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IMPROVED TRANSFORMATION EFFICIENCY OF DEXTRAN GENE(S) IN *Saccharomyces cerevisae* USING THE COMBINED EFFECT OF TWO DIFFERENT DIRECT TRANSFORMATION METHODS

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ABSTRACT

Intact yeast cells *Saccharomyces cerevisiae* can be efficiency transformed by combined effect of polyethylene glycol and $CaCl_2$. This study had been undertaken to determine the effect of using 24% (PEG) in 0.4M manitol and 30 mM $MgCl_2$, 0.3% $CaCl_2$ and the combination of the two individuals.

The results showed that (PEG) or $CaCl_2$ alone caused a few transformants and a low of transformation percentage. In addition, (PEG) gave higher transformants and transformation percentages more than that of $CaCl_2$ as it facilitates the entrance of DNA into the yeast cell walls. In contrast, the combined effect of (PEG) and $CaCl_2$ in the same condition reproducibly yield the maximum number of transformants and transformation percentages. Data also revealed that the transformation percentage was 2.5 fold higher than values given in individual treatment.

It is obvious that higher culture viscosity were produced by transformants isolates from combined effect and $CaCl_2$ experiments. This observation was due to Ca^{++} activated dextran sucrose by increasing the V_{max} and decrease the K_m of sucrose polymerase. In this study, transformants isolates seem to been differ in their ability to express dextran gene(s). This means that the events at one molecule can affect event at a distant location by different ways on the same molecule.

INTRODUCTION

An important problem of modern biotechnology is the biosynthesis of valuable polysaccharides using highly effective recipient system containing cloned genes. A candidate for the role of such recipient is a culture of yeast cells, which in the first place, possess eukaryotic organization, which permits adequate expression of the genes of higher eukaryotes to be achieved and the second place, yeasts are simple and economical to culture (Granovskii et al., 1984). The major products usually associated with the fermentation industry are alcoholic beverages, baker's yeast, antibiotics, amino acids, vitamins, enzymes and polysaccharides. The main influence exerted by genetics in these industries has been shown in strain improvement programs, increases in yield having the criterion which has been applied in judging success and the genetic input have often been minimal. The strain improvement program was mutation, screening operations which were extremely successful in producing large increases in yield cost-effectiveness (Byrom, 1988).

Klebe et al. (1983) reported that polyethylene glycol (PEG) can induce genetic transformation in both bacteria (*E. coli* and yeast, *Saccharomyces cerevisiae*) without cell wall removal, PEG-mediated transformation of *E. coli* is technically simple and yield transformation with an efficiency of 10^{-6}-10^{-7}

transformants. Detailed analysis of the promotors involved in (PEG) mediated of *E. coli* revealed basic differences between (PEG) and standard $CaCl_2$ methods for transformation of *E. coli*. PEG-mediated transformation of yeast is far simpler than existing protoplast method, is comparable in efficiency.

Bushell (1983) reported that the first microbial polysaccharide to be developed commercially was dextran which is produced by various *Acetobacter and Leuconostoc* species. The recent work with dextran has been conducted with various strains of *Leuconostoc mesentroides*. *Alsop (1983)* noted that the following potential uses for native dextrans have been described, secondary recovery of petroleum oil drilling muds, sterilization of soil aggregates, protective coating for seeds, deflocculans in paper products, metal plating process, surgical sutures, food industry for stabilizing and impairing viscosity to syrup and in pharmaceutical industry as blood plasma extenders and blood flow improves.

This study was carried out to improve the transformation efficiency of dextran gene(s) in Saccharomyces cerevisiae from *Leuconostoc mesentroides* using the combined effect of two chemical agents 24% polyethylene glycol in 0.4M mannitol and 0.3% $CaCl_2$.

MATERIALS AND METHODS

A. Strain:

A diploid type *Saccharomyces cerevisiae* strain obtained from the Egyptian Sugar Distillation Company, Hauamdia, Giza, Egypt.

B. Media:

1. (YEP) medium: It consists of 0.3% yeast extract, 2% glucose, 2% peptone and tap water up to 100 ml, it was used for propagation and maintenance culture.

2. Dextran medium: Sucrose 2% was added instead of glucose in the formentioned medium. This medium was used to select the yeast transformants (Kurt and Curt, 1983).

C. The isolation of DNA:

DNA was isolated, purified and fragmented from *Leuconostoc mesentroides* R15 by the methods of Bendish and Bolten (1967).

E. Transformation conditions:

I. *Saccharomyces cerevisiae*: recipient strain was grown in 50ml of (YEP) medium. Cultures were incubated at 30-32°C on a reciprocal shaker at 100 strike/min. for 2 days (exponential phase).

II. For direct transformation: The cell were washed three times in distilled water at room temperature and they were resuspended in:

i. 0.3% $CaCl_2$.

ii. 24% polyethylene glycol (PEG) in 0.4% mannitol and 30mM $MgCl_2$.

The cells were frozen for 30 minutes (freezing enhances the transformation efficiency) (Francoise et al., 1989). After thawing, the suspension was diluted 10 times with distilled water and incubated at 30-32°C for the expression of dextran gene(s).

III. The combined effect: the yeast cells were transformed using (PEG) plus $CaCl_2$. The suspension was diluted and incubated to the optimum temperature of *Saccharomyces cerevisiae* (30-32°C).

IV. Characterization of the transformed cells: The transformants samples were scored and the means were calculated. Transformants which express the gene(s) of dextran (dextran producing transformants) were isolated using high viscosity of colony as a criterion of production of polysaccharides.

V. Fermentation process (batch culture): Propagation of dextran producing yeast transformants in shaking culture did not provide constant environments and rotary shaker (160 rpm) was used.

The fermentation conical flasks (250 ml) containing sterile medium and standard inoculum were added to the fermentation flasks to give a final working volume 50ml. Initial pH was adjusted at 6.7±0.1 by addition of NaOH (1N) or HCl (1N). Temperature was kept at 32°±0.1 during batch cultivation.

VI. Standard inoculum: Standard inoculum was prepared by inoculation of 50ml dextran medium (Kurt and Curt, 1983) in conical flask with a loop of yeast transformants. Inoculated flask was incubated at 32°C for 24h as a stand batch culture. Certain volume depending on the experiment was used as a standard inoculum.

VII. Dextran determination: Dextran was determined either viscometrically or coloremetrically. Culture viscosity as an index for dextran production was measured by the method described by Bourne (1982). Falling ball viscometer (Barnant Company, Gilmant Instruments, Barrington, USA) was used as one of the most effective and accurate instruments for the determination of viscosity.

The viscosity was calculated using the following formula:

$$\mu = K (Pt-Po)t$$

where:

μ: viscosity in centipoise (cP) (one centipoise = 0.01 poise, one poise is defined as the viscosity in which a velocity gradient of 1cm sec^{-1} is obtained when a force of 1 dyne is applied to two surfaces 1cm a part that encompass the liquid that is flowing)

K: viscometer constant (3.3)

Pt: density of tantalum ball (16.6)

Po: density of liquid (grams/ml)

t: time of descent (minutes).

RESULTS AND DISCUSSION

The alkali cation used for cells transformation and gene expression experiments has relied upon the discharge of cellular suspension supplemented with the alkali cations to generate the required results. Attempts to use this type of experiments to permeablize yeast cells have met with success.

Table (1) gives the total number of competents, the number of transformants and the percentages of tranformants obtained from (PEG) alone, $CaCl_2$ alone and the combined effect between (PEG) and $CaCl_2$ in three experiments.

Results revealed that direct transformation in three experiments using (PEG) or $CaCl_2$ alone showed a few transformants and a low of transformation percentage. In addition in the same experiments, (PEG) showed higher transformant percentages than that of ($CaCl_2$) as it facilitates the entrance of DNA into the yeast cell walls. Percentages of transformation in this case was also higher than that obtained with $CaCl_2$ method. In contrast, the combined effect of (PEG) and $CaCl_2$ in the same condition reproducibly yielded the maximum number of transformants and transformation percentage (9 and 2.083 respectively). These results were in agreement with Teresa and Haryoug (1989). They transformed the filamentous *Cyanbacterium, Anabaena sp.* strain M131 with the shuttle vector PR16 by combined effect of Li^+ and (PEG).

A method for transformation might be used of combined effect of alkali ion for the generation of membrane distortions, allowing the uptake for the donors DNA. The method for combined effect induced fusion of membranes termed chemifusion and the generation of small localized holes in biological membrane.

Results also showed that (PEG) gave transformation percentage similar to that obtained by the combined treatment in one experiment (9 and 1.685). However, the addition of such compound did respectively increase transformation efficiency.

Although an effect of other alkali cations such as Ca^{2+} and Zn^{2+} which induce competence in *E. coli* cells *(Mandel and Higa, 1970)* and plant protoplasts (Suzuki and Tanab, 1979) respectively were inert with yeast cells. The anionic moiety has either no effect or only a slight one since (PEG) did show higher efficiency than $CaCl_2$.

Various hypothesis explained the uptake mechanisms of polynucleotides into microorganisms are presented by *Grinium (1980)*. The chemiosomatic mechanisms of DNA transport predicts the obligatory dependence of the DNA transport process chemical potential gradient of (H^+) ions across the plasma membrane and the rearrangement of phospholipid bilayers, through which polynucleotides or polynucleoproteins can enter the cells. Hisao et al. (1982) showed that the uptake of DNA by cation treated yeast cells was also driven by the membrane potential.

Table (1): Number of competents, number of transformants and their percentages obtained following the transformants of yeast strain with *Leuconostoc mesentroides* R15 fragmented DNA using (PEG) and $CaCl_2$ and the combined effect:

	No. of competents	% survival	No. of transformants	% transformation
PEG				
(Exp.1)	762	49.8	4	0.525
(Exp.2)	256	46.4	3	1.170
(Exp.3)	435	52.3	9	1.685
$CaCl_2$				
(Exp.1)	394	66.2	2	0.500
(Exp.2)	389	66.1	2	0.510
(Exp.3)	383	65.1	2	0.520
Combined				
effect	628	50.1	7	1.115
(Exp.1)	432	52.6	9	2.083
(Exp.2)	235	57.8	6	2.553
(Exp.3)				

Data also revealed that the greater number of transformants and the transformation percentage were obtained in the case of combined effect treatment (PEG plus $CaCl_2$) (9 and 2.083 respectively). The transformation percentage was 2.5 fold higher than the values given in individual treatment. *Shivarova et al. (1983)* described the use of electroporation of *Bacillus cereus* in the presence of (PEG). They found a 10-fold increase in transformation efficiency when they used (PEG) combined with the electric field method.

Table (2) gives colonies showing high viscosity and surrounded by clear zones (acid producers) on dextran's medium (Med. 2) plates were picked and purified. A number of 63 of these transformants isolates from different experiments under investigation were obtained. All transformants isolates were characterized by forming round, convex and slimy colonies with smooth margins.

Results in table (2) clearly showed that viscosity of the transformed cultures varied from 439 to 2557 cP. Highest viscosities were detected in the transformant Y45 and Y80, Y92 being 2557, 2495 and 2248 cP respectively which isolated from combined effect (PEG plus $CaCl_2$ experiment). Within transformants isolated from $CaCl_2$ experiments Y50 and Y72 give higher viscosity being 1652 and 1186 cP respectively but lower than combined effect experiments. The lowest viscosity was recorded for transformants isolates Y15 and Y84 being 439 cP for both isolates.

It is obvious therefore, that higher culture viscosities were produced by trabsformants isolates from combined effect and $CaCl_2$ experiments. The present results are in line with Miller and Robyt (1986) who observed that Ca^{++} activated dextran sucrose by increasing the V_{max} and decreasing the Km of sucrose. Craeger and Crarger (1990) reported that dextran sucrose was able to carry out transformation of sucrose into dextran in cell free nutrient solution at pH 5.8-6.5 and a temperature of 25-30°C.

In conclusion, combined effect of (PEG) plus $CaCl_2$ is a powerful tool for transformation of yeast cells, as well as individual (PEG) and $CaCl_2$. Because of the cell membrane and the size of the yeast cells theoretically require a much large treatments to induce portion than do on plant cells or the even mammalian cells.

Data also showed that transformants isolates seemed to be differ in their ability to express dextran gene(s). This means that an event at one location on a DNA molecule can affect on different event at a distant location on different ways on the same molecule. First tracking or translocation of a protein along a DNA, second the association of two protein bound at separate sites to form a DNA loop inbetween and third distal interaction that are affected by the topology of the DNA. The basic characteristics of each type are in terms of the known physicochemical properties of DNA.

Gene transformation or gene transfer is experimental process for modifying the cell to take up and express gene sequences of purified DNA that has been isolated from donor cells.

Table (2): Culture viscosity of different yeast transformants isolates after 7 days at 30-32°C:

Combined effect transformants isolates	Viscosity of culture cP*	PEG transformants isolates	Viscosity of culture (cP)*	$CaCl_2$ transform-ants isolates	Viscosity of culture (cP)*
Y59	2214	Y9	466	Y10	466
Y38	1871	Y22	467	Y3	500
Y92	2248	Y13	500	Y12	500
Y16	1837	Y15	439	Y50	1652
Y2	1625	Y17	528	Y4	562
Y18	2125	Y37	562	Y55	843
Y49	2036	Y48	658	Y24	809
Y60	2029	Y46	610	Y45	686
Y45	2557	Y36	747	Y72	1186
Y29	1652	Y3	782	Y58	1090
Y15	1494	Y30	809	Y19	1028
Y27	1028	Y21	610	Y105	843
Y80	2495	Y84	439	Y98	843
Y8	1309	Y51	596	Y83	720
Y6	1371	Y39	562	Y62	658
Y41	1028	Y26	596	Y58	685
Y1	989	Y34	528	Y77	685
Y56	1027	Y53	466	Y89	895
Y32	1494	Y54	466	Y109	842
Y20	1556	Y35	500	Y111	658
Y18	1034	Y40	528	Y121	596
Y27	1817	Y17	610	Y142	809

*** CP centipoise viscosity of medium 15cP**

REFERENCES

Alsop, R.M. (1983): Industrial production of dextrans; In: Progress in industrial microbiology, Vol. 18: Microbial polysaccharides, Bushell, M.E. (1983) (Ed.),pp. 1-45.Elsevier Publ., Amsterdam, Oxford, New York, Tokyo.

Bendish, A.J. and Botten, E.T. (1967): Rebtendess among plants as measured by DNA agar technique.Plant. physiol.; 42: 959-966.

Bourne, M.C. (1982): Food texture and viscosity: Concept and measurement.Academic Press Inc.; USA: p. 233-243.

Bushell, M.E. (1983): Progress in industrial microbiology, Vol. 18: Microbial polysaccharides, pp. V.XI. Elsevier Publ., Amestrdam, Oxford, New York, Tokyo.

Byrom, D. (1988): Impact of genetics on fermentation technology. J.Appl. Bacteriol.; Vol. 63: No. 3.

Cryeger, W. and Crueger, A. (1990): Biotechnology: A text book of industrial microbiology, 2nd. Ed.Science Tech. Publishers, Sundeland, USA; 33.

Francoise, B.; Lucie, F. and Jean, C.H. (1989): Characterization cloning, curing and distribution in lactic acid bacteria of PLP1, a plasmid from Lactobacillus plantarum CCM and its use in shuttle vector constraction. Plasmid 22, 193-202.

Granovskii, N.N.; Ashlyankerish, M. and Zhdanv, V.M. (1984): Cloning and expression of the Sv40 viras genome in cells of yeast Saccharomyces cervisiae. Bioche.; Vol. 274: No. 1-6.

Grimium, L. (1980): Nucleic acid transport driven by ion gradient across cell membranes.FEBS Lett.; 113: 1-10.

Hisao, L.; Yasaki, F.; Fousaku, M. and Akira, K. (1982): Transformation of intact yeast cells treated with alkali cations.J. Bact., Vol. 153: No. 1, p. 163-168.

Klebe, R.G.; Harriss, J.V.; Sharb, Z.D. and Douglas, M.G. (1983): General method for polyethylene glycol induced genetics transformation of bacteria and yeast.Gen.; Vol. 25: 233-341.

Kurt, G. and Curst, E. (1983): Dextran production by microorganisms or enzymes. Europian Pat., No. 87: 404 AZ, CCF Chem. (Abst.).

Mendel, M. and Higa, A. (1970): Calcium-dependent bacteriophage DNA infection. J. Med. Biol.; 53: 159-162.

Miller, A.W. and Robyt, J.F. (1986): Activation and inhibition of dextran sucrose by calcium.Biochem. Biophys. Acta; 88: 32-39.

Shivarova, N.; Forster, W.; Jacob, H.E. and Grigorova, R.(1983): Transformation of bacteria with DNA plasmid.Z. Allg. Microbiol.; 23: 595-599.

Suzuki, M. and Tanabe, I. (1976): Uptake of single strand bacteriophage DNA by isolated tobacco protoplasts.Z. Phlanzenphiol.; 78: 421-433.

Teresa, T. and Hargoung, P. (1989): Transformation of filamentous cyanobacterium by electroporation. J. Bacteriol.; Vol. 171, No. 10: 5734-5746.

تحسين كفاءة التحول الوراثى لخميرة السكاروميسيس سرفيسيا لإكسابها جينات الدكستران بإستخدام التأثير المشترك لطريقتين مختلفتين من الطرق المباشرة للتحول الوراثى

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يمكن زيادة المحولات الوراثية لخميرة السكاروميسيس سرفيسيا الغير معاملة بكفاءة وذلك بإستخدام التأثير المشترك للبولى إثيلين جليكول وكلوريد الكالسيوم وذلك بإستخدام 0.3% كلوريد كالسيوم، 24% من البولى إثيلين جليكول مذابات فى محلول 0.4 وزن جزى مانيتول، 3مليمول من كلوريد الماغنسيوم كما أستخدم مزيج من كل منهما.

وقد أوضحت النتائج أن كل من البولى إثيلين جليكلول وكلوريد الكالسيوم بمفرده قد أعطى نسبة قليلة من المحولات الوراثية. بالإضافة إلى ذلك فإن البولى إثيلين جلكيلول أعطى نسبة محولات وراثية أعلى من كربونات الكالسيوم. وبعكس ذلك فقد أعطى التأثير المشترك لكلا المادتين نسبة أعلى من المحولات الوراثية عن إستخدام كل مادة على حدة حيث بلغت 5و2 ضعف.

ومن الواضح أيضا من النتائج أن المحولات الوراثية الناتجة من التأثير المشترك لكلا المادتين (البولى إثيلين جليكلول وكربونات الكالسيوم) والناتجة أيضا من المقارنة بكربونات الكالسيوم أعطت لزوجة عالية وهذه الملاحظة ترجع إلى أن Ca^{++} تنشط إنتاج الديكستران من السكروز بزيادة اللزوجة القصوي وإنخفاض K_m للسكروز.

وقد أيدت المحولات الوراثية قابلية مختلفة للتعبير عن جينات الدكستران بواسطة طرق مختلفةعلى نفس الجزى.