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Studies on Physiological Effects and Plasmid Stability of JM109 Host System

Praveen Kumar Vemuri[1*], Shaik Mohammad Anjum[1], Lakshmi Saranya Medida[1], P. V. Hemanth Sai[1], Mahathi Gummavajjala[1], Braja Kishori Panigrahi[1], Swati Dash[2] and Swati Senapati[3]

[1]Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Andhra Pradesh, India.
[2]Department of Genetic Engineering, SRM Institute of Science and Technology, Tamil Nadu, India.
[3]Department of Biotechnology, SRM Institute of Science and Technology, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors PKV and SMA taken responsibility in the conception and design of the study. Authors LSM and PVHS contributed substantially in development of methods and its optimization. Authors MG and BKP have provided critical revision of the article for important intellectual content. Author SD has checked the references. Author SS have given final approval of the version to be published. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The purpose of the study delineates the growth and plasmid stability of *E. coli* JM109 host system.
Methods: 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.
Results: Among chemicals, acridine orange showed highest effect on growth of DH5a, while among the drugs, dantron 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.

**Corresponding author: E-mail: vemuripraveen@gmail.com;*

Conclusion: 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.

Keywords: JM109; dantron; acridine orange; radio frequency; microwave.

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

2. MATERIALS AND METHODS

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

2.1 Preparation of Bacterial Culture

Stock culture of *E coli* JM109 was sub-cultured on LB agar at 37°C for 24 h [13]. A total of 45 sterile falcon tubes were taken and grouped into three categories, every tube had a cell density of $2.25*10^7$ cells/ml, inoculated a flask containing 250 ml of sterile culture medium and labelled with varying concentrations from 1 mg to 5 mg of chemicals (Sodium acetate, Benzene, Acridine orange) [14-15] from 1 mg to 5 mg, drugs (Tacrolimus, Sodium bisulphate, Dantron) [16], radiation (Radio waves - 0.5 to 2, Microwave - medium, medium-low, low, UV rays – 212 nm, 253 nm, 365 nm) [17], inoculated aseptically, incubated for overnight at 37°C for 120rpm to obtain a concentration of 1.5x 10 cells/ml.

2.2 Bacterial Plasmid DNA Isolation

Cell pellet was harvested by centrifugation at 6,000 rpm for 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].

3. RESULTS AND DISCUSION

3.1 Bacterial Plasmid DNA Isolation

Plasmid DNA was isolated from 0.5-5 mL of overnight *E. coli* culture grown in LB or rich growth medium (OD_{600} = 3-5). Plasmid DNA was isolated usually by alkaline-lysis method, quantity and quality of isolated DNA was evaluated spectrophotometrically and by agarose gel electrophoresis, respectively. The isolated plasmid DNA has shown an A_{260}/A_{280} ratio of 1.8±0.2, indicating relative purity. 10 ul of the plasmid DNA is used for the detection on ethidium bromide stained agarose gels.

In addition, we were carried out further studies to increase the yield of the plasmid concentration. Varying proportions of chemicals and drugs with concentrations from 1 mg/ml to 5 mg/ml were used. Maximum yield was observed for cells

treated with sodium acetate at 5 mg/ml, tacrolimus at 3 mg/ml. Cells were also exposed to various radiations and yield was observed in significant increase over use of chemicals and drugs. Maximum yield is observed with microwave radiation with medium-low frequency at 9 seconds, 1 GHz radio wave frequency for 15 minutes and UV-A radiation for 3 minutes were kept respectively.

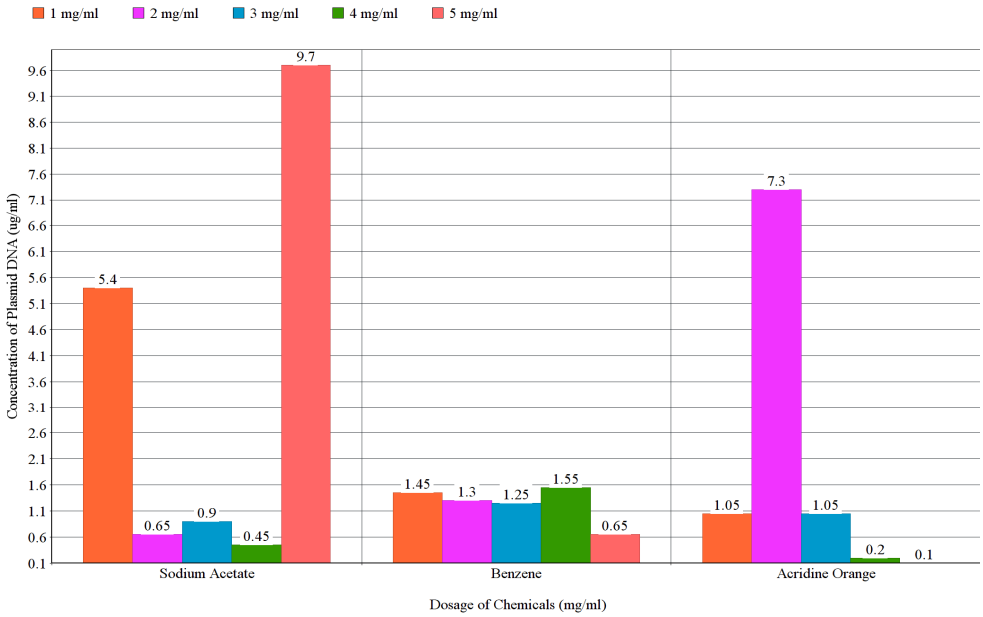

Fig. 1. Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying concentration of chemicals. High quantity of plasmid is observed when treated the cells with sodium acetate of 5 mg/ml concentration

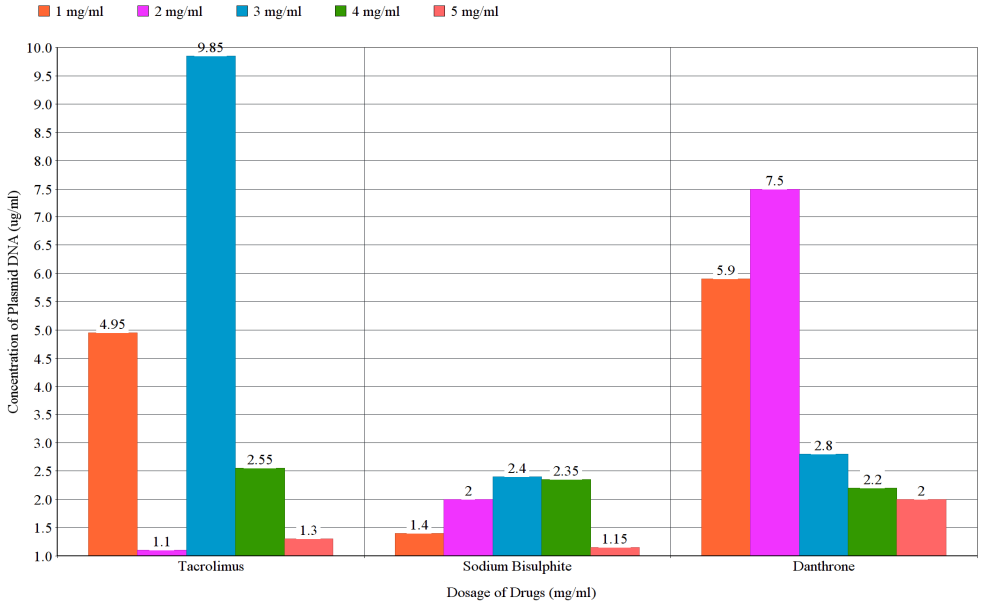

Fig. 2. Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying concentration of drugs. High quantity of plasmid is observed when treated the cells with tacrolimus of 3 mg/ml concentration

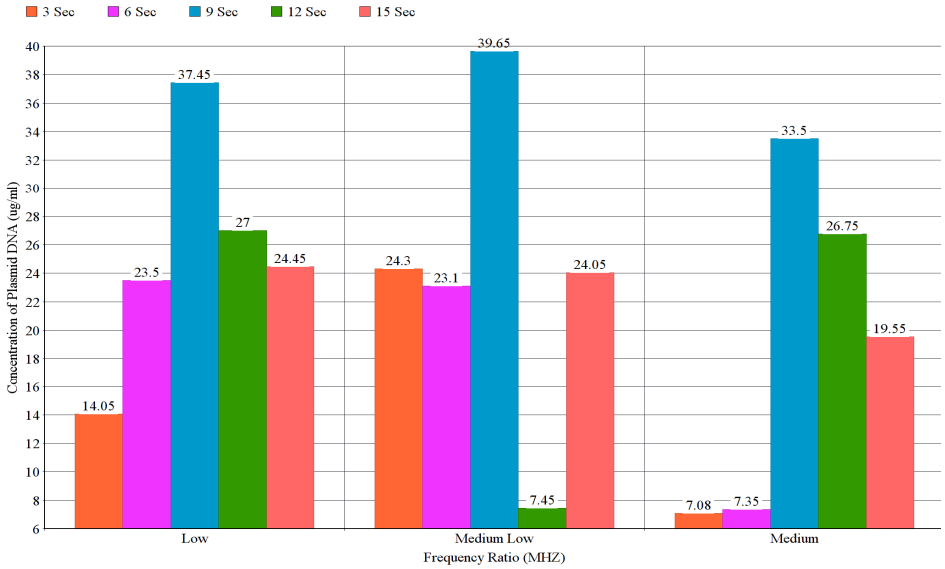

Fig. 3. Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying exposure of microwave radiations. High quantity of plasmid is observed when exposed the cells with microwave radiation of medium-low frequency for 9 seconds

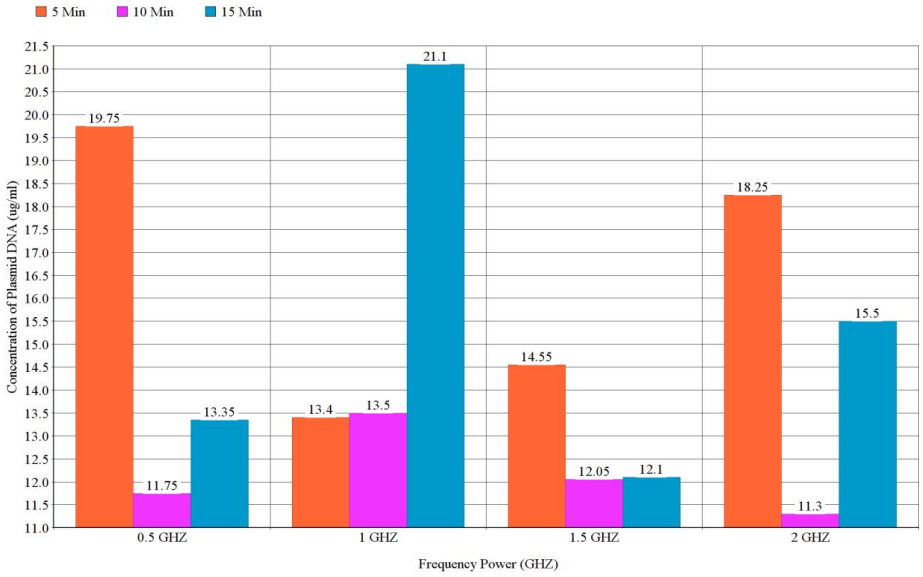

Fig. 4. Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying exposure of radio wave frequency. High quantity of plasmid is observed when exposed the cells with radio wave frequency of 1GHz for 15 minutes

3.2 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 1 kb ladder DNA for reference and checked for the purity. The concentration of the plasmid DNA obtained was 39.65 µg/ml.

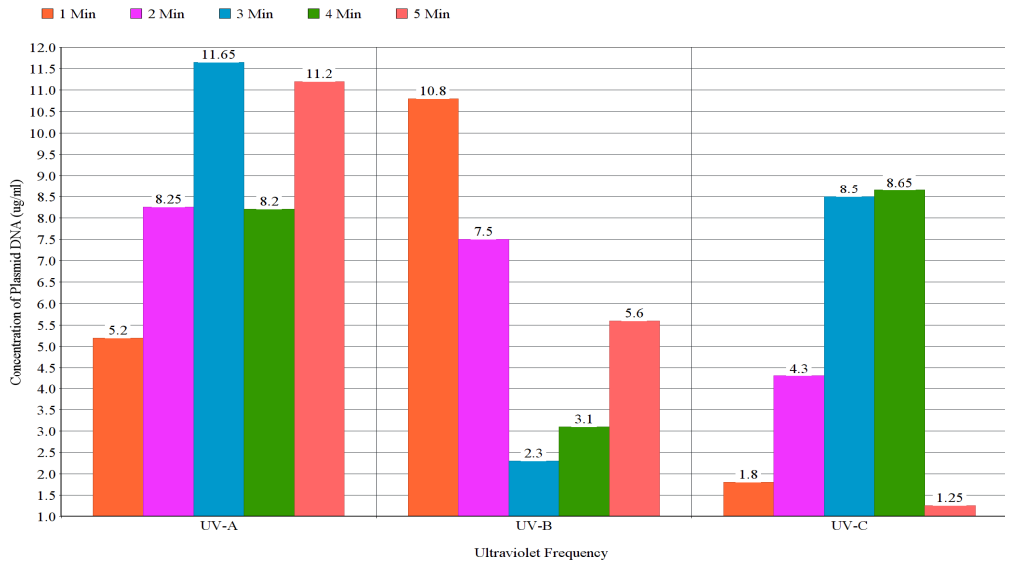

Fig. 5. Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying exposure of UV radiations. High quantity of plasmid is observed when exposed the cells with UV-A frequency for 5 minutes

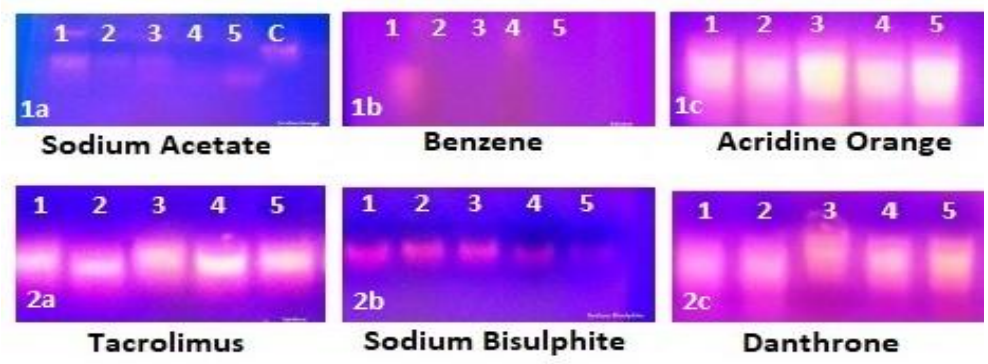

Fig. 6. High-copy number plasmid DNA was isolated from overnight bacterial culture 1a) treated with sodium acetate (1-5 mg/ml) 1b) treated with benzene (1-5 mg/ml) 1c) treated with acridine orange (1-5 mg/ml) 2a) treated with tacrolimus (1-5 mg/ml) 2b) treated with sodium bisulphite (1-5 mg/ml) 2c) treated with dantron (1-5mg/ml) and purified plasmid DNA was analyzed by agarose (1%) electrophoresis

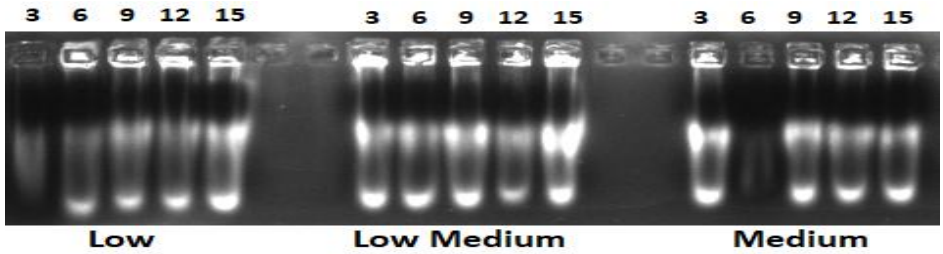

Fig. 7. High-copy number plasmid DNA was isolated from overnight bacterial culture exposed at low, low-medium, medium microwave radiation ranging from 3 to 15 seconds and purified plasmid DNA was analysed by agarose (1%) electrophoresis

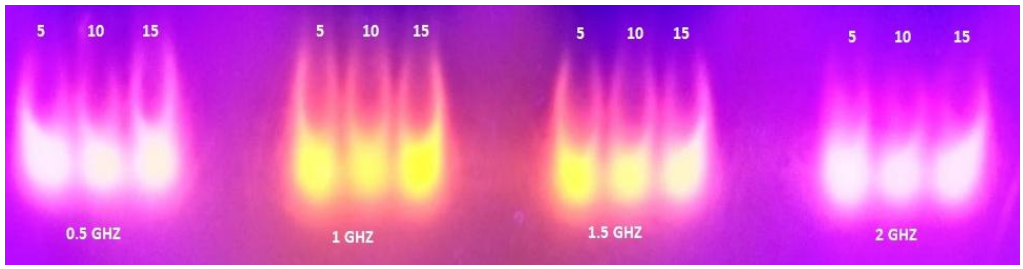

Fig. 8. High-copy number plasmid DNA was isolated from overnight bacterial culture exposed with radio wave radiation ranging from 5 to 15 minutes and purified plasmid DNA was analysed by agarose (1%) electrophoresis

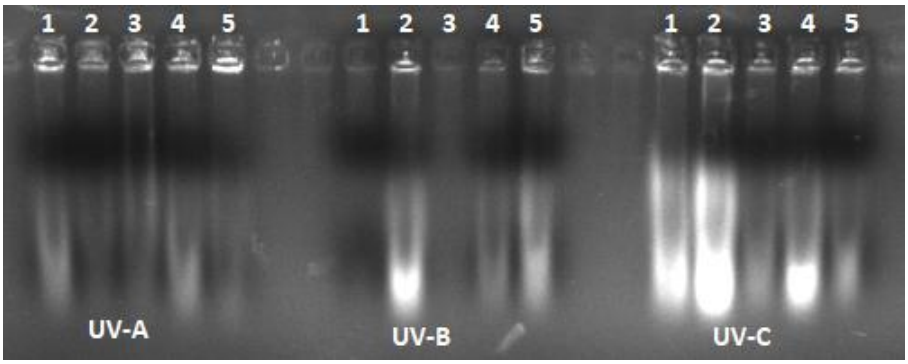

Fig. 9. High-copy number plasmid DNA was isolated from overnight bacterial culture exposed with ultraviolet radiation ranging from 1 to 5 minutes and purified plasmid DNA was analysed by agarose (1%) electrophoresis

4. CONCLUSION

Microorganisms have played an important role in human society, especially in the preparation of foods, beverages, and also in the treatment of infectious diseases. Here we focused on plasmid stability in at molecular and cellular engineering levels using various drugs, chemicals and radiation. With improved understanding, plasmid stability may be enhanced by manipulating plasmid composition and structure, modifying genetic and physiological properties of host system.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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