ORIGINAL ARTICLE

Effect of Plasmid Presence and Stability on Growth of *Escherichia coli* DH5α with Use of Drugs, Chemicals, and Radiation

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Abstract

Objective: The purpose of the study delineates the growth and plasmid stability of *E. coli* DH5α host system. **Materials and 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 the highest effect on growth of DH5α, while among the drugs, danthrone showed maximum effect on the growth of the organism. Radiofrequency of 2 GHz and low-intensity microwave radiation were recorded as the highest inhibitory effects. However, there is no significant effect in growth observed in exposure to UV rays. **Conclusion:** The present work discussed that drugs, chemicals, radiofrequency, and microwave radiation have a huge effect on the growth of organism and also on the concentration and stability of plasmid.

Key words: DH5-alpha, Danthrone, Acridine orange, Radiofrequency, Microwave

INTRODUCTION

Multidrug treatments, exposure to radiations, and effect of chemicals are increasingly important in medicine and for probing microbial systems.[1-4] Enterohemorrhagic *E. coli* produces cytotoxins or Shiga-like toxin which is responsible for hemorrhagic colitis.[5] Studies suggest that the effect of radiation on microbes varies from one organism to another, as follows: *Klebsiella pneumonia*[6] greater than *E. coli* greater than *Salmonella typhimurium*, *Streptococcus faecium*, *Enterobacter aerogenes*, and *Erwinia herbicola*.[7] For over a decade, geneticists have been interested in the prospect of inducing mutations with chemicals,[8] particularly, it has been hoped that mutagenic compounds might be discovered, through their specificity of action which would lead to some understanding of the chemical basis of mutation and ultimately of the structure[9] and organization of the gene.[10] Bacterial cells transferred to rich medium without toxic chemicals, growth can be resumed and plasmid bands may be detectable.[11] In general, bacteria express one or at most two homologous from each of the families,[12] while eukaryotic cells have multiple homologous localized in different intracellular compartments and regulated in response to different signals.[13] Homologues within each family share a high degree of sequence homology, with almost 50% amino acid identity between corresponding bacterial and mammalian proteins.[14] Plasmids are foundational tools for biotechnology, an understanding of the basic biology of plasmids is required for improved applications.[15] In this study, the concentration and stability of plasmid were improved using chemicals, drugs, and exposure to various radiations.

MATERIALS AND METHODS

All molecular biology kits were procured from HiMedia Laboratories, Mumbai or Puregene, UK. *E. coli* DH5α (MTCC:483) was procured from MTCC, Chandigarh. All solvents and reagents were of analytical grade, and all experiments were performed with deionized water.

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Preparation of bacterial culture

Stock culture of *E coli* DH5α was sub-cultured on LB agar at 37°C for 24 h.[16] 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 250 ml of sterile culture medium and labeled with varying concentrations from 1 mg to 5 mg of chemicals (sodium acetate, benzene, and acridine orange)[17-19] and from 1 mg to 5 mg of drugs (tacrolimus, sodium bisulfate, and danthrone)[20-22] and radiation[23-25] (radio waves – 0.5 GHz–2 GHz; microwave – medium, medium-low, and low; and UV rays – 212 nm, 253 nm, and 365 nm), inoculated aseptically, incubated for overnight at 37°C for 120 rpm to obtain a concentration of 1.5×10 cells/ml.

Bacterial colony-forming units

The main culture (500 mL LB medium) was inoculated with preculture, approximately generating a start OD_{600} of 0.1.

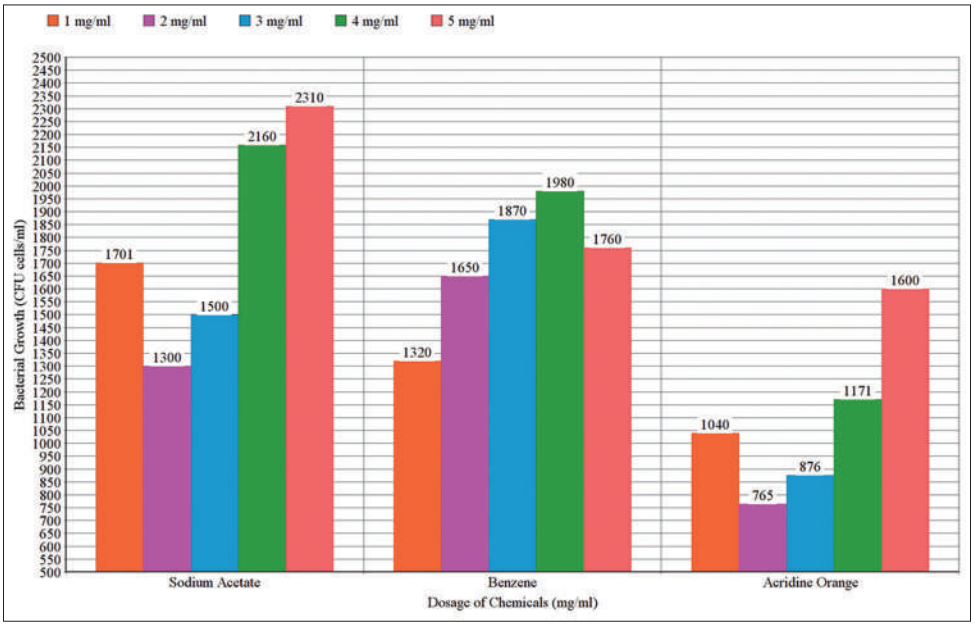

Figure 1: Viable cell count of DH5α treated with sodium acetate, benzene, and acridine orange (1–5 mg/ml). Sodium acetate has major influence on the increase in growth of bacterial cells, while acridine orange has a high influence in limiting the growth of bacterial cells

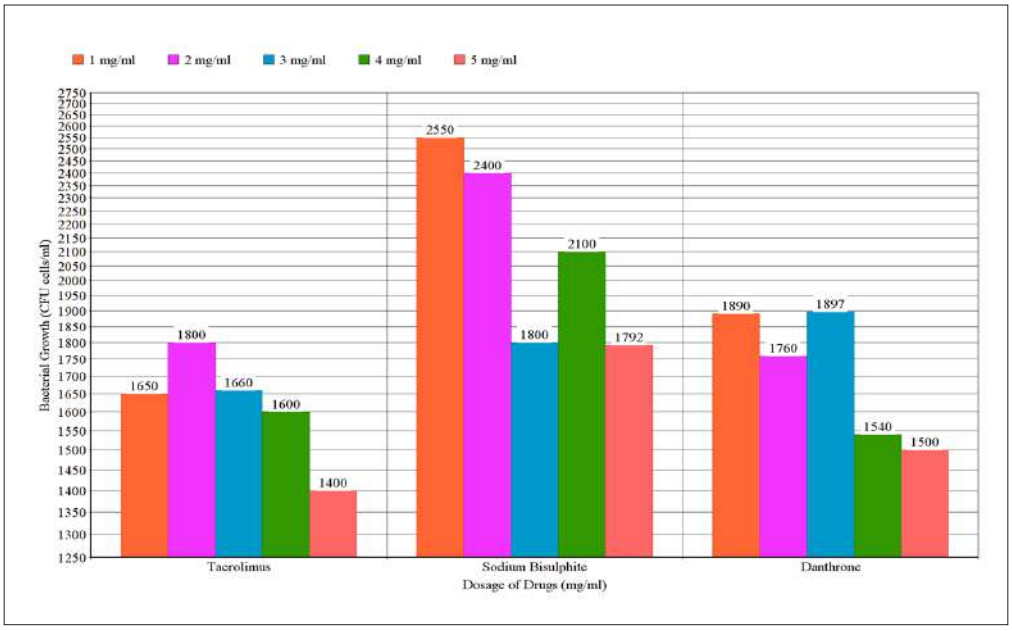

Figure 2: Viable cell count of DH5α treated with tacrolimus, sodium bisulfite, and danthrone (1–5 mg/ml). High viable cell count was observed for tacrolimus at 2 mg/ml, sodium bisulfite at 1 mg/ml, and danthrone at 1 mg/ml, respectively, while tacrolimus at 5 mg/ml has a high influence in limiting the growth of bacterial cells

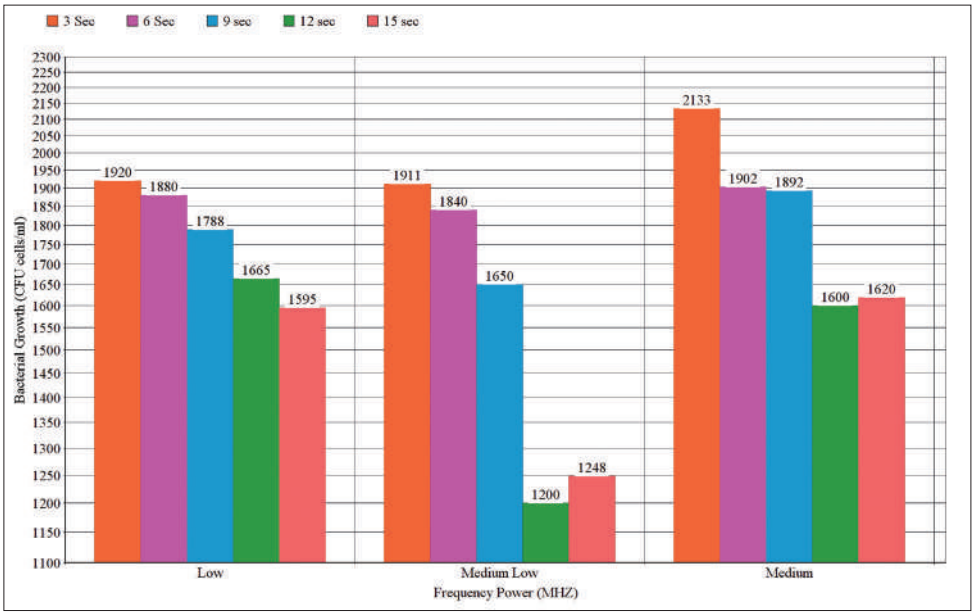

Figure 3: Viable cell count of DH5α exposed with microwave radiation at frequency of low, medium-low, and medium at 3, 6, 9, 12, and 15 s. High viable cell count was observed for low frequency at 3 s, medium-low at 3 s, and medium at 1 s, respectively

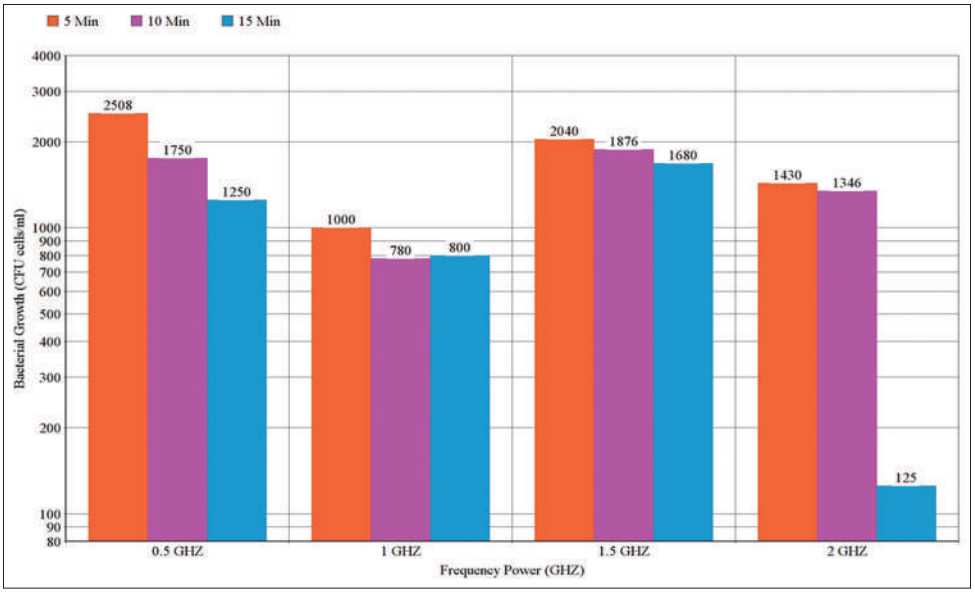

Figure 4: Viable cell count of DH5α exposed with 0.5, 1, 1.5, and 2 GHz radio wave frequency for 5, 10, and 15 min. High viable cell count was observed for 0.5 GHz, 1 GHz, 1.5 GHz, and 2 GHz at 5 min

After growing at 37°C for several hours to a final OD_{600} of 1, the culture was transferred into a sterile, precooled centrifuge tube, and put on ice bath for 10 min. Aliquots of 100 µL of bacteria cultures (10^6 cells/mL) grown in 10 mL of LB broth for 6 h and were spread over LB agar plates supplemented with the respective drugs, chemicals, and radiation. After overnight period, the growth of each sample was documented and compared to those of wild organism to verify any synergistic effect among the mutant organism. Bacterial colony-forming units of each plated were enumerated by manual counting and/or by automated plate counter.

Bacterial plasmid DNA isolation

Cell pellet was harvested by centrifugation at 6000 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 or by alkaline lysis method.[26] Plasmids were eluted in 1 mL 1 mM Tris/HCl pH 8 or sterile ddH_2O and plasmid concentration was measured (NanoDrop 2000, Thermo Scientific) or determined by comparing the DNA concentration of 1 µL linearized plasmid with 5 µL DNA Marker (Puregene).

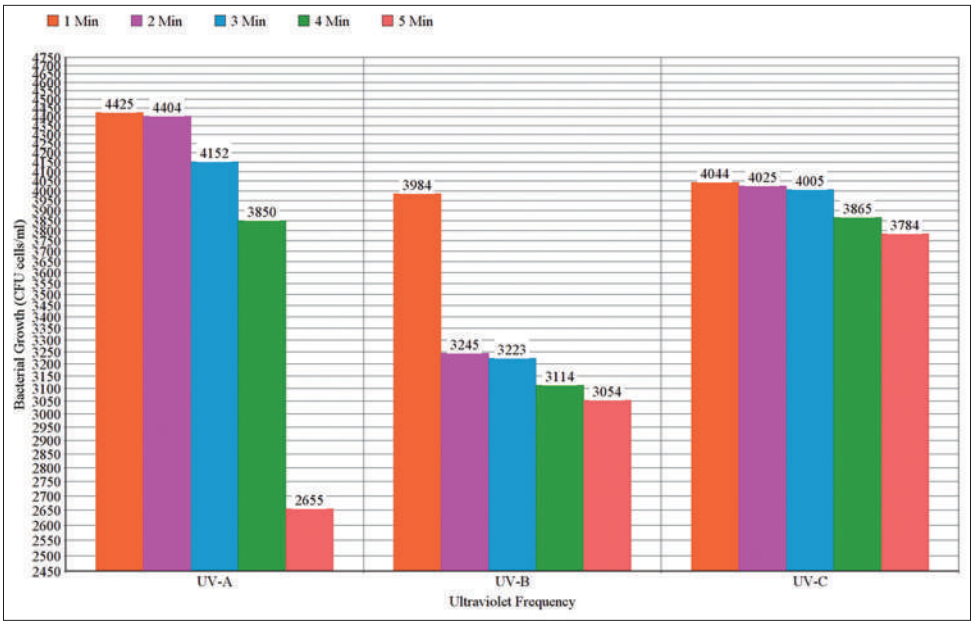

Figure 5: Viable cell count of DH5α exposed with ultraviolet range of A, B, and C for 1, 2, 3, 4, and 5 min. High viable cell count was observed for UV-A, UV-B, and UV-C at 1 min

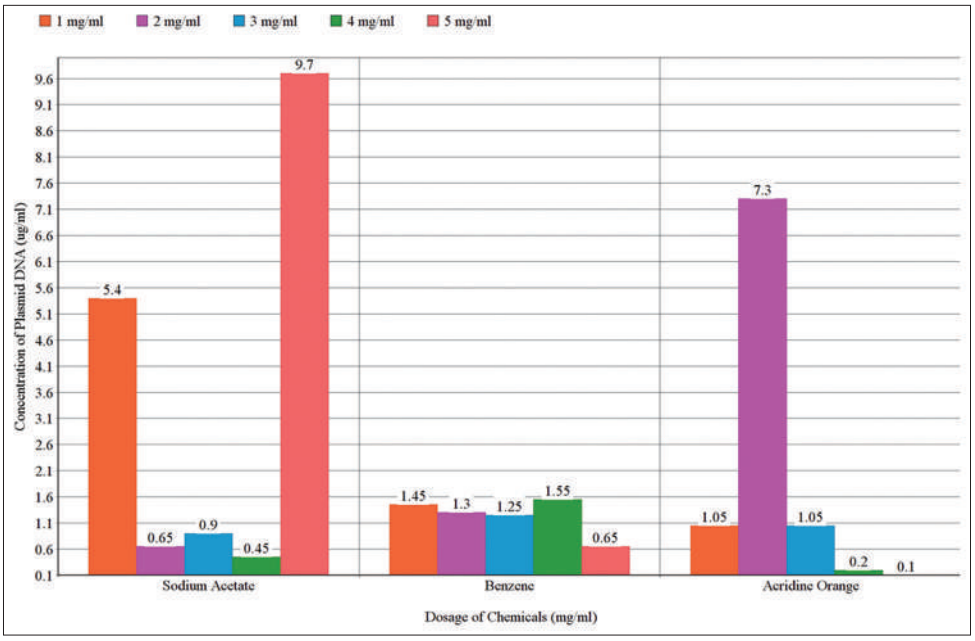

Figure 6: Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying concentration of chemicals. High quantity of plasmid is observed when treated the cells with sodium acetate of 5 mg/ml concentration

RESULTS AND DISCUSSION

Determination of colony-forming units from *in vitro* cultures

Viable cell counts of cultures were determined by plating 100 µl of 10^6 dilution of the appropriate culture grown in LB broth on LB agar plates and counting the colonies after aerobic incubation at 37°C for overnight period. Bacterial culture was exposed with varying concentrations of chemicals, drugs, and exposed to several rays. High viable cell count was observed for sodium acetate at 5 mg/ml, benzene at 4 mg/ml, and acridine orange at 5 mg/ml, respectively.

To verify the influence of bacterial cell count, chemicals and drugs with a variable concentration from 1 mg/ml to 5 mg/ml were used. High count was observed for cells treated with sodium acetate at 5 mg/ml as shown in Figure 1 and sodium bisulfite at 1 mg/ml as shown in Figure 2. Cells were also exposed to various radiations and yield was observed in significant increase over the use of chemicals and drugs.

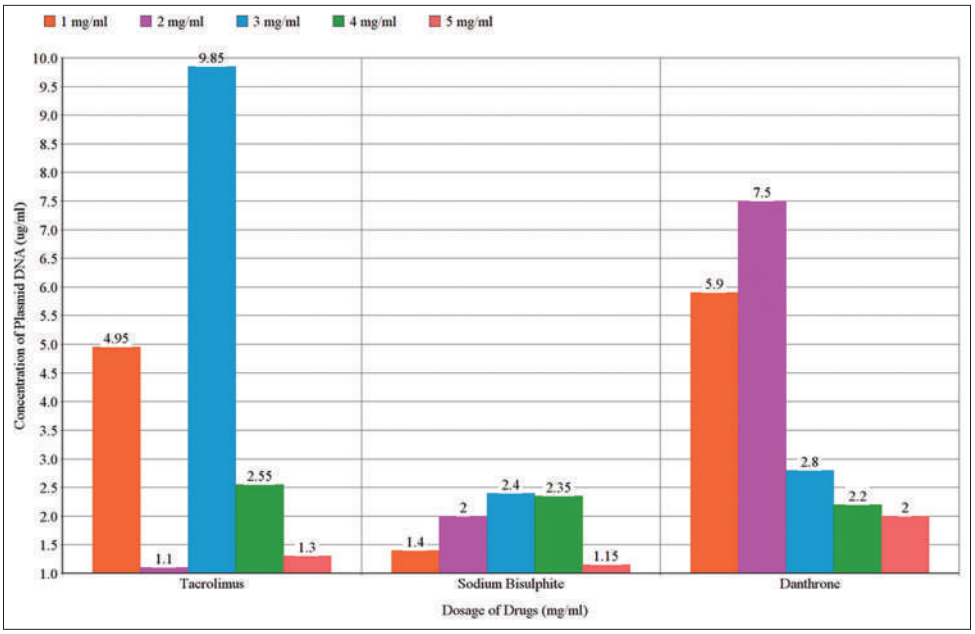

Figure 7: Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying concentration of drugs. High quantity of plasmid is observed when treated the cells with tacrolimus of 3 mg/ml concentration

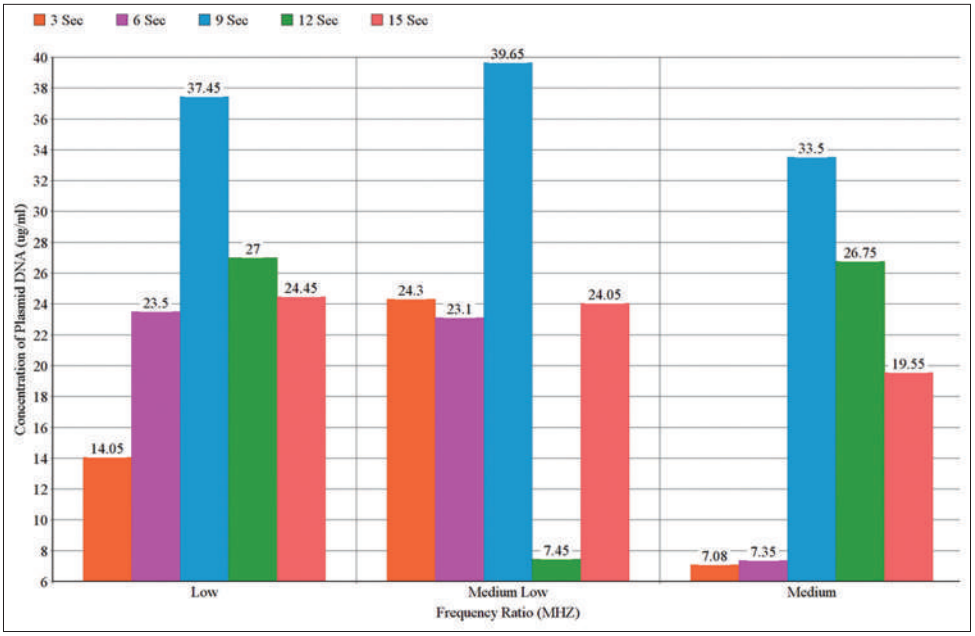

Figure 8: Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying exposure of microwave radiations. High quantity of plasmid is observed when exposed the cells with microwave radiation of low frequency for 9 s

Maximum yield is observed with microwave radiation with medium frequency at 3 s, as shown in Figure 3 0.5 GHz radio wave frequency for 5 min, as shown in Figure 4 and UV-A radiation for 1 min, as shown in Figure 5 respectively.

Bacterial plasmid DNA isolation

Plasmid DNA was isolated from 0.5 to 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. About 10 ul of the plasmid DNA is used for the detection on ethidium bromide-stained agarose gels.

Further studies were carried out 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

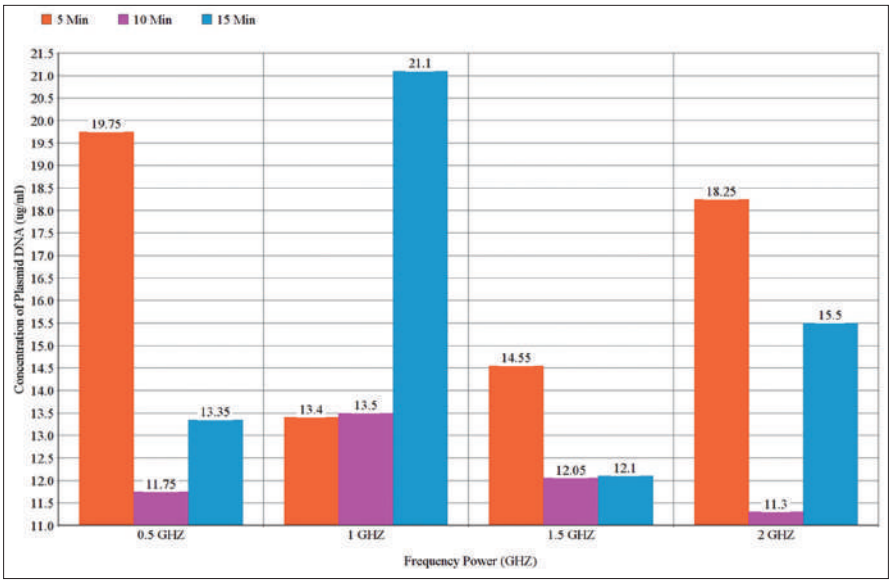

Figure 9: Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying exposure of radio wave frequency. High quantity of plasmid is observed when exposed the cells with radio wave frequency of 1 GHz for 15 min

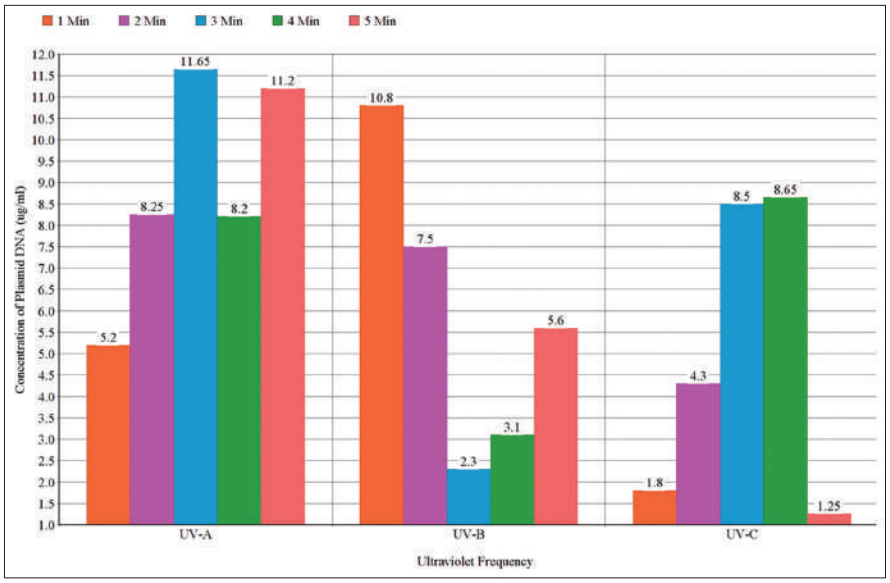

Figure 10: Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying exposure of UV radiations. High quantity of plasmid is observed when exposed the cells with UV-A frequency for 15 min

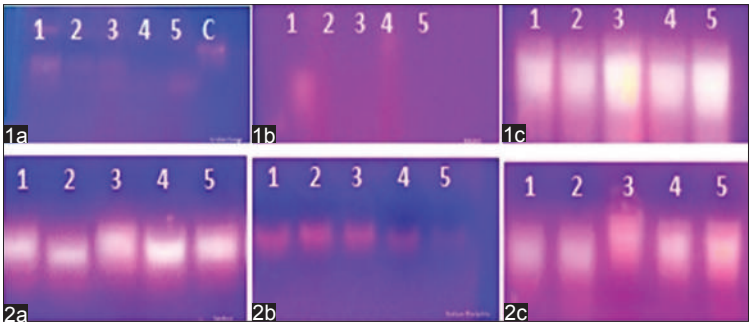

Figure 11: High copy number plasmid DNA was isolated from overnight bacterial culture (1a) treated with sodium acetate (1–5 mg/ml); (1b) treated with benzene (1–5 mg/ml); (1c) treated with acridine orange (1–5 mg/ml); (2a) treated with tacrolimus (1–5 mg/ml); (2b) treated with sodium bisulfite (1–5 mg/ml); (2c) treated with danthrone (1–5 mg/ml) and purified plasmid DNA was analyzed by agarose (1%) electrophoresis

with sodium acetate at 5 mg/ml as shown in Figure 6 and tacrolimus at 3 mg/ml as shown in Figure 7. Cells were also exposed to various radiations and yield was observed in significant increase over the use of chemicals and drugs. Maximum yield is observed with microwave radiation with medium-low frequency at 9 s, as shown in Figure 8 1 GHz radio wave frequency for 15 min as shown in Figure 9 and UV-A radiation for 3 min as shown in Figure 10 were kept, respectively.

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 the use of 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 as shown in Figures 11-14 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.

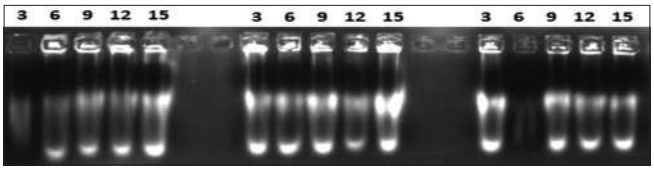

Figure 12: High copy number plasmid DNA was isolated from overnight bacterial culture exposed at low, low-medium, and medium microwave radiation ranging from 3 to 15 s and purified plasmid DNA was analyzed by agarose (1%) electrophoresis

CONCLUSION

Comparative studies between wild and mutant *E. coli* strains have further elucidated for the viability and stability of host system and its plasmid. However, further studies such as 16s rRNA sequencing and knock out technology have to be done to verify the stability of plasmid in the host system. Our study proved that mutant host system has maximum yield of plasmids than wild system which can retain stability over a multitude of generations both *in vitro* and *in vivo* without antibiotic selection. With these plasmids, studies requiring genetic complementation, protein expression, or genetic reporter systems would not only overcome the burden of antibiotic usage but also eliminate the side effects of these antibiotics. Thus, our approach in generating plasmid recovery can be used as

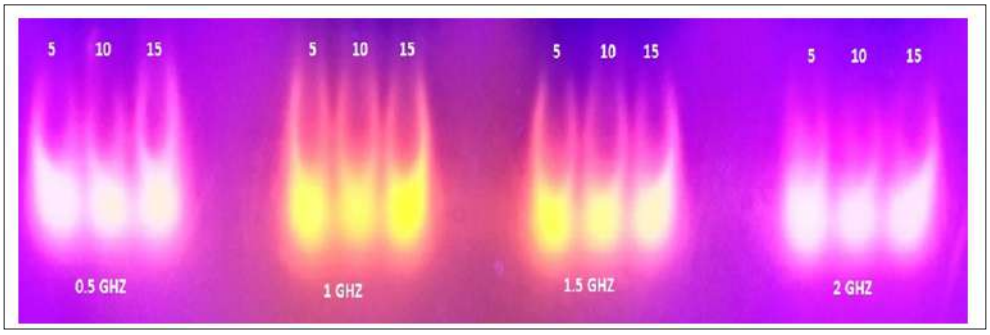

Figure 13: High copy number plasmid DNA was isolated from overnight bacterial culture exposed with radio wave radiation ranging from 5 to 15 min and purified plasmid DNA was analyzed by agarose (1%) electrophoresis

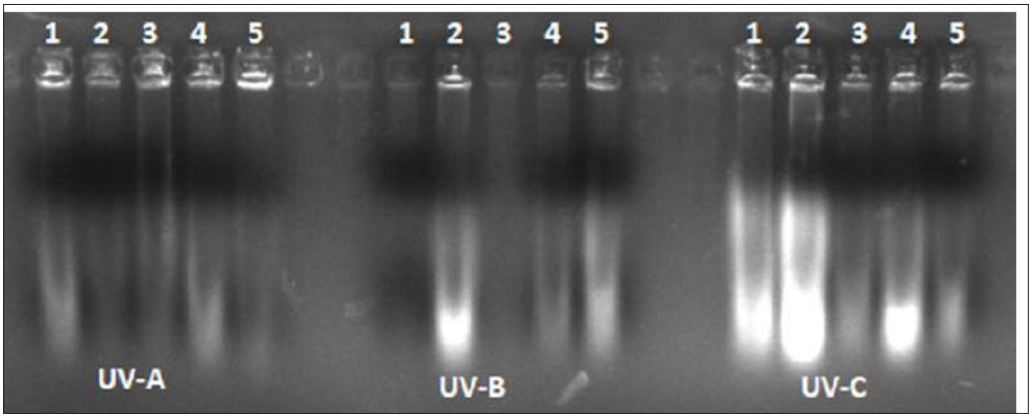

Figure 14: High copy number plasmid DNA was isolated from overnight bacterial culture exposed with ultraviolet radiation ranging from 1 to 5 min and purified plasmid DNA was analyzed by agarose (1%) electrophoresis

a powerful genetic tool for obtaining maximum yield and stability of plasmids.

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AUTHORS' CONTRIBUTIONS

Praveen Kumar Vemuri designed the study, took lead in writing the manuscript, and provided final approval of the version to publish. Tegegnework Mekonnen Gizaw carried out the experiments, contributed to the interpretation of the results, drafted, and provided critical revision of the manuscript.

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