmid. All transconjugants approximately clearly indicate the presence of corresponding bands to donor DNA plasmid. These results confirm that a conjugative transfer of plasmid from *E. coli* to yeast may be occur. The transfer may allow the spread of virulence factors and resistance to medical drugs even between distantly related organisms and could probably help in the transformation of harmless saprophytes into potential causative agents of human infections (Mentel *et al.*, 2006).

Many studies have reported this observation, Nishikawa *et al.* (1990) suggested that conjugative pAY 101 plasmid can be transferred from *E. coli* to yeast cells. Inomata *et al.* (1994) clearly indicated the conjugal transfer of 3 constructed plasmids from *E. coli* to *Saccharomyces Kluyveri.*

Timing of *Gus* Transfer

The time course of *gus* appearance in yeast cells after mating presented in Fig. 4. The used yeast species did not contained selectable markers, and they were resistant to tetracycline and spctenamycin that contained in donor plasmids so, the detection of transconjugants was dependent on detection of *gus* gene using specific PCR. After intervals mating time (0.0, 0.5, 1.0.2.0, 3.0, 4.0, 8.0.12.0.16.0 and 20.0 h.), the mating mixture was diluted and plating on YPD medium for 48 h. Random yeast colonies were subjected to isolation of DNA and specific PCR. The results were displayed disapperance of *gus* gene in yeast cells at zero and 0.5 h., and it appeared in 50% of cells at 1.0 h. After two to 20h, all tested yeast colonies were contained the *gus* gene. These results indicated that, transfer of plasmids from *E. coli* to yeast was started at 1.0 h, but the high frequency was after two to 20h. Nishikawa *et al.* (1990) reported that Trp^+ gene was appeared in yeast cells after 6 h. and the maximum was around 12 h.

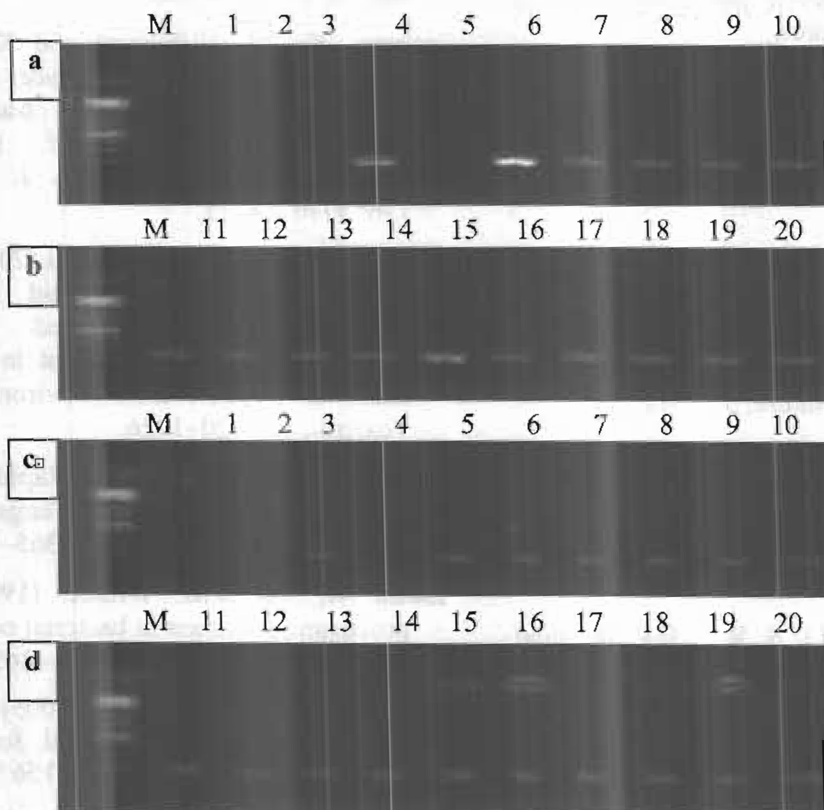

Fig. 4. Course time of mating:(a,b) transconjugants of *E. coli* (pJan25) x *Rhodotorula sp* ,(c,d) transconjugants of *E. coli (pGWB533)* x *Candida sp.* lane 1-2:0 h, 3-4: 0.5 h, 5-6:1 h,7-8:2 h, 9-10:3 h,11-12: 4 h, 13-14: 8 h, 15-16:12h, 17-18:16 h,19-20:20h.

Host ranges of promiscuous plasmids are not limited to prokaryote kingdom. As the best known example among trans-kingdom gene transmission, the transfer of T-DNA on Ti-plasmid of *Agrobacterium* to dicot plant have been extensively studied. Buchanan *et al.* (1987) reported a direct transfer of a broad- host rang plasmid from *Agrobacterium tumefaciens* to *Nicotiana plumbaginifolia* cells. Bundock *et al.* (1995) and Piers *et al.* (1996) demonstrated that *A. tumefaciens* was able to transfer their T-DNA into *S. cerevisiae*. Heinemann and Sprague (1989) and Nishikawa *et al.* (1990) clearly indicated that conjugative plasmid can be transferred from *E.coli* to *S. cerevisie* yeast. Fitzpatrick *et al.* (2008) indicated that, PR and PhzF genes were transferred from bacteria to *candida parapsilosis*.

The present study clearly indicate that a conjugative plasmids (Pgwb533 and Pjan25) can transfer from *E.coli* to *Rhodotorula spp.* and *candida spp.* yeasts. This is another successful evidence for conjugative gene transfer from prokaryotes to eukaryotes.

Acknowledgment

I would like to thank Safy M. Shebl for helping me in this study.

REFERENCES

Buchanan, W.V., J.E. Passiatore and F. Cannon (1987). The *omb* and *oriT* mobilization functions of a bacterial plasmid promote its transfer to plants. Nature, 329 : 172-175.

Bundock, P., A.D. Dulk-Ras, A. Beijersbergen and J.J. Hooykaas (1995). Trans-kingdom T-DNA transfer from *Agrobacterium tumefaciens* to *Saccharomyces cerevisiae*. The EMBO Journal, 14 : 3206 - 3214.

Dodsworth, J.A., L. Li, S. Wei, B.P. Hedlund, J.A. Leigh and P. Figueiredo (2010). Interdomain Conjugal Transfer of DNA from Bacteria to Archaea. Applied and Environmental Microbiology, 76: 5644–5647.

Fitzpatrick, D.A., M.E. Logue and G. Butler (2008). Evidence of recent interkingdom horizontal gene transfer between bacteria and *Candida parapsilosis*. BMC Evolutionary Biology, 8 : 181-195.

Hassan, A.A. (2010). Characterization of bacterial isolates (*Rhizobium leguminosarum* and *Bacillus thuringiensis*) able to degrade of malathion and methomyl pesticide. J. of Agricultural Chemistry and Biotechnology, Mansoura Univ., 11: 575-588.

Hassan, A.A. and A.M. Alzohairy (2012). Detection and behavior study of some genetically engineered microorganisms released in soil using plate counting, gene transfer assay and specific pcr methods. J. Agric. Chem. and Biotechn., Mansoura Univ., 3 (8): 329 – 344.

Hassan, A.A. and S.A.M. Mahgoub (2011). Salt inducible-proteins and conjugal gene transfer of halotolerant *Staphylococcus* isolated from salinity soil. Egyptian J. of Genetics and Cytology, 40: 263-280.

Heinemann, J.A. and G.F.J. Sprague (1989). Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. Nature, 340: 205-209.

Inomata, K., M. Nishikawa and K. Yoshida (1994). The yeast *Saccharomyces kluyveri* as a recipient eukaryote in transkingdom conjugation: behavior of transmitted plasmids in transconjugants. J. Bacteriol., 176: 4770 – 4773.

John, R.C. and G.C. Okpokwasili (2012). Crude Oil-Degradation and Plasmid Profile of Nitrifying Bacteria Isolated from Oil Impacted Mangrove Sediment in the Niger Delta of Nigeria. Bull. Environ. Contam. Toxicol., 88:1020–1026.

Kado, C.I. and S.T. Liu (1981). Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol., 145: 1365–1373.

Lanka, E. and B.M. Wilkins (1995). DNA processing reactions in bacterial conjugation. Annu. Rev. Biochem., 64: 141–169.

Losa, M. and F.D.L. Cruz (2005). Bacterial conjugation: a potential tool for genomic engineering. Res. Microbiol., 156 : 1–6.

Mentel, M., M. Spırek, D.J. Ramberg and J. Piskur (2006). Transfer of Genetic Material between Pathogenic and Food-Borne Yeasts. Applied and Environmental Microbiology, 72: 5122–5125.

Nakagawa, T., S. Nakamura, K. Tanaka, M. Kawamuka, T. Suzuki, K. Nakamura, T. Kimura and S. Ishiguro (2008). Development of R4 Gateway Binary Vectors (R4pGWB) Enabling High-Throughput Promoter Swapping for Plant Research. Biosci. Biotechnol. Biochem., 72 : 624–629.

Nishikawa, M., K. Suzuki and K. Yoshida (1990). Structure and function stability of IncP plasmids during stepwise transmission by trans-kingdom mating: promiscuous conjugation of *Escherichia coli* and *Saccharomyces cerevisiae*. Jpm. J. Gent., 65: 323-334.

Picardeau, M. (2008). Conjugative transfer between *Escherichia coli* and *Leptospira spp.* as a new genetic tool. Appl. Environ. Microbiol., 74:319–322.

Piers, K., J.D. Heath, X. Liang, K.M. Stephenst and E.W. Nestert (1996). *Agrobacterium tumefaciens*-mediated transformation of yeast. Proc. Natl. Acad. Sci. USA, 93: 1613-1618.

Waters, V.L. (2001). Conjugation between bacterial and mammalian cells. Nat. Genet., 29: 375–376.

Wolk, C.P., A. Vonshak, P. Kehoe and J. Elhai (1984). Construction of shuttle vectors capable of conjugative transfer from *Escherichia coli* to nitrogen-fixing filamentous cyanobacteria. Proc. Natl. Acad. Sci., U.S.A. 81: 1561–1565.

خميرة الرودوتوريولا والكانديدا كمستقبل حقيقى النواة فى التزاوج بين أوليات وحقيقيات النواة

أمينة أحمد حسن

قسم الوراثة – كلية الزراعة – جامعة الزقازيق – مصر

عادة ما يكون التزاوج فعال بين أعضاء أو أفراد نوع أو جنس محدد ويمكن أن يحدث أيضاً بمعدل منخفض بين كائنات متباعدة ومختلفة فى تركيب سطح الخلية. هذه الدراسة هى محاولة لإنجاز التزاوج بين بكتيريا اشيريشيا كولا كمعطى، خمائر الرودوتوريولا والكانديدا كمستقبل. تم استخدام بلازميدات مولفة هى (pJan25, pGWB533) والمشتقة من بلازميد R4pGWB المعروف بأنه Conjugative. مخلوط التزاوج كان يتكون من سلالة *E.coli* محتوية على أحد البلازميدين وخميرة الرودوتوريولا أو الكانديدا. تم عزل DNA البلازميد من خلايا الخميرة الناتجة من التزاوج وتفريدة كهربائياً، وقد تبين وجود حزم من DNA مقابلة لـ DNA البلازميد المعزول من خلايا اشيريشيا كولاى المعطية. هذه النتائج تدل بوضوح أن كلا البلازميدين كانا لهما القدرة على الانتقال إلى خلايا الخميرة. تم أيضا دراسة زمن الانتقال وذلك بعمل PCR باستخدام primers متخصصة للكشف عن جين الـ *gus* فى خلايا الـ Transconjugants على فترات زمنية مختلفة وقد وجد أن الانتقال قد بدأ بعد ساعة واحدة من التزاوج ولكن زاد معدل الانتقال بزيادة زمن التزاوج من ساعتين إلى ٢٤ ساعة. هذه الدراسة تعتبر دليل على نجاح النقل الجينى التزاوجى بين أوليات وحقيقيات النواة.