Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(1): 337-344. **OPEN ACCESS**

Pulsed Electric Field Effects on Cell Viability of the Human Fungal Pathogen *Candida albicans*

Amr A. Abd-Elghany[1,2]*, Mohamed A. El-Sakhawy[3,4]and Ashraf T .Abul-Hamd[3,5]

[1]Radiology and Medical Imaging Department, College of Applied Medical Sciences, Prince Sattam Bin Abdul-Aziz University, Al-Kharj, **Kingdom of Saudi Arabia**
[2]Biophysics Department, Faculty of Science, Cairo University, Cairo University St., Giza, **Egypt**
[3]Medical Laboratory Sciences Department, College of Applied Medical Sciences, Prince Sattam Bin Abdul-Aziz University, Al-Kharj, **Kingdom of Saudi Arabia**
[4]Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, **Egypt**
[5]Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, **Egypt**

***Correspondence:** amrabdelghany25@gmail.com **Received 07-12-2020, Revised: 26-1-2021, Accepted: 01-02-2021 e-Published: 09-02-2021**

This work evaluates the lethal effects of the main electroporation parameters (field strength, [2.92 - 9.15 kV/cm], pulse duration [0.01 - 0.6 ms], and number of pulses [1 - 10]) on the human pathogen *Candida albicans.* The latter is chosen as a biological model for microorganisms. Electroporation is applied to a suspension of *Candida albicans* via a square pulse generator. Plate count method is used to determine the viability of yeast cells. Results show a threshold field strength value, above which, cell viability sharply decreases by increasing field intensity down to zero viability at very high field intensities. This threshold value decreases from 5 to 3 kV/cm by increasing the pulse duration from 10 to 600 µs. The electric field intensity needed to kill 50% of cells (E_{50}) is shown to decrease at long pulse durations (up to 600 µs). This can be attributed to the observed increase in delivered charge to 0.58 C and energy to 197 kJ with increase in pulse duration. The present data is essential for the optimization of pulsed electric field protocols for food preservation. It provides a quantitative insight on the origin of the fungicidal effects of pulsed electric fields.

Keywords: Square electric pulses; *Candida albicans*; Electroporation; Cloning efficacy test.

INTRODUCTION

Yeasts are unicellular fungi that typically reproduce by budding (Scheinfeld and Lambiase, 2010). For thousands of years ago, yeasts have been used to make fermented foods and beverages. These include beer, wine, sake, and bread. Some bioactive compounds, such as second generation biofuels, β-glucans, mono-oligosaccharides, vitamin B, proteins and minerals are produced by yeast (Sergi Maicas, 2020; Choi and Chung, 1998; Chae et al. 2001, Waszkiewicz-Robak, 2013; Steensels et al. 2014). On the other hand, yeasts may spoil foods and beverages (Walker and Stewart, 2016). *Candida,* is a species of yeast that causes both superficial infections (e.g. skin infections, vaginitis and oropharyngeal infections, that are frequent but not life threatening) and invasive infections (e.g. the invasion of blood stream, that is systemic and is often lethal), (Nur Yapar, 2014). *Candida albicans* is responsible for ~50% of all invasive fungal infections in humans. It causes death for about 49% of blood stream infections (Wisplinghoff et al. 2004).

Yeasts can serve as a useful model for eukaryotic biology. This originates from the simplicity with which the relation between gene structure and protein function can be established

(Botstein and Fink, 1988; Botstein and Fink, 2011).

High intensity electric pulses are becoming an increasingly important tool in many biotechnological and medical applications due to both reversible and irreversible breakdown of cell membrane in a process called electroporation or electropermeabilization. Reversible electroporation causes an increase in the transmembrane potential above a threshold value and the formation of transient electropores which could facilitate the transition of non permeant drugs and DNA into cells and tissues (Kranjc et al. 2017; Rosazza et al. 2016). Irreversible electroporation causes rupture of membranes and cell death; therefore it finds applications in tumor ablation (Stehling et al. 2016), food preservation and water purification (Chong, 2014; Cebrian et al. 2016).

The application of Pulsed Electric Fields (PEF) to yeasts may cause a lethal effect at field intensities above a threshold or at long times of exposure. For example, the PEF treatment at optimal values of energy consumption (186 kJ/kg) and electric field strength (29 kV/cm) permitted reduction of the population of spoilage flora in must and wine up to 99.9% (Puértolas et al., 2009). In contrast to other methods employed for microbial cell inactivation, non-thermal PEF treatment selectively removes the membrane barriers in cells and does not cause any visible damage of cells (El Zakhem et al. 2006a; 2006b; Sale and Hamilton 1967). PEF treatment of *S. cerevisiae* yeast improves the accumulation of zinc and magnesium in the yeast biomass (Pankiewicz and Jamroz, 2010; 2011). The application of PEF with restrictions of electric field intensity and exposure time within certain reasonable ranges allows the preservation of functionality of cell membranes (Fologea et al., 2004). PEF technique improves the efficiency of both bacterial and fungal cell transformation (Costaglioli et al., 1994; Shen et al., 2013; Teissié et al. 2002) and cell electrofusion (Hu et al., 2013). Appropriate adjustment of the electrical parameters is required to establish a specific protocol for a certain fungal species (Ruiz-Diez, 2002).

Therefore this work investigates the effects of PEF on the yeast species, *Candida albicans,* which represents a common source of opportunistic infection. Emphasis is given to elucidating the possible dependence of cell viability of this species on the electrical parameters used in electroporation protocols.

MATERIALS AND METHODS

Test organisms

The quality control strain of *Candida albicans*; ATCC 40193 is obtained from the Medical Microbiology Lab. (College of Applied Medical Sciences, Prince Sattam Bin AbdulAziz University, Al-Kharj, KSA). The strain is stored on yeast malt agar slants.

Media

Yeast malt agar contains dextrose 10 g, peptone 5 g, malt extract 3 g, yeast extract 3 g, agar 20 g and distilled water 1000 ml (Scharlau, Barcelona, Spain).Yeast malt broth contains dextrose 10 g, peptone 5 g, malt extract 3 g, yeast extract 3 g and distilled water 1000 ml (Scharlau, Barcelona, Spain).

Preparation of fungal suspension

Fungal suspension is prepared by taking 4-5 colonies (>1 mm diameter) from a 24 h yeast agar growth culture (Scharlau, Barcelona, Spain) at 35±2°C. *Candida Albicans* cells are suspended in 5 ml of sterile saline solution (0.85% NaCl). Cell suspension is shaken for 15 s and fungal cell concentration is adjusted spectrophotometrically (OD_{530}) to produce 0.5 McFarland Standard equivalent to 1-5 x 10^6 CFU/ml. Fungal inoculum suspension is diluted to 1:1000 in sterilized yeast malt broth medium (Scharlau, Barcelona, Spain) to give a final cell concentration equivalent to 0.5 - 2.5 x 10^3 CFU/ml. The number of viable organisms/ml is tested by the plate count method (using Symbiosis aCOLade colony counter) to ensure optimal dispersion.

Exposure of yeast cells to square electric pulses

400 µl of final fungal inoculum suspensions containing 1-5 x 10^6 CFU/ml are transferred aseptically to 2 mm gap width electroporation cuvette (Model No. 620, BTX Harvard Apparatus, USA). Fungal suspensions are exposed to high intensity monopolar electric pulses with field strength of 2.92 - 9.15 kV/cm, pulse duration of 0.01 - 0.6 ms, and number of pulses of 1 - 10.

Determination of charge and energy due to electric pulses

The resistance of the exposure medium R is calculated using Eq. 1,

$$\sigma = L / R A \quad (1)$$

Where σ is the electrical conductivity (measured using a conductivity meter) of the

exposure medium, A is the electrode surface area and L is the length of the electrode. The charge corresponding to E_{50} (electric field intensity required to kill 50% of the cell population) is calculated using Eq. 2 (Wanda et al., 2003),

$$Q = E_{50}\, t / R \quad (2)$$

Where Q is the charge due to the electric pulses; and t is the total exposure time calculated as the pulse duration multiplied by the number of pulses. The energy E delivered to the sample corresponding to E_{50} is determined by Eq. 3 (Wanda et al., 2003),

$$E = E_{50}^2\, t / R \quad (3)$$

Determination of the number of viable yeast cells

The viable yeast cells are determined by spreading 100 µl of electrically treated fungal suspensions on yeast agar plates (90 mm) using sterilized glass rod. The plates are incubated at 35±2 °C for 24 h and the number of viable organisms/ml is determined by the plate count method (using Symbiosis aCOLade colony counter) to ensure optimal dispersion.

RESULTS

Response of *Candida albicans* to electric pulses

To investigate the interaction of pulsed electric field with *Candida albicans*, Candida cells are electroporated using a collection of electrical parameters. Figures 1-3 show the dependence of cell viability on field intensity and number of pulses at three different pulse durations of 10, 100, and 600 µs, respectively. The number of pulses used in each figure is 4, 6, 8, and 10. One can notice that, for a certain pulse duration and number of pulses, there exists a threshold field strength value, above which, cell viability sharply decreases by increasing field intensity down to a zero viability at very high field intensities.

The threshold field strength values are probably around 5, 4 and 3 kV/cm for pulse durations of 10, 100 and 600 µs as shown in figures 1, 2 and 3, respectively. Therefore, the threshold field strength value seems to decrease with increase in pulse duration for a certain number of pulses.

From the presented data, one can suggest boundary limits that are possibly optimal for irreversible electroporation (which is highly important for food preservation).

Effect of pulse duration on E_{50}

The relationship between the electric field intensity required to kill 50% of cell population (E_{50}) and pulse duration is examined by determining E_{50} at defined number of pulses (4, 6, 8, and 10) as a function of pulse duration (figure 4). It can be seen that, except for 4 and 6 pulses at 100 and 600 µs, respectively, E_{50} shows a gradual decrease with increase in number of pulses and pulse duration. In other words, the electric field intensity needed to kill 50% of cells decreases when using higher number of pulses or longer pulse durations.

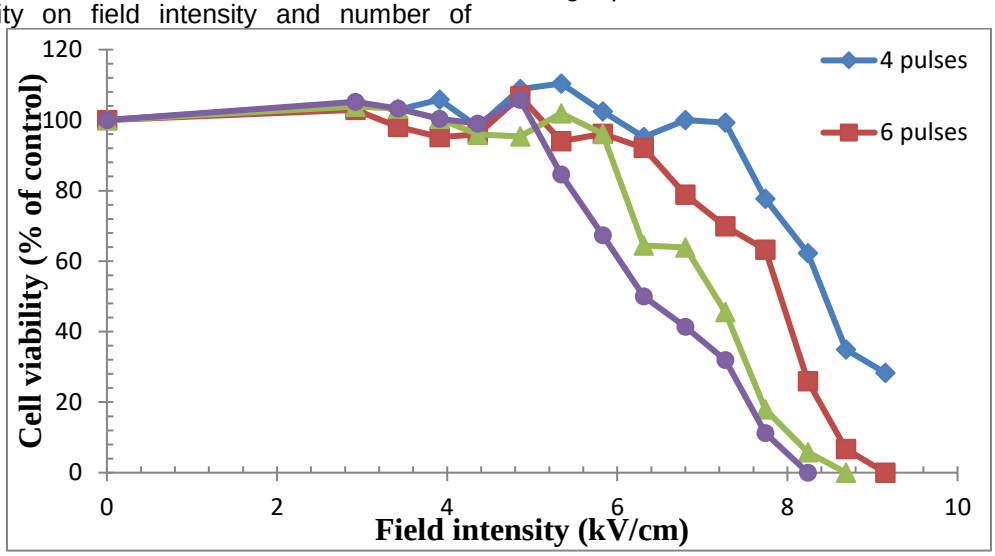

Figure 1: Cell viability of *Candida Albicans* as a function of field intensity and number of pulses at 10 µs pulse duration.

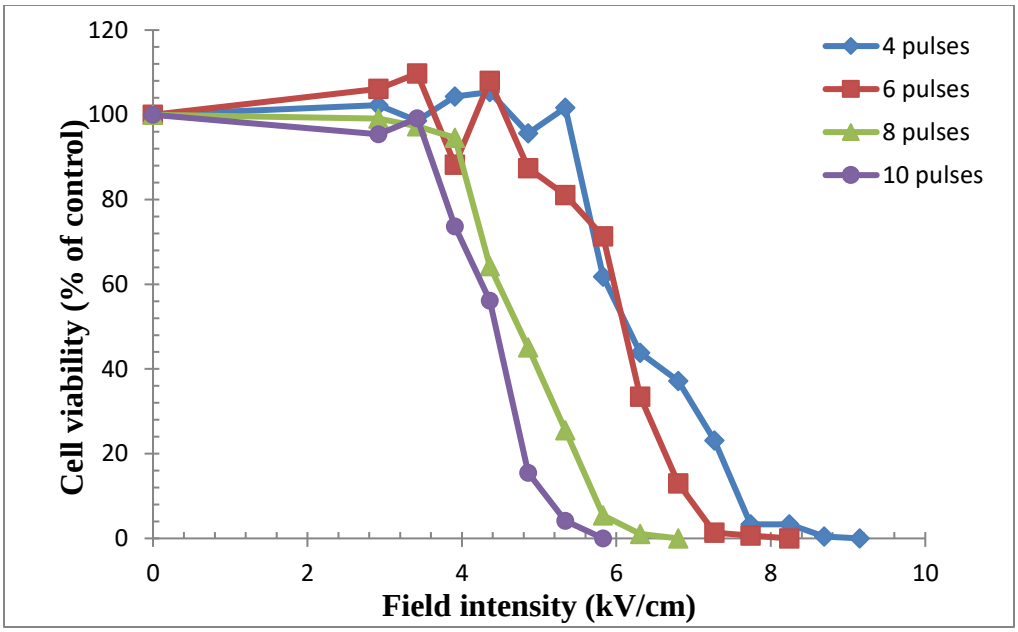

Figure 2: Cell viability of *Candida Albicans* as a function of field intensity and number of pulses at 100 µs.

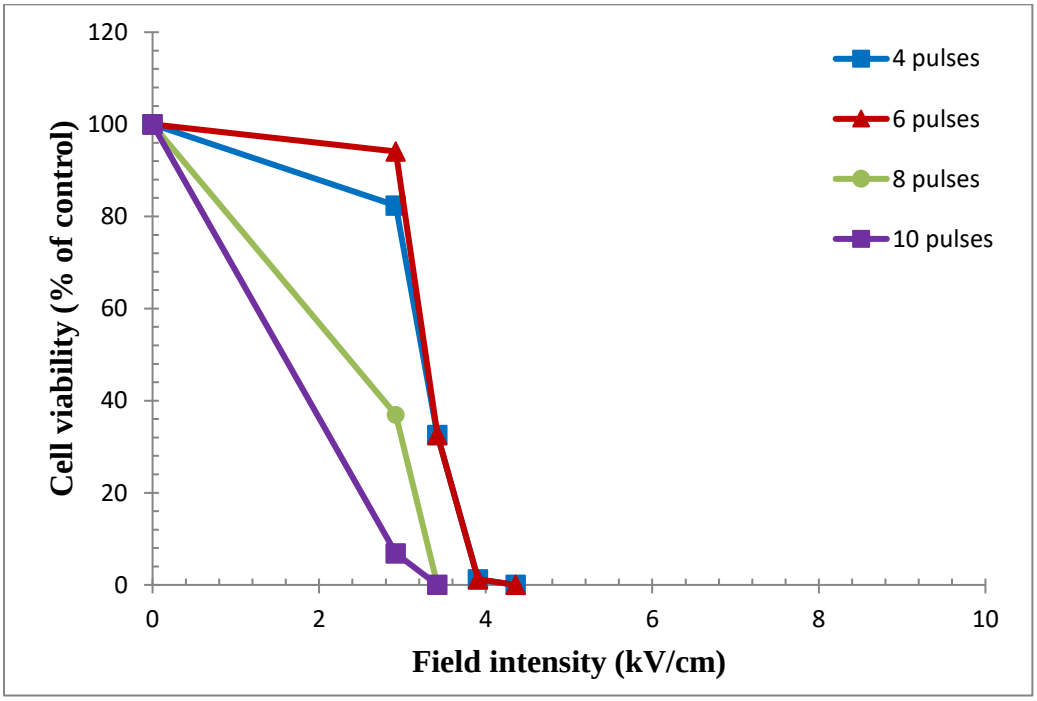

Figure 3: Cell viability of *Candida Albicans* as a function of field intensity and number of pulses at 600 µs.

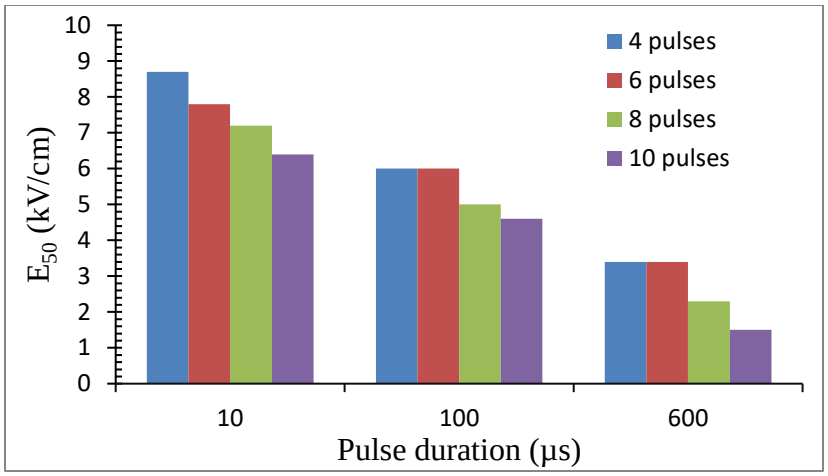

Figure 4: The dependence of E_{50} on pulse duration at constant pulse numbers.

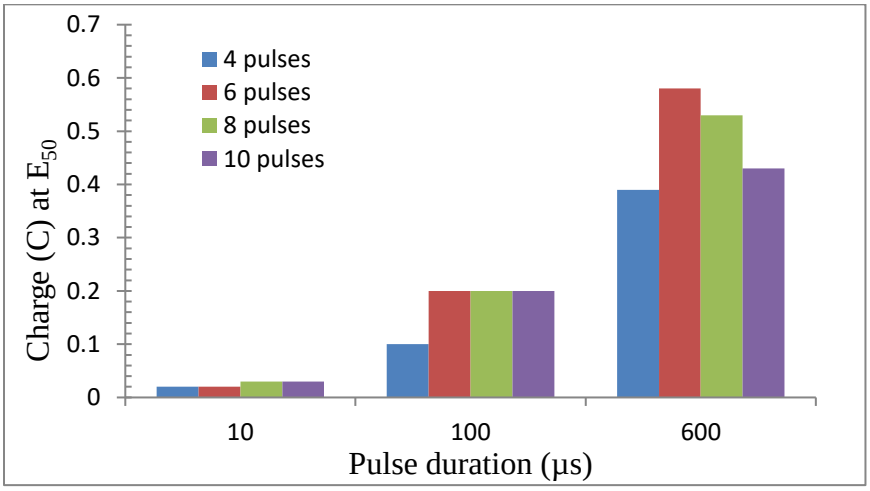

Figure 5: The total charge delivered by electric pulses at E_{50} plotted as a function of pulse duration at different number of pulses.

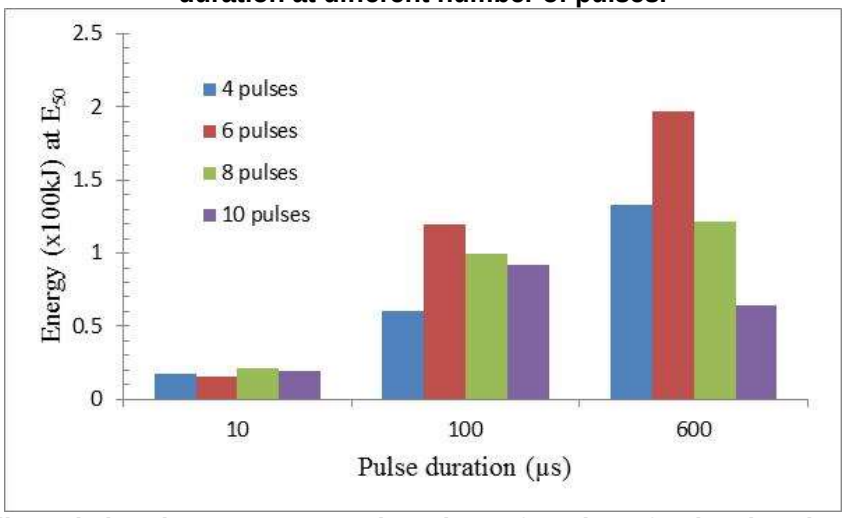

Figure 6: Delivered electric energy at E_{50} plotted as a function of pulse duration for different number of pulses.

Effect of pulse duration on delivered charge and energy

For the same number of pulses, one can notice that the total charge delivered by electric pulses increases by increasing pulse duration (figure 5). The variation of total charge with number pulses is either small (at 100 µs) or does not seem to have a constant pattern (at 600 µs). On the other hand, the delivered electric energy corresponding to E_{50} increases by increasing pulse duration at a defined number of pulses (figure 6). The number pulses has nearly no effect on delivered charge and electric energy at a pulse duration of 10 µs (figures 5 and 6, respectively).

DISCUSSION

The ability to optimize electroporation parameters to combat pathogens via electroporation offers a new horizon in this context. In this work it is shown, quantitatively, that there exists a threshold electric field strength above which there is a sharp decrease in the viability of *Candida Albicans*, used as a good model for human pathogen yeast cells. Moreover, it is shown that it is possible to reach a zero viability or, in other words, complete eradication of pathogen. This is a potentially valuable result for future application of the technique. The role of combinations of electric field strengths, pulse duration and number of pulses are extensively investigated. The decrease in E_{50} with increase in pulse duration is well explained by the corresponding measured increase in each of induced charge and deposited energy.

Some of the previous studies on mammalian cells reported the dependence of cell viability on electrical charge (Wanda K. et al. 2003, Sasa Haberl et al. 2013, and Moshin A. et al. 2017). Unfortunately, these studies had data sets on mammalian cells having only one phospholipid bilayer membrane. On the other hand, the choice of *Candida Albicans*, in this work, offers a better model for human pathogen yeast cells, with two membranes which are separated by the cell wall.

The field intensity required to decrease the viability of *Candida* is considerably higher than that needed for mammalian cells. This is because the electric field must affect the two membranes and the cell wall.

Three possible mechanisms of cell death are being postulated: irreversible electroporation, joule heating, and toxicity due to electrochemical products. Irreversible breakdown and rupture of the cell wall and the membranes are the most likely mechanism for lethal effects due to the loss of cellular contents, or due to the activity of reactive species associated with electropermeabilization (Kekez M. et al. 1996; Miller et al. 2005). In this work, irreversible electroporation begins at 4 pulses with field intensity of 7.74kV/cm and pulse duration of 10 µs. It is shown that it is possible to reduce the threshold value of irreversible electroporation by increasing the number of pulses. Joule heating can be excluded because the temperature of the samples in the present measurements is no more than 40^{o}C which can be tolerated by yeast cells. For mammalian cells, a temperature of 43^{o}C must be applied for 30 min to induce cell death (Nakajima K., Hisazumi H., 1983; Pogorzala L., 2013). Electrochemical contamination has toxic effects on mammalian cells in suspension due to electrolysis of the media and release of ions from aluminum electrodes (Youxing F. et al. 2015). In this work, the lethal effects due to electrochemical deposits can be excluded due to the presence of cell walls, which protect yeast cells from osmotic stress and toxic compounds (Arunas et al. 2014).

The present *in vitro* study utilizes pulsing protocols that can be used for fermentation in beverages and microbial fuel applications. In case of *in vivo* protocols, such as in food preservation, yeast cells may have different geometry and would respond differently to environmental factors. Based on this study, fungicidal effects of pulsed electric fields can be examined for patients with fungal infections that are resistant to chemical drugs.

CONCLUSION

The lethal effect of main electroporation parameters (field intensity, pulse duration and number of pulses) used in the electroporation protocols of the human pathogen *Candida Albicans* are comprehensively evaluated using plate count method. The dependence of the viability of *Candida* on electroporation parameters is quantitatively established. The field intensity and pulse duration are two crucial parameters for such effect. The decrease in E_{50} with increase pulse duration is attributed to the corresponding measured increase in each of induced charge and deposited energy. The present results pave the way for wide range of applications in gene transfection experiments using yeast cells, fungicidal effects of pulsed electric fields on patients with fungal infections that are resistant to chemical drugs and fungicidal effects of pulsed electric fields for food and beverages industry.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The present work was supported by Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam Bin Abdul-Aziz University, Alkharj, SA.

AUTHOR CONTRIBUTIONS

Amr A. Abd-Elghany designed the study and conducted the data search and was the major contributor in drafting, writing, and editing of the manuscript. Mohamed A. El-Sakhawy co-designed the study and performed the experimental part. Ashraf T. Abu El-Hamd assisted in writing and reviewing of the manuscript. All authors read and approved the final manuscript.

REFERENCES

Arunas S., Aurelijus Z., Almira R., Saulius B., Nerija Z., Gintautas S., Larisa C., Voitech S., Arunas R. (2014). Electric field induced effects on yeast cell wall permeabilization. *Bioelectromagnetics* 35, 136-144.

Botstein D., Fink GR (2011). Yeast: An Experimental Organism for 21st Century Biology, YEASTBOOK PERSPECTIVES, Genetics, Genetics Society of America Vol. 189, 695–704

Botstein D., Fink GR. (1988). Yeast: an experimental organism for modern biology. *Science* 240, 1439–1443.

Cebrian G, Marias P, Condon S (2016). Comparative resistance of bacterial foodborne pathogens to non-thermal technologies for food preservation. *Front Microbiol.* 7, 734.

Chae HJ, Joo H, In MJ (2001). Utilization of brewer's yeast cells for the production of food-grade yeast extract, Part 1: Effects of different enzymatic treatments on solid and protein recovery and flavor characteristics. Bioresource Technology 76, 253-258.

Choi SJ, Chung BH (1998). Simultaneous production of invertase and yeast extract from baker's yeast. Biotechnology and Bioengineering 13, 308-311.

Chong L, Xing X, Wenting Z, Jie Y, Desheng K., Alexandria B., Yi C. (2014). Static electricity powered copper oxide nanowire microbicidal electroporation for water disinfection. Nanoletters 14(10), 5603-5608.

Costaglioli P, Meilhoc E, Masson JM (1994). High-efficiency electrotransformation of the yeast Schwanniomyces occidentalis. Current genetics 27 (1), 26–30.

El Zakhem H, Lanoisellé JL, Lebovka NI, Nonus, M., Vorobiev, E. (2006a). Behavior of yeast cells in aqueous suspension affected by pulsed electric field. Journal of Colloid and Interface Science 300 (2): 553–563.

El Zakhem H, Lanoisellé JL, Lebovka NI, Nonus M, Vorobiev E (2006b). The early stages of Saccharomyces cerevisiae yeast suspensions damage in moderate pulsed electric fields. Colloids and Surfaces B: Biointerfaces 47 (2), 189–197.

Fologea D, Vassu T, Stoica I, Csutak O, Sasarman E, Smarandache D, Ionescu R (2004). Efficient electrotransformation of yeast using bipolar electric pulses. Romanian Biotechnological Letters 9, 1505–1510.

Hu N, Zhang X, Yang J, Joo S, Qian S (2013). A cell electrofusion microfluidic chip with micro-cavity microelectrode array. Microfluidics and Nanofluidics 15 (2), 151–160.

Kekez M, Savic P, Johnson B (1996). Contribution to the biophysics of the lethal effects of electric field on microorganism. Biochim. Biophys. Acta 12;1278(1), 79-88.

Kranjc S, Cemazar M, Sersa G, Scancar J, Grabner S (2017). In vitro and in vivo evaluation of electrochemotherapy with trans-platinum analogue trans-[$PtCl_2$-(3-Hypy)$_2$]. Radiol. Oncol. 51(3), 295-306.

Miller L, Leor J, Rubinsky B (2005). Cancer cells ablation with irreversible electroporation. Technol Cancer Res Treat 4, 699–705.

Moshin AK, Rumana A, Srivastava AN (2017). Cancer cell viability and survival in vitro as function of cell surface electric charge: An analytical study. ERA JOURNAL OF MEDICAL RESEARCH 2, 67-77.

Nakajima K, Hisazumi H (1983). An experimental

study of enhanced cell killing by hyperthermia and bleomycin. Urol. Res. 11, 43-46.

Nur Yapar. (2014). Epidemiology and risk factors for invasive candidiasis. Ther. Clin. Risk Manag. 10, 95-105.

Pankiewicz U, Jamroz J (2010). Effect of pulsed electric fields upon accumulation of magnesium in Saccharomyces cerevisiae. European Food Research and Technology 231 (5), 663–668.

Pankiewicz U, Jamroz J (2011). Effect of Pulsed Electric Fields upon Accumulation of Zinc in Saccharomyces cerevisiae. Journal of microbiology and biotechnology 21 (6), 646–651.

Pogorzala L, Mishra S, Hoon M (2013). The cellular code for mammalian thermosensation. J. Neurosci. 33(13), 5533-5541.

Puértolas E, López N, Condón S, Raso J, Álvarez I (2009). Pulsed electric fields inactivation of wine spoilage yeast and bacteria. International Journal of Food Microbiology 130 (1), 49–55.

Rosazza C, Meglic S, Zumbusch A, Rols MP, Miklavcic D (2016). Gene electrotransfer : a mechanistic prospective. Curr. Gen. Ther. 16(2), 98-129.

Ruiz-Diez B (2002). Strategies for the transformation of filamentous fungi. J. Appl. Microbiol. 92:189–95.

Sale AJ, Hamilton WA (1967). Effects of high electric fields on microorganisms: I. Killing of bacteria and yeasts. Biochimica et Biophysica Acta , BBA - General Subjects 148(3), 781–788.

Sasa H, Masa K, Karel F, Dusa H, Vladimir BB, Damijan M, Jean-Micheal E, Marie-Pierre R, Mojca P (2013). Effect of different parameters used for in vitro gene electrotransfer on gene expression efficiency, cell viability and visualization of plasmid DNA at the membrane level. The Journal of Gene Medicine 15 (5), 169-181.

Scheinfeld NS, Lambiase MC (2010). Candidiasis, Cutaneous http://emedicine.medscape.com/ article/ 1090632- followup. updated oct 2010.

Sergi Maicas (2020). The role of Yeasts in Fermentation processes. Microorganisms 8(8), 1142.

Shen X, Chen Y, Liu T, Hu X, Gu Z (2013). Development of a high-efficient transformation system of Bacillus pumilus strain DX01 to facilitate gene isolation via gfptagged insertional mutagenesis and visualize bacterial colonization of rice roots. Folia Microbiologica 58 (5), 409-417.

Steensels J, Snoek T, Meersman E, Nicolino MP, Voordeckers K and Verstrepe KJ (2014). Improving industrial yeast strains: exploiting natural and artificial diversity. FEMS Microbiol Rev 38, 947–995.published by John Wiley & Sons Ltd on behalf of Federation of European Microbiological Societies.

Stehling MK, Guenther E, Mikus P, Klein N, Robinsky L, Robinsky B (2016). Synergistic combination of electrolysis and electroporation for tissue ablation. PLoS One 11(2), e0148317.

Teissié J, Eynard N, Vernhes MC, Bénichou A, Ganeva V, Galutzov B, Cabanes PA (2002). Recent biotechnological developments of electropulsation. A prospective review. Bioelectrochemistry 55 (1–2), 107–112.

Walker GM, Stewart GG (2016). Saccharomyces cerevisiae in the production of fermented Beverages. Beverages 2(4), 30.

Wanda K, Gurvinder SN, Melissa BA, Sukhendu BD, Dietmar PR (2003). Viability of cancer cells exposed to pulsed electric fields: The role of pulse charge. Annals of Biomedical Engineering 31, 80-90.

Waszkiewicz-Robak B (2013). Spent Brewer's Yeast and Beta-Glucans Isolated from Them as Diet Components Modifying Blood Lipid Metabolism Disturbed by an Atherogenic Diet: Lipid Metabolizm. InTech Pub, Rijeka, Croatia.

Wisplinghoff H, et al. (2004). Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. 39(3):309–317.

Youxing F, Kazuki Y, Xueguang J, Xiao-Guang S, Tetsuya T, Nada M, Sheng D (2015). An AlCl3 based ionic liquid with a neutral substituted pyridine ligand for electrochemical deposition of aluminum. Electrochimica Acta 160, 82-88.