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ENHANCEMENT OF CELLS FUSION USING MAGNETIC FIELD IN SEXUALLY HOMOGENEITY *Saccharomyces cerevisiae* STRAINS

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ABSTRACT

Most methods involving the hybridization of yeasts aimed to producing new organisms with sets of desirable characteristics and frequently requires the integration of properties from two parental strains. Protoplast fusion has emerged as an important yeast breeding technique Fusion between two homothallic strains do not occurred because cell wall involved similarly pheromones (related to the same mating type). Novozym is one of lytic enzymes used herein for protoplast formation. The flux density of Magnetic Field (MF); 0.7mT (millitesla or 7Gauss) was played an important role to arise protoplast formation and fusion. MF was decreased 75% of lytic enzyme concentration used in protoplast formation. Yeast cell cultures exposed to MF were achievement for single cell separated by micromanipulator unit. The Cells grown in YPD medium for 24hr were transferred into starvation medium for 4hr. Exposure time of MF were 5,10 and 15 min to detected cell viability. As soon as, the exposure time for 5min and 10 min were used for protoplast formation and fusion, respectively. The prtoplasts formation percentages were 33 and 50% in GT160 and GM3 yeast strains, respectively. The protoplast volumes were related with responsibility of yeast strains in protoplast formation. The diploid phases were selected on selective medium contained Phloxin-B stain which appeared each haploid, diploid and dead cells. The diploid phases were encouraged to entire meiotic division by asci formation. The ascospores were transferred into complete medium supplemented auxotrophic requirements and $CuSo_4$ to aide obvious visible color. The genetic analysis were detected the dominant and recessive alleles. All dominants alleles were exist with high percentages. Only the *leu* gene which was defected in the two strains emerged very low dominant percentage.

Ethanol production from two haploid strains and diploids were determined for example of biological activity. Diploids have been record the highest production after 2hr. Ethanol production values were significant after 6hr. Percentage of cells viability measured from unbudding / budding numbers was the other side of biological activity. MF decreased formation of buds exposing for 5 or 10 min through 1, 2 and 3 hr from the fermentations. While, exposed for 15 min did not rform any buds in two strains.

RAPD-PCR reaction was conducted using three primers; two from them were attached with genomic DNA extracted. The exposure time of MF due to disappeared DNA bands, the exposing for 10 min was high effect than 5 min. This work assumed that MF might be utilizes for genetically improvement of economic microorganisms have default sexual cycle.

Keywords: Magnetic field -Protoplast fusion –Yeast

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INTRODUCTION

Baker's yeast can be maintained as either haploid cells or diploid cells that make them attractive to geneticists electrical energy involves voltage and current. Voltage, the pressure behind the flow of electricity (measured in volts), produces an electric field. Current, the rate of electricity flowing (measure in amperes or amps) produces a magnetic field. The higher current, stronger is high magnetic field. Magnetic fields are different to screen and will pass readily through the fabric of a building, but trees and building materials for example will screen electric fields are measured in voltages / meter (V/m). and magnetic fields in microtesla (μT) ,milligauss (mG), or amperes /meter (A/m(1 μT =10mG=0.8 A/m) (Molder, 2000).

The static magnetic field (SMF) and electromagnetic field (EMF) caused inhibition of the cell division in *Escherichia coli* K-12 Ion mutant. The low-frequency EMF 4 Hz led to the 20% survival, but EMF at 50 Hz increased the survival of cells up to 53%. After exposure to magnetic field cells lost capacity for division and grow as filaments, unable to form the colonies on the solid media (Stepanian *et al*, 2000)

The factors controlling the pattern of cell division planes in an organism have been under intense scrutiny because of their importance to cell survival and lineage. Much attention has focused on processes that actively orient the mitotic apparatus after its formation, but early-acting processes that lead it to form in a particular orientation have also been implicated (Valles *et al*., 2002). Biomagnetic group team was working in NASA project, ingoing experiments on the influences of MF on life sciences, to study the action of MF in microgravitational conditions Li, *et al*., 1997). Yeast culture was chosen as the primary specimen of focus for the microgravity project, because various aspects of yeast growth can be studied within short time duration. Interest in the effects of pulsed magnetic fields has led to numerous studies of phenotypic changes in exposed cell populations. Most cell types have a distinct coat (glycocalyx) which presents a highly negatively charged surface to the environment. The effects of exposure to PEMF on this cell coat by exposing a monocyte-like, non-adherent, mammalian cell line (U937) to a 25 Hz pulse-burst field from a Helmholtz coil pair (7.5 cm radius). Peak flux densities of 0.63 mT were used, with a rise time of 200 μs and a fall time of 20 μs. [The induced electric field intensity at the periphery of the culture vessel was estimated at 160mV/m.] For sham exposure, the Helmholtz coils were not energized. Surface charge density was assayed by partition chromatography. Cells exposed for 48 hr had a significantly higher partition coefficient than control cells ($p < 0.03$), consistent with an increase in the negative surface charge density on the cells. In the context of previous experiments undertaken by this group, the investigators concluded that the observed effect was due to the induced electric field (Chan *et al*., 1997).

Following cell division (i.e. mitosis), the daughter cells confront two possible fates. They may decide to enter immediately into another round of growth and division, thereby remaining in the active growth cycle. This leads

to repeated rounds of cell division and results in turn in an exponentially increasing cell population (Kennedy and Pierson, 1996).

Regenfuss *et al.* (1985) appeared, emphasis in research had radically shifted from "classical biophysics" (e.g., kinetics, reaction dynamics, thermodynamics) to "modern biology" (e.g., protein structure and molecular biology). This shift in emphasis may have "diluted" the impact of the development of this important new kinetic tool. The research "pendulum," however, is beginning to swing back, with renewed interest being placed on obtaining a more detailed understanding of how protein structures "actually work" and are assembled (i.e., folded). To answer mechanistic questions of this type, kinetic studies are required, and renewed interest in rapid kinetic methods is once again surfacing (Takahashi *et al.*, 1997).

This work is aimed to observe the bio effects of magnetic fields (MF) on cell activities for example, budding percentage, cell wall especially how MF exposure could modify the responses of eukaryotic cell through protoplast formation and fusion, and their natural production *i.e.* alcohol. The two yeast strains used in this study possess the same mating type (a). *S.cerevisiae* is a model organism because they well-known genetics which make it ideal for biological studies. DNA PCR technique was used for analyzed parental strains and their fusion that exposed to MF for 5 and 10 min.

MATERIALS AND METHODS

I. Microbial strains and growth conditions:

S.cerevisiae strains used in this study are listed in Table 1.

Table 1 : Constructive yeast strains

Strains	Description	Source
GT160-34B	a/ade$_1$,leu$_2$,his$_6$,met$_{14}$,lys$_9$	YGSC*
GM3	a/gal$_{10}$,trp$_1$,met$_8$,ura$_3$,ura$_4$,ade$_{5,7}$,leu$_2$,lys$_1$,ilv$_1$,aro$_1$D,Can$_1$,S uc,mal ,Cupr.	" " " "

• **Yeast Genetic Stock Center ,USA**

Media

1-YPD: Yeastextract,1%; peptone,2%; d H_2O adjust pH to 5.8, autoclave 30-40 min, and cool to 65°C,then add 50 ml of sterile 40% glucose and 20 gm Bacto agar was added before autoclaving when needing to solidify the medium (Sherman *et al.*,1986).

2-YPGS: Yeast extract, 1%; peptone,2%; glucose, 2% and sorbitol, 18.2%.

3-SD (a selective medium): Yeast nitrogen base, 0.67%; glucose, 2%; supplemented with auxotrophic requirements (225 mg/l adenine, histidine, methionine, uracil, lysine with 200µM $CuSo_4$. Cupper sulfate was added to emerge visual color.

4-PSP2(pre sporulation medium): Yeast nitrogen base, 6.7 g; yeast extract, 1g potassium acetate, 10g Mix in 1L of 50 mM potassium phthalate buffer (pH5.0).

5-SPM (sporulation medium): potassium acetate, 3g raffinose, 0.2g, Mix in 1L of dsH_2O, media from2-5 according to Sherman (1991).

Buffer Solution & Lytic enzyme

KCl, 0.65 M; Sorbitol, 1M; $CaCl_2$,100mM and Novozym (an enzyme preparation which digests the cell wall).

II. Methodology

Cells Synchronized and Magnetic field exposure

All cell yeast cultures which exposed to MF were as single cells separated by micromanipulator unit on 5cm YPD plates. They were incubated for 24hr at 30°C. Then they transferred into 10cm plates for another 24hr at 30°C. Single colony was transferred into liquid YPD medium with shaking at 200rpm on the same conditions for 24hr. The cells were centrifuged and washed twice with phosphate buffer. Suspended pellets were transferred into water as a starvation medium and incubated at 30°C for 4hr to insure that most cells closed their divisions or the storage is consumption. A suitable dilution (10^{-4}) of pellets was transferred into eppendorf tubes. Each two strains, immediately, 50µl were transferred into cuvette placed in MF center between magnetic units. The magnetic system consists of two iron cores of the same size. Two units of magnets were contact one opposite the other with electric power, current AC 220 V. Flux densities of magnetic field was 0.7mT (7 G). The exposure time of MF was 5, 10 and 15 min to detect MF effects on cell viability through budding cells (Cooper, 1998).

Protoplast formation

From the storage previous dilutions in eppendorf tubes, 50µl was transferred into 100ml conical flask MM containing low glucose and grew to mid-exponential phase (approx. 5 hr . The cultures were centrifuged and washed twice with water and once with 0.65 M KCl. Pellet was resuspend in 0.65 M KCl containing 0.25 mg/ml Novozym (low conc. enzyme enhances cell wall digestion with MF). Each culture, took place 50µl in cuvette, exposed to MF for 5 min. and incubated at 30°C .After approx 15 min check spheroplasts have formed. Spin protoplasts at 200 rpm for 5 min. and resuspend in small volume of sorbitol buffer (1M).The protoplasts might determined and count by haemacytometer (Evans and Conrad, 1987).

Protoplast fusion

Traditional protocols for protoplast fusion according to Zimermann & Sipiczki (1996) were used as modified. Two equal volumes (50µl) from protoplasts were mixed in cuvette and exposed to MF for 10 min. The Mixture was incubated for 20-45 min at 30°C and washed three times with sorbitol buffer and microscopically was continued.

Protoplast regeneration and selection

The Mixture was plated on sorbitol agar medium with a lower adenine concentration for cell wall regenerate. Colonies were grown on SD as selective medium. The visual colonies were transferred on other liquid YPD and incubated for 24 hr at 30°C (Sherman, 1991). Plated 50µl on YPD with Phloxin-B stain to check the colonies for diploid or ploidy formations.

Genetic analysis

The visual dark pink colonies that selected with Phloxin-B stain were diploid phase. They were encouraged to entire meiotic division by growing on PSM and SM media. The asci were dissected by micromanipulator unit with β-Glucuronidase enzyme. The ascospores have been grown were transferred into YPD and SD medium with requirement supplements and 100μM $CuSo_4$ as a physiological marker to accurate selection (Sherman, 1991).

Ethanol assay

Ethanol concentration was determined using simple and quick chemical. Ethanol concentration calculated for the sample using the following formula: Ethanol (g/l) = A (Sample)/ A (Standard) X 0.8 (for the undiluted samples), the absorbance was recorded with a spectrophotometer at 575nm. according to the modified method of Dhinakar, (1996). Ethanol determination was used as a parameter of fermentation activities.

RAPD-PCR analysis

Genomic DNA was extracted from the two yeast strains used and from that exposed to MF. The exposure was for 5 and 10 min. The yeast samples using "Genomic DNA Purification Kit, Fermentas, Germany", and according to its accompanied protocol RAPD-PCR reaction was conducted using three arbitrary 10-mer primers as shown in Table 2. RAPD-PCR conditions and gel electrophoresis were applied according to Maniatis *et al.*, (1982).DNA Marker ladder 100bp+1.5kbp Biron, Germany.

Table 2: Primer names and sequences of the three arbitrary 10-mer primers

Primers	Sequence (5´to3´)
B-08	GTCCACACGC
B-09	TGGGGGACTC
B-11	GTAGACCCGT

RESULTS AND DISCUSSIONS

MF and cell budding

There are variations of responsive to MF exposure especially for low flux intensity (0.7mT). Two *S.cerevisiae* strains were varied in reproduction percentage. This variability was measur as percentage of unbuddding/budding. Fig 1, A and B showed the relations between MF exposure time and cell budding. The variability of GT160 strain (Fig1, A) after 1hr from cell arresting was 10.6, 40 ,21.4%, and 0.00; as a control group, 5, 10 and 15min, respectively. The same strain after 2hr from cell arresting, bud variability was 23.3, 18.4, 11.5% and 0.00; as a control, 5, 10 and 15min, respectively. These variations were high significant when cells exposed after 3hr since cell arresting, the bud variability was 52, 10.4, 6.0% and 0.00; as a control, 5, 10 and 15min, respectively. The second strain, GM3 (Fig1, B) was appeared that budding activity was low in starting case. However, the

substantial number affects by MF exposing times. They were 2.5, 28, 16.7% and 0.00; as a control, 5, 10 and 15 min, respectively. After 2hr, they were 25, 16.3, 15.6% and 0.00; as a control, 5, 10 and 15 min, respectively. But after 3hr they were 53.6, 2.5, 2.4% and 0.00; as a control, 5, 10 and 15 min, respectively. These results elucidate the MF effects on cell reproduction. In addition, these effects were not depended only on MF exposure time or flux intensity or the distance from the MF source, but also dependence on the genetic constructive strains. Most mutants fall broadly into only three of four easily recognized arrest types: no bud, small bud, large bud, and doublet. The increase and decrease in the cyclic changes of the relative permittivity correspond to the increase in cell length and bud size and to the septum formation between mother and daughter cells, respectively. In the second cycle starting from -180 min, the low-frequency demonstrated increased with the cell elongation and then decreased. However, the decrease in Bud scars (BS) no longer coincided in time with the increase in the septation index because of the low synchrony in the second cell division cycle (Asami *et al.*, 1999).

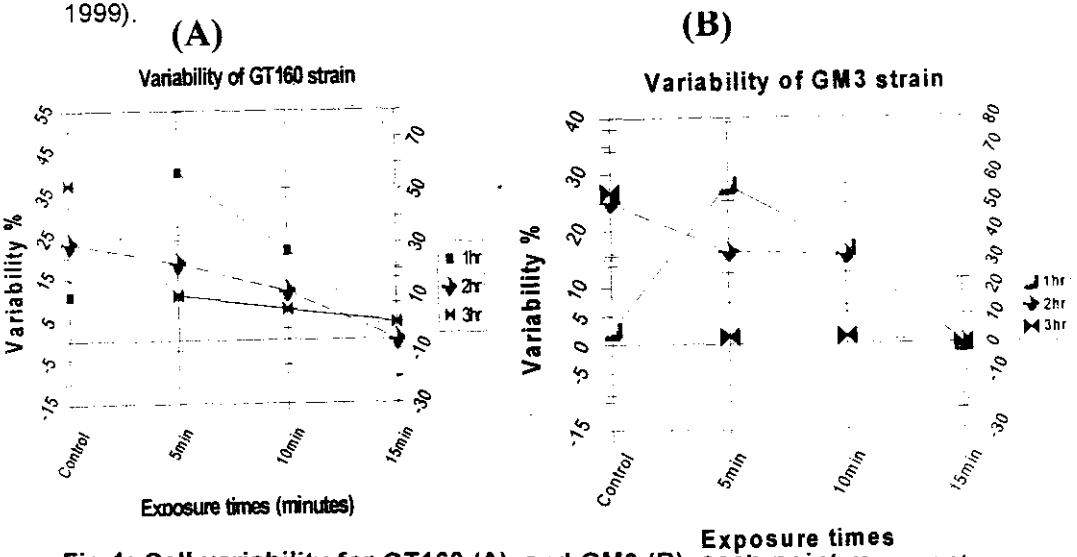

Fig 1: Cell variability for GT160 (A), and GM3 (B), each point represents the mean ± SD.

In addition, cell intensity and high volume after MF exposure are shown in Fig.2. The dark pink of the cells with the Phloxin –B stain illustrated they are diploid phase. Biological cells are polarized in an Ac field owing to interfacial polarization that is the accumulation of charges at the boundaries between the membrane and aqueous phases of different electrical properties (Asami, 1998).Although this does not rule out the possibility that the cells were influenced by the MFs, it does limit the number of possible physicochemical changes that might have occurred to their cell walls and membranes (Zhou *et al.*, 1998). Yeast cells exposed to 0.5 and 1.0 T for 5, 10 and 15 min were fluctuated of viable cell number or the survival percent through 72hr (Sharaf El-Deen, 2005).

Formation of protoplasts and their fusion

The concentration of cells was $2x10^6$approximately at OD_{600} = 0.1. The protoplasts formation dependence on the genotypes of *S.cerevisiae* strains especially them following the same protoplast protocol. Table 3 showed that the GT160 strain was lower competence protoplast formation than the GM3 strain. The GT160 strain formed 33% and GM3 strain formed 50%. Where, low concentration of lytic enzyme (0.25mg/ml) was used with MF. On other work, the GT160 yeast strain was formed 34.09% protoplasts with high concentrated lytic enzyme (1mg/ml), (Sharaf El-Deen & Ahmed, 2004). This result may be verified that MF affect on enzyme activity for spheroplasts or protoplasts formation. On the other hand, the other strain (GM3) formed 50% protoplast .The Fig 2, b appeared protoplasts formation from GM3 and GT160 strains that exposed to MF for 10 min to fusion occurred. The results obtained in numerous studies seem to confirm the significance of protoplast fusion as a method of improving industrial yeast strains, despite difficulties in introducing specific genes and low stability of recombinants (Dziuba and Chmielewska, 2002). Diploid protoplast formation was accounted as each two adhesive protoplasts with different size. The log and stationary phases after24hr at 30°C, OD_{600} was 0.8-1.2 (approximately $2x10^7$.Table 3 showed that protoplast regeneration was 10% from total numbers of the protoplasts. When cells were exposing to MF, cell wall affected energy of MF. An electromagnetic field (H), 0.1 T, cell resistance (R) increased from 0.158MΩ to 0.200 MΩ through 5min exposing MF. Therefore, MF was increasing the potential of cell surface (Sharaf El-Deen and Terra, 1999).

Table 3 : Account of protoplast by haemacytometer/ml

Strains	Colonies No.	Protoplast No.	Protoplast formation%	Protoplast regeneration%
GT160-34B	$3x10^5$	$1x10^5$	33	-----
GM3	$2x10^5$	$1.x10^5$	50	-----
GT160/GM3	------	------	$0.5x10^3$	10

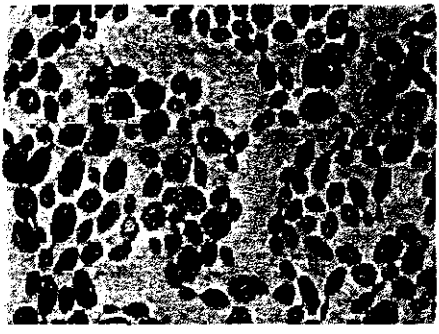

Fig 2 a: Cell aggregating after MF exposure for 5 min without enzyme

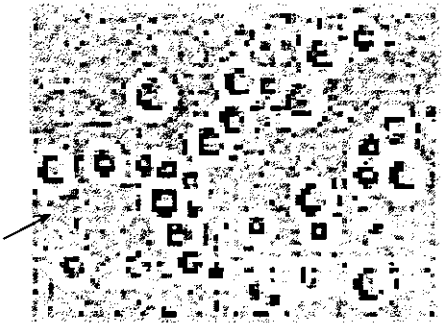

Fig 2 b: Two protoplasts through exposed to MF for 10 min with enzyme.the arrow showing big protoplast (GM3).

Tetrad genetic analysis

Haploids due to ascus dissection have been genetically variations. Genotypes test of tetrads were detected from 25 asci. Fig 3 demonstrated the genotypes of these spores. The ade_1 sited on chromosome 1, and $ade_{5,7}$ sited on chromosome 7 (Cherry *et al.*, 1997). After fusing, the dominant case of this gene was presented in 88 colonies from 100 ascospores, but the recessive was presented in 12 colonies. Here, MF may be induces alleles complementation through protoplast fusing because dominance cases were raised. The leu_2 sited on chromosome 3, this site was defected in the two strains. Therefore, the recessive *leu* allele was presented in 98 colonies. Fortunately, the two visible colonies of *leu* dominants is evidence to DNA repairing It is generally accepted that ELF EMF do not transfer energy to cells in sufficient amounts to directly damage DNA; however, it is possible that certain cellular processes altered by exposure to ELF EMF, such as free radicals and indirectly affect on the structure of DNA. Most investigators have looked for strand breaks and other chromosomal aberrations, including sister chromatid exchange, formation of micronuclei, and/or effects on DNA repair The genotoxic effects of EMF have been extensively reviewed by (Markkanen, *et al.*, 2001).

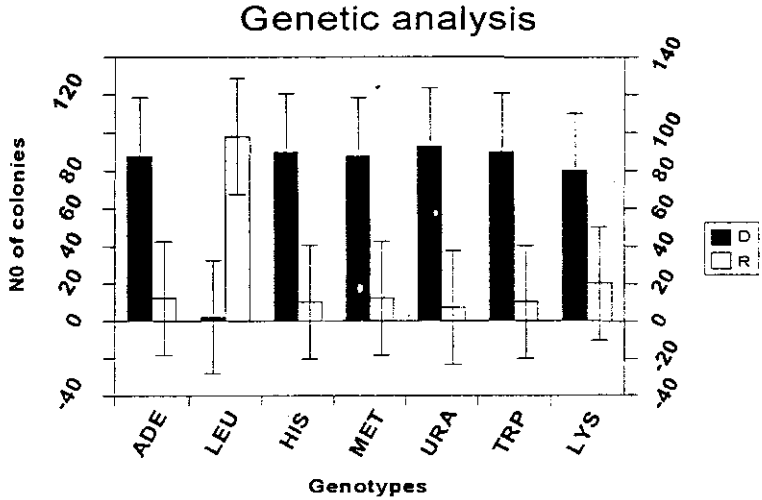

Fig 3: Genetic analysis of ascospores ensue from diploid phase GT160/GM3).Where D; dominants and R; recessive.

Complementation is the process whereby two mutations in different genes, when combined in a diploid, supply one another's deficiency to produce a wild type phenotype. Thus, gene complementation takes place when heterozygote is formed between parents of different mating types with mutants in two different genes. A normal phenotype will occur if a single dose of each gene is sufficient for normal catalytic function. When two individuals are homozygous for mutations within the same gene (i.e. allelic mutants), however, complementation will not usually take place and the F1 will have a mutant phenotype (Kawa-Rygielsska, 2004).

Ethanol assay

The two parental strains; GT160 and GM3 and their diploids appeared different values of ethanol production (Fig 4). In spite of, there are differed in genotypes between two types of haploid strains and diploid phase; they were assembly in ethanol production at 4 and 24hr. Ethanol production raise was significant after 6hr. The diploid phase; GTM were increased approximately 1.4 fold than the two haploid strains. This result is relatively consistent; the diploids arisen from two different genetically haploid strains were produced higher values of ethanol over their parental haploids. This is due to the effect of heterosis. .

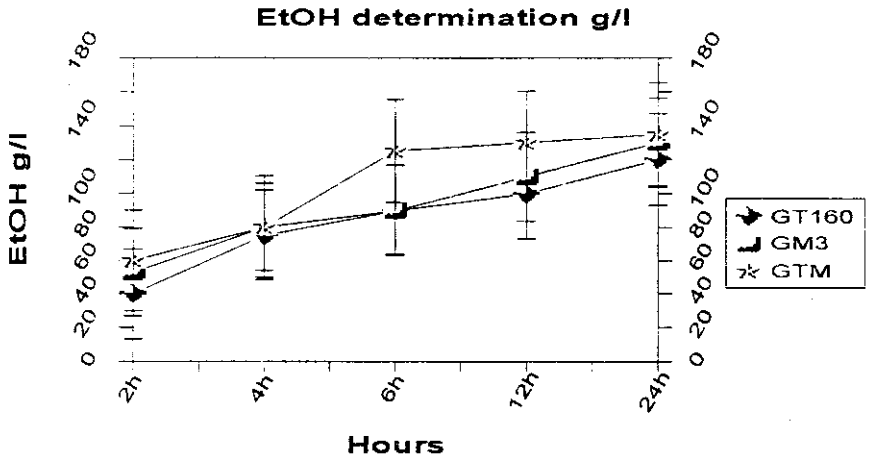

Fig 4 . Ethanol determination of two fermented cultures for 2, 4,6,12 and 24h.

Magnetic effects induced in ethanolic fermentation by *S.cerevisiae* strain DAUFPE-1012 were studied during a 24 h exposure to 220 mT steady magnetic fields (SMF) at 23 +/- 1 °C, As a result, increased ethanol concentration 3.4-fold in magnetized cultures. Glucose consumption was higher in magnetized cultures, which correlated to the ethanol yield (Da Motta *et al.*, 2004). The effect of EMF on the general transcriptional level has been studied in the yeast *S. cerevisiae* and in cultured human cells. The relatively large variations between experiments are possibly due to the unusually long exposure (several generations), thus reflecting changes in properties that biological materials undergo with time).In spite of, budding cells affected by exposing MF, natural product i.e. ethanol was not affected. Researchers have uncovered a strategy that cells may have evolved to prevent oxidative damage to DNA during replication. New findings suggested that the majority of DNA synthesis takes place during the reductive phase of the cell cycle when these damaging oxidative reactions have temporarily ceased. Until now, scientists assumed that respiratory oscillation (the alternation of respiratory and reductive phases) in yeast cells was unrelated to the timing of DNA synthesis (McKittrick *et al.*, 2004).

RAPD-PCR analysis

Two arbitrary 10-mer primers; B-9 and B-11 appeared different bands in original and exposed yeast strains. But the other primer B-08 did not appear all bands. Fig 5 emerged two variable bands with primer B-09 GT-160 strain, (original strain) appeared DNA band about 1300bp and the other between 700-800bp. On the other hand, the big band disappeared when strains exposing to MF either 5 or 10min, but small band which take place between 700-800bp not differ. In GM3 strain two DNA bands were different than the GT-106. In GM3 strain, the large DNA band (1300bp) was light and small band (700-800) was relative with the other strains. Disappeared DNA bands of samples that exposed to MF are refers to partial damage in DNA. The DNA damage affected with exposure time of MF. It was increased with exposing for10 min. While a very large number of cellular components, cellular processes, and cellular systems can conceivably be affected by EMF, mechanistic studies are essential to interpret and help guide the experimental work. Because evidence from previous theoretical and experimental studies suggested that EMF is unlikely to induce DNA damage directly, most studies have been conducted to examine MF effect on the cellular membrane, general and specific gene expression, and signal transduction pathways. More recently, studies specifically addressing the genotoxic effect of exposure to magnetic fields have been pursued (Fingerhut *et al.*, 1980). The MF exposing was high effects on GM3 than GT-160 yeast strain. The variable effects of MF on the strains may be return to their genotypes. Mutation is an area of molecular research in which consistency among results appears to be developing. In numerous studies, 48-h exposures to flux densities below approximately 0.1-1 mT have consistently shown no effect on mutagenesis in *Salmonella typhymurium*. As discussed elsewhere, there is little evidence that EMF below 0.1 mT damage DNA or induce cytogenetic damage; these effects are usually associated with mutation.

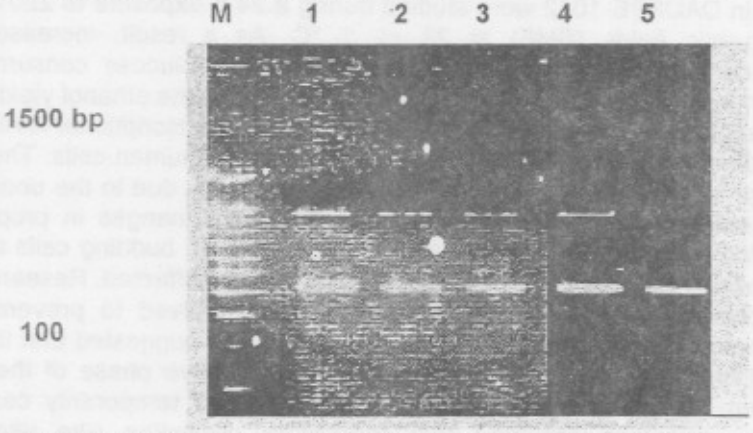

Fig 5: RAPD patterns of two *Saccharomyces cerevisiae* strains primed by B-09 (TGGGGGACTC) Lane1; (M)marker, Lane2;GT-160 an original strain, lane 3; GT-160 exposing for 5 min, Lane 4; GT-160 exposing for 10 min, Lane 5; GM3 original strain, Lane 6;GM3 exposing for 5 min.

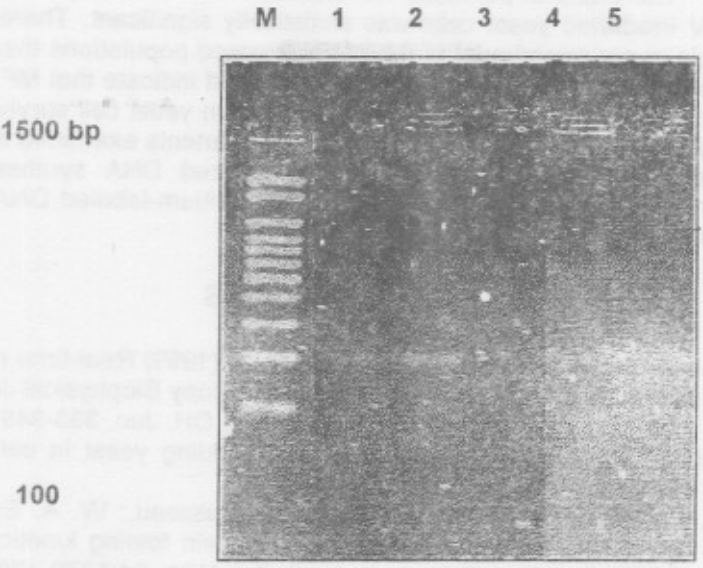

Fig 6: RAPD patterns of two *Saccharomyces cerevisiae* strains primed by B-11 (GTAGACCCGT)Lane1;(M)marker,Lane2;GT-160 an original strain, lane 3; GT-160 exposing for 5 min, Lane 4; GT-160 exposing for 10 min, Lane 5; GM3 original strain, Lane 6;GM3 exposing for 5 min.

In yeast, genes controlling ribosome biogenesis and protein translation have been identified as critical regulators of cell growth and cell size (Jorgensen *et al.*, 2002 and Zhang *et al.*, 2002). Indeed, for yeast this mode of cell-growth control is thought to provide a mechanism to link growth to the cell cycle. For example, the translation of the mRNAs for key yeast cell-cycle regulators is critically dependent on ribosome numbers within a cell (Polymenis and Schmidt, 1997 and Daga and Jimenez, 1999). The cells arrest in the G1 phase of the cell cycle and grow a projection towards one another forming a shmoo projection. Imaging of microtubule dynamics with green fluorescent protein (GFP) fusions to dynein or tubulin revealed that the nucleus and spindle pole body (SPB) became oriented and tethered to the shmoo tip by a Mt-dependent search and capture mechanism (Maddox *et al.*, 1999).Cells goes through four different phases: G1, S, G2, and M, which are the traditional subdivisions of the standard cell cycle. For most of the cells (both prokaryotes and eukaryotes), the time of M and S phases does not vary too much, whereas G1 and G2 phases highly fluctuate and the cells stay in different phases for different time(Qian *et al*, 2001). In this study, the results supposed that the MF affected cell cycle division especially after starvation period, and they are return to start a new cycle. Once cells have passed START phase, they were expose to MF. The effect of MF on cell growth or their budding may be due to events occurring in G1 as phase for pre-synthesis of DNA.

The effect of particular MF exposure on the colony forming ability of the UV irradiated yeast cells was statistically significant. There were fewer CFUs in every experiment in (UV+MF) exposed populations than in only UV exposed yeast populations. These results could indicate that MF exposure in conjunction with UV may have some effects on yeast cell survival or growth (Markkanen *et al.*, 2001). In preliminary experiments examined the effects of magnetic field exposure on enzyme-catalyzed DNA synthesis, reaction mixtures containing 67-mM KH_2PO_4 buffer, tritium-labeled DNA (Harada *et al.*, 2001).

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تحسين دمج خلايا خميرة السكاروميسيس المتجانسة جنسيا باستخدام المجال المغناطيسي

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استخدم في هذا البحث سلالتين من خميرة الخباز (السكاروميسيس) ذات تركيب وراثي مختلف و ذات طرز تزاوجي واحد (a) ومن الطبيعي أن لا يحدث تزاوج بينهما لتشابه بما يعرف بشبيه الهرمون (Pheromone) . لذا تتجه الأبحاث إلى تقنية نزع الجدار الخلوي (Protoplast) . ذلك حين الحاجة الاقتصادية لدمج هذه السلالات. والتحلل الإنزيمي للجدار الخلوي هي أحد هذه الطرق. وقد استخدم إنزيم التحلل Novozym بتركيز 1mg/ml للعينة في العديد من الأبحاث . وفي هذا البحث قد استخدم تركيز منخفض من هذا الإنزيم بالإضافة إلى التعرض للمجال المغناطيسي شدته 0.7mT(7G) . وقد كان التعريض للمجال المغناطيسي لمدة ٥دقائق . وكانت هذه المدة مع هذا التركيز من الإنزيم مناسب للحصول علي نسب تتراوح من ٣٠ إلى ٥٠ % من عدد الخلايا الكلي، حيث أعطت السلالة GT160 ٣٣% والسلالة GM3 ٥٠% . مما يبين أن للتركيب الوراثي دور في مدي الاستجابة للإنزيم والمجال المغناطيسي. والذي قد أظهر الفحص الميكروسكوبي، ان الخلايا المنزوعة الجدار للسلالة GM3 كانت الأكبر حجما هي التي أعطت النسبة الأعلى في الاستجابة، بينما الخلايا المنزوعة الجدار للسلالة GT160 الأقل حجما هي التي أعطت النسبة الأقل للاستجابة للمجال المغناطيسي. وقد تم تعريض الخلايا المنزوعة الجدر للمجال المغناطيسي لمدة ١٠دقائق تحت نفس الظروف، وقد أظهرت الاختبارات تكون الحالة الثنائية وباستخدام صبغة الPhloxin-B تبين أن نسبة حدوثها ١٠% . وقد تم إدخال الخلايا الثنائية في مراحل تكوين الأكياس الجرثومية واختبر ٢٥ كيس أسكي وراثيا طبقا لاحتياجات كلا الأباء المشاركة في هذه الحالة. وقد أشارت النتائج إلى زيادة نسبة المستعمرات الخلوية ذات الأليلات السائدة لكل من Ade (88%) – (90%) His – (88%) Met – (90%) Trp – (80%) Lys –. (93%) Ura . عدا جين ال Leu فكانت نسبته ٢% .

وقد تم اختبار السلالتين أحادية التركيب الوراثي والحالة الثنائية الناتجة بالنسبة لإنتاجها من الكحول كصورة من صور النشاط الحيوي للسلالات عند تعرضها للمجال المغناطيسي. فكانت الزيادة معنوية عند تقديره بعد٦ ساعات في كل من الأباء والهجين.حيث انه بعد ٢ساعه زاد إنتاجية الهجين (GTM) ٥٠%و ١٣,٢% عن السلالة GT160-GM3 علي التوالي. وبعد ٢٤ ساعة كانت زيادة الهجين ١٢,٥%و ٣,٨٥% عن السلالة GT160-GM3 علي التوالي.

وقد كان للمجال المغناطيسي أثر أيضا علي حيوية الخلايا في صورة معدل التبرعم(عدد الخلايا غير المتبرعمة/ عدد الخلايا المتبرعمة) . فقد كانت السلالة GT160 كمجموعة مقارنة ٥٢،٢٣،٣،١٠،٦% بعد ١،٢،٣ ساعة علي التوالي. أما التعرض للمجال المغناطيسي لمدة ٥دقائق فقد أدي إلى لانخفاض حيويتها حيث كانت ٤٠،١٨،٤،١٠،٤ % بعد ٣،١،٢ ساعة علي التوالي. أما المعرضة ل ١٠دقائق فقد انخفضت حيويتها بنسبة واضحة فكانت ٥،١١،٥،٢١،٤ % بعد ٣،١،٢ ساعة علي التوالي. والسلالة GM3 كان الانخفاض بنسبة أعلي هذا بالإضافة إلى أنها أقل من السلالة GT160 في معدل التبرعم وعلي ذلك فكانت نسبة الانخفاض في معدل التبرعم متقاربة في حالة التعرض لمدة ٥ أو ١٠دقائق. أما في كلتا السلالتين بعد التعرض للمجال المغناطيسي لمدة ١٥ق لا يوجد تبرعم في خلايا المستعمرات .

وبتحاليل ال PCR الذي استخدم فيه ثلاث بادئات ذات ترتيب قواعد معينة ، أعطي اثنين منها نتائج تشير إلى وجود فروق متمثلة في وجود واختفاء بعض حزم من DNA التي توضح أن المعاملة بالمجال المغناطيسي قد يؤدي إلى إحداث بعض التلف في DNA أو تغيير في بعض التتابعات والتي قد ينتج عنها طفرات.ويخلص هذا العمل إلى أنه يمكن الاستفادة من المجال المغناطيسي في تحسين الأداء الحيوي للكائنات ، خاصة التي لها أهمية اقتصادية.