Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01
DOI URL: http://dx.doi.org/10.21474/IJAR01
ISSN NO. 2320-5407

RESEARCH ARTICLE

International Conference on Recent Advances in Biotechnology, Biomolecules and Pharmacy RABBP – 2020 (Organized in Virtual Mode due to COVID-19 Pandemic) during 17th to 19th December 2020 at KL University, Vijayawada, Andhra Pradesh, India.

PLASMID STABILITY STUDIESOF JM109 HOST SYSTEM

PhavethraSago, Vineethanand J, Lakshmi Naga Bhavani Alluri, Sai Priya Avuthu, Sai DhanushaPalagiri and Praveen Kumar Vemuri
Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Andhra Pradesh,India.

Manuscript Info

Key words:-
JM109, Danthrone, Acridine Orange, Radio Frequency, Microwave

Abstract

The purpose of the study delineates the growth and plasmid stability of *E.coli*JM109host system. Different concentrations of drugs, chemicals and various frequency of radiations were subjected to the host system to verify the colony forming units along with plasmid concentration and stability. Among chemicals, acridine orange showed highest effect on growth of DH5a, while among the drugs, danthrone showed maximum effect on the growth of the organism. Radio frequency of 2GHz and low intensity microwave radiation were recorded as highest inhibitory effects. However, there is no significant effect in growth was observed in exposure to UV rays. The present work discussed that, the effect of drugs, chemicals, radio frequency and microwave radiation have a huge effect not only on growth of organism but also concentration and stability of plasmid.

Introduction:-

Drug treatments, effect of chemicals and exposure to radiations are very important in research and medicine [1]. For over a decade, geneticists were interested in inducing mutations with chemicals and radiations targeted to discover the mutagenic compounds through their specific activity which can probably give some understanding and knowledge of chemical basis of mutation and gene structure [2-4]. Plasmids are important tools for biotechnology, an understanding of the biology of plasmids is highly needed for improved industrial applications [5]. Many expression systems exploit plasmids as the vectors for production of recombinant proteins or non-proteinous recombinant components [6]. Such plasmids show an essential impact on productivity. Thus, studying plasmid stability and colony forming units is necessary both at industry and research level [7]. JM109 competent cells are generally a E. coli strain for cloning and plasmid maintenance [8]. Bacterial Strain JM109 is an important host for transforming pGEM vectors and for producing single-stranded DNA from M13 or phagemid vectors [9]. The strain can be transformed effectively by different techniques. As JM109 is recA-and does not have the E. coli K restriction system [10], undesirable restriction of cloned DNA and recombination with host DNA are not allowed [11]. JM109 is lacking in beta-galactosidase action because of deletions in both genomic and episomal duplicates of the lacZ

Corresponding Author:-Phavethra Sago
Address**:-**Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Guntur, Andhra Pradesh, India.

gene. [12].In this study, the concentration and stability of plasmid were analysed using drugs, chemicals and exposure to various radiations.

Materials And Methods:-

All molecular biology kits were procured from Thermo Fischer, India. *Ecoli*JM109(Promega: P9751) was procured from Promega - India. All solvents and reagents were of analytical grade, and all experiments were performed with deionized water.

Preparation of bacterial culture

Stock culture of *E coli*JM109was sub-cultured on LB agar at 37^0C for 24 h [13]. A total of 45 sterile falcon tubes were taken and grouped into three categories, every five tubes were added having density of $2.25*10^7$ cells/ml, inoculated a flask containing 250ml of sterile culture medium and labelled with varying concentrations from 1mg to 5mg of chemicals (Sodium acetate, Benzene, Acridine orange) [14-15] from 1mg to 5mg, drugs (Tacrolimus, Sodium bisulphate, Danthrone)[16], radiation (Radio waves - 0.5ghz to 2ghz, Microwave - medium, medium-low, low, UV rays - 212nm, 253nm, 365nm) [17], inoculated aseptically, incubated for overnight at 37^0C for 120rpm to obtain a concentration of 1.5x 10 cells/ml.

Bacterial Plasmid DNA Isolation

Cell pellet was harvested by centrifugation at 6,000 rpmfor 15 min at RT. The supernatant was removed and plasmids were extracted using HiPurA™ Plasmid DNA Miniprep purification (HiMedia), as per the manufacturer's instructions [18].Plasmids were eluted in 1 mL 1 mM Tris/HCl pH 8 or sterile ddH2O and plasmid concentration was measured (NanoDrop 2000, Thermo Scientific) [19] or determined by comparing the DNA concentration of 1 µL linearized plasmid with 5 µL DNA Marker (Puregene) [20].

Results And Discusion:-

Plasmid stability studies

Plasmid stability has been problematic in bacterial studies, and historically antibiotics have been used to ensure plasmid stability. This has been a major limitation during *in-vivo* studies, in which, providing antibiotics for plasmid maintenance is difficult and has confounding effects. In the present study, we used different chemicals, drugs and exposed with various radiations to construct stable plasmids that obviate antibiotic usage. The samples were then run on 1% agarose gel together with 1kb ladder DNA for reference and checked for the purity. The concentration of the plasmid DNA obtained was 39.65 µg/ml.

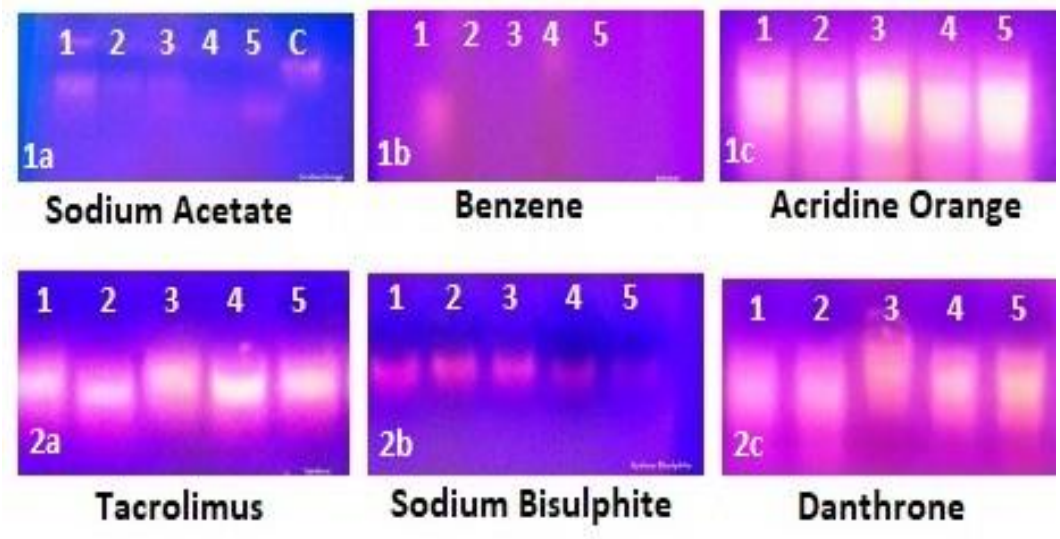

Figure 1:- High-copy number plasmid DNA was isolated from overnight bacterial culture 1a) treated with sodium acetate (1-5mg/ml) 1b) treated with benzene (1-5mg/ml) 1c) treated with acridine orange (1-5mg/ml) 2a) treated with tacrolimus (1-5mg/ml) 2b) treated with sodium bisulphite (1-5mg/ml) 2c) treated with danthrone (1-5mg/ml) and purified plasmid DNA was analyzed by agarose (1%) electrophoresis.

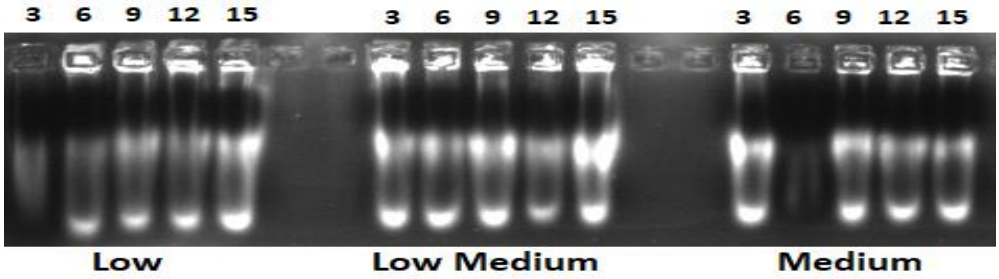

Figure 2:- High-copy number plasmid DNA was isolated from overnight bacterial culture exposed at low, low-medium, medium microwave radiation ranging from 3 to 15 seconds and purified plasmid DNA was analysed by agarose (1%) electrophoresis.

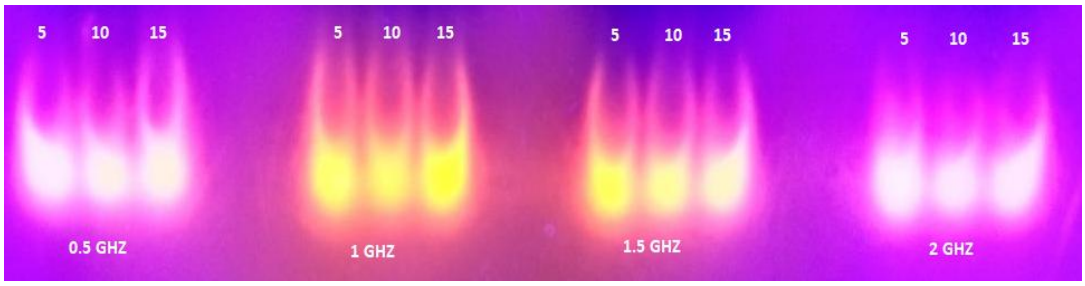

Figure 3:- High-copy number plasmid DNA was isolated from overnight bacterial culture exposed with radio wave radiation ranging from 5 to 15 minutes and purified plasmid DNA was analysed by agarose (1%) electrophoresis.

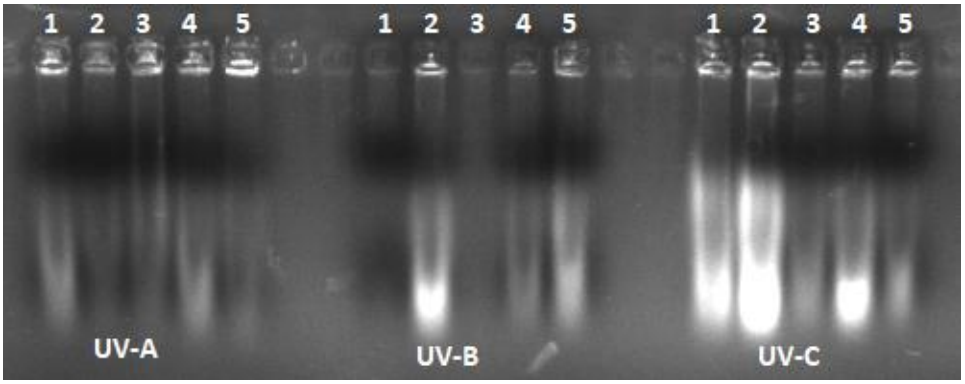

Figure 4:- High-copy number plasmid DNA was isolated from overnight bacterial culture exposed with ultraviolet radiation ranging from 1 to 5 minutes and purified plasmid DNA was analysed by agarose (1%) electrophoresis.

Conclusion:-

Although the molecular details of plasmid constructs and biology of file cell areof interest, biochemical engineers are concerned primarily with maintaining plasmidstability with environmental manipulations including media composition andselection pressures, dissolved oxygen, temperature, pH and mode of cultivation.Here we focused on plasmid stability in at molecular and cellular engineering levels using various drugs, chemicals and radiation. With improvedunderstanding, plasmid stability may be enhanced by manipulating plasmidcomposition and structure, modifying tile genetic and physiological properties of host.

References:-

1.Vemuri PK. Effect of Plasmid Presence and Stability on Growth of Escherichia coli DH5α with Use of Drugs, Chemicals, and Radiation. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm. 2019 Jul 27;13(3).

2. HAMKALO BA. The Effects of Ultraviolet Radiations on Bacteria. InConcepts in Radiation Cell Biology 1972 Jan 1 (pp. 91-121). Academic Press.

3.Ribo JM, Kaiser KL. Effects of selected chemicals to photoluminescent bacteria and their correlations with acute and sublethal effects on other organisms. Chemosphere. 1983 Jan 1;12(11-12):1421-42.
4. Al-Kandari F, Al-Temaimi R, van Vliet AH, Woodward MJ. Thymol tolerance in Escherichia coli induces morphological, metabolic and genetic changes. BMC microbiology. 2019 Dec;19(1):1-1.
5.Tolmasky ME, Alonso JC, editors. Plasmids: biology and impact in biotechnology and discovery. John Wiley & Sons; 2020 Jul 24.
6. Chen R. Bacterial expression systems for recombinant protein production: E. coli and beyond. Biotechnology advances. 2012 Sep 1;30(5):1102-7.
7.Ahmetagic A, Pemberton JM. Stable high-level expression of the violacein indolocarbazole anti-tumour gene cluster and the Streptomyces lividansamyA gene in E. coli K12. Plasmid. 2010 Mar 1;63(2):79-85.
8.Hanahan D, Jessee J, Bloom FR. [4] Plasmid transformation of Escherichia coli and other bacteria. InMethods in enzymology 1991 Jan 1 (Vol. 204, pp. 63-113). Academic Press.
9. Wood TK, Peretti SW. Construction of a specialized-ribosome vector or cloned-gene expression in E. coli. Biotechnology and bioengineering. 1991 Oct 20;38(8):891-906.
10. Narumi I, Satoh K, Kikuchi M, Funayama T, Kitayama S, Yanagisawa T, Watanabe H, Yamamoto K. Molecular analysis of the Deinococcusradioduransrec A locus and identification of a mutation site in a DNA repair-deficient mutant, rec30. Mutation Research/DNA Repair. 1999 Dec 7;435(3):233-43.
11. Rhee JI, Bode J, Diaz-Ricci JC, Poock D, Weigel B, Kretzmer G, Schügerl K. Influence of the medium composition and plasmid combination on the growth of recombinant Escherichia coli JM109 and on the production of the fusion protein EcoRI:: SPA. Journal of biotechnology. 1997 Jun 13;55(2):69-83.
12.Borloo J, De Smet L, Vergauwen B, Van Beeumen JJ, Devreese B. A β-Galactosidase-Based Bacterial Two-Hybrid System To Assess Protein− Protein Interactions in the Correct Cellular Environment. Journal of proteome research. 2007 Jul 6;6(7):2587-95.
13. Chung WH, Wong KY, Leung KT, Sheldon JR, Yim MS, Saliba JH. in Escherichia coli O157: H7 rpoS Role of.
14.Sun WJ, Lee C, George HA, Powell AL, Dahlgreir ME, Greasham R, Park CH. Acetate inhibition on growth of recombinant E. coli and expression of fusion protein TGFα-PE40. Biotechnology letters. 1993 Aug 1;15(8):809-14.
15. Qu XH, Chen JC, Ma QX, Sun SY, Chen GQ. Biotransformation of benzene to cis-1, 2-dihydroxycyclohexa-3, 5-diene using recombinant Escherichia coli JM109 (pKST11). Sheng wu gong chengxue bao= Chinese journal of biotechnology. 2003 Jan 1;19(1):74-80.
16. Connelly PR, Thomson JA, Fitzgibbon MJ, Bruzzese FJ. Probing hydration contributions to the thermodynamics of ligand binding by proteins. Enthalpy and heat capacity changes of tacrolimus and rapamycin binding to FK506 binding protein in deuterium oxide and water. Biochemistry. 1993 Jun 1;32(21):5583-90.
17.Ramabhadran TV, Jagger J. Mechanism of growth delay induced in Escherichia coli by near ultraviolet radiation. Proceedings of the National Academy of Sciences. 1976 Jan 1;73(1):59-63.
18.Padhan M, Pattnaik S. A Correlation Study Between Drug Resistance and Plasmid Profiling. InApplications of Biotechnology for Sustainable Development 2017 (pp. 35-43). Springer, Singapore.
19. Al-Shaibani A, Naidoo D, Pan K, Tran M. Construction of pANPT, a novel pBR322-derived plasmid with deletion of the tetracycline resistance gene. Journal of Experimental Microbiology and Immunology (JEMI) Vol. 2014;18:102-7.
20.Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. Analytical biochemistry. 1995 Apr 1;226(2):235-40.