

# Improving Physiological Factors and Plasmid Stability of DE3 Host System

Maheswara Reddy Mallu<sup>1\*</sup>, Rajeswara Reddy Erva<sup>2</sup>, Anupama Ammulu Manne<sup>3</sup>,  
D. S. N. B. K. Prasanth<sup>4</sup>, T. C. Venkateswarlu<sup>5</sup>, Supriya Chadalavada<sup>1</sup>,  
Arun Kumar Kurpati<sup>6</sup>, Nikhitha Chava<sup>1</sup>

<sup>1</sup>Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Guntur, Andhra Pradesh, India,

<sup>2</sup>Department of Biotechnology, National Institute of Technology, Tadepalligudem, Andhra Pradesh, India,

<sup>3</sup>Department of Civil Engineering, PVP Siddhartha Institute of Technology, Vijayawada, Andhra Pradesh,

India, <sup>4</sup>Department of Pharmacognosy, KVSRR Siddhartha College of Pharmaceutical Sciences, Vijayawada,

Andhra Pradesh, India, <sup>5</sup>Department of Biotechnology, Vignans Foundation for Science, Technology and

Research, Guntur, Andhra Pradesh, India, <sup>6</sup>Department of Biotechnology, Osmania University, Hyderabad,

Telangana, India

## Abstract

**Objective:** The purpose of the study delineates the growth and plasmid stability of DE3 host system. **Methods:** Different concentrations of drugs, chemicals, and various frequencies 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, danthron 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 that was observed in exposure to UV rays. **Conclusion:** The present work discussed that, the effect of drugs, chemicals, radio frequency, and microwave radiation has a huge effect not only on growth of organism but also concentration and stability of plasmid.

**Key words:** DE3, Drugs, Microwave, Plasmid, Radio frequency, Stability

## INTRODUCTION

Plasmid instability frequently hinders the use of recombinant organisms for the large-scale, commercial synthesis of foreign proteins.<sup>[1]</sup> Plasmid DNA is commonly used as a simpler substitute for a cell in studies of early effects of ionizing radiation, because it allows to determine yields of primary DNA lesions.<sup>[2]</sup> Numerous methods for enhancing the stability of recombinant organisms have been discovered.<sup>[3]</sup> Stable host-vector connections can be attained by adjusting the genetic makeup of recombinants as well as the environment under which the organisms are cultured.<sup>[4]</sup> Plasmids have an essential impact on productivity; related factors are plasmid copy number, structural plasmid stability, and segregational plasmid stability.<sup>[5-8]</sup> Plasmid copy number determines the gene dosage accessible for expression and many plasmids lead generally to a high productivity.<sup>[9]</sup> To analyze an expression system, the quantification of plasmid copy number is very helpful for its expression level

in any host system.<sup>[10]</sup> The previous studies on strains of *Escherichia coli* containing aggregates of plasmids were shown to be stable over 5–6 months in continuous cultivation under carbon limited conditions at a growth rate of 0.1 h<sup>-1</sup> in the presence of drugs which select for the maintenance of both plasmids.<sup>[11]</sup> Almost all kinds of antibiotics and a large range of Gram-positive and Gram-negative organisms have shown plasmid-encoded resistance; as a result, many antibiotics are no longer effective.<sup>[12]</sup> Bacteria would become re-sensitive to common antibiotics if these resistance-mediating plasmids were removed from them in a systematic manner.<sup>[13]</sup> Plasmids, thus, present unique targets that have not yet been used clinically.<sup>[14]</sup>

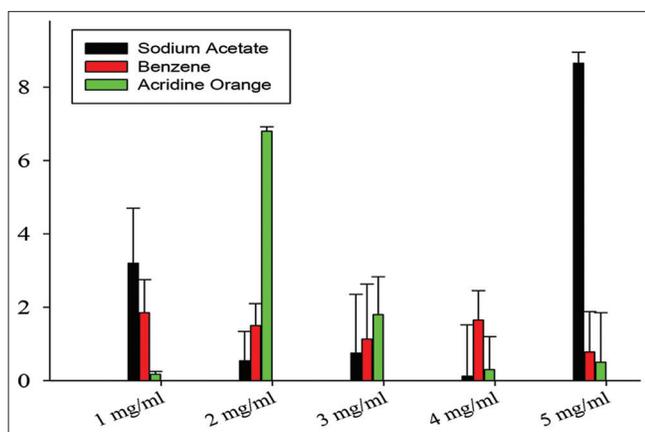
### Address for correspondence:

Maheswara Reddy Mallu, Department of Biotechnology,  
Koneru Lakshmaiah Education Foundation,  
Vaddeswaram, Guntur District, Andhra Pradesh, India.  
E-mail: mahesh\_bt@kluniversity.in

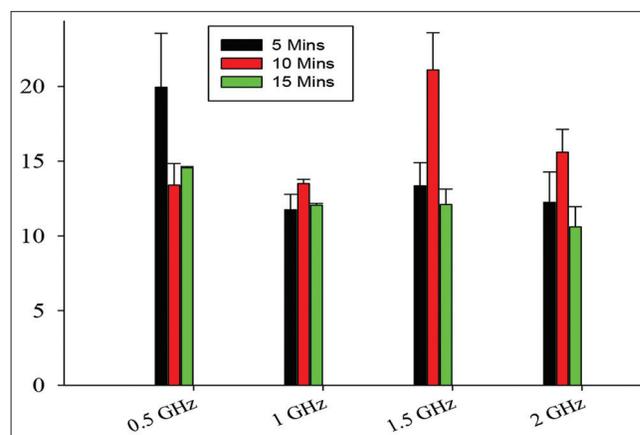
**Received:** 22-08-2022

**Revised:** 12-10-2022

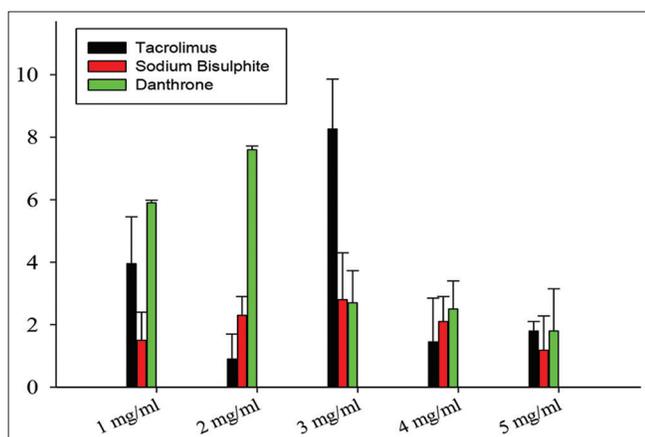
**Accepted:** 21-10-2022



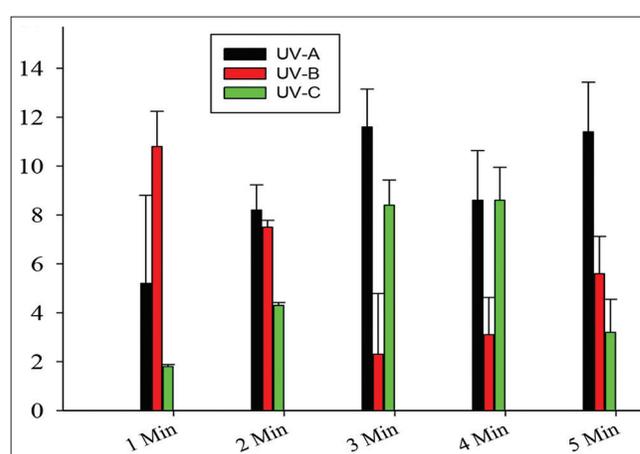
**Figure 1:** Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying concentration of chemicals. High quantity of plasmid is observed when treated the cells with sodium acetate of 5 mg/ml concentration



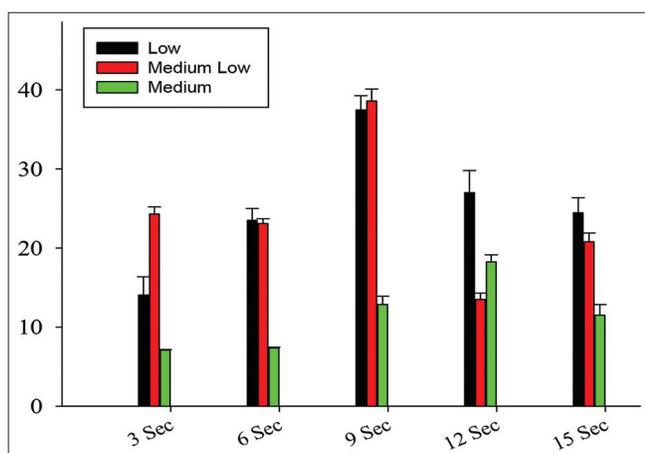
**Figure 4:** Bar graph showing quantity of plasmid DNA isolated from bacterial cells treated with varying exposure of radio wave frequency. High quantity of plasmid is observed when exposed the cells with radio wave frequency of 1 ghz for 15 min



**Figure 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



**Figure 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 15 min



**Figure 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 low frequency for 9 s

## MATERIALS AND METHODS

All molecular biology kits were procured from Thermo Fischer, India. *E. coli* DE3 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* DE3 was sub-cultured on LB agar at 37°C for 24 h. A total of 45 sterile falcon tubes were taken and grouped into three categories, every five tubes were added having density of  $2.25 \times 10^7$  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) from 1 mg to 5 mg, drugs (Tacrolimus, Sodium bisulphate, and Danthron), radiation (Radio waves – 0.5–2 ghz, Microwave-medium,

medium-low, low, UV rays-212 nm, 253 nm, and 365nm), inoculated aseptically, and incubated for overnight at 37°C for 120 rpm to obtain a concentration of  $1.5 \times 10^8$  cells/ml.<sup>[15]</sup>

### 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 Plasmid DNA Miniprep purification (Genei), as per the manufacturer's instructions.<sup>[16]</sup> Plasmids were eluted in 1 mL 1 mM Tris/HCl pH 8 or sterile ddH<sub>2</sub>O and plasmid concentration was measured (NanoDrop 2000, Thermo Scientific) or determined by comparing the DNA concentration of 1  $\mu$ L linearized plasmid with 5  $\mu$ L DNA Marker (Genei, Bangalore).

## RESULTS AND DISCUSION

### Bacterial plasmid DNA isolation

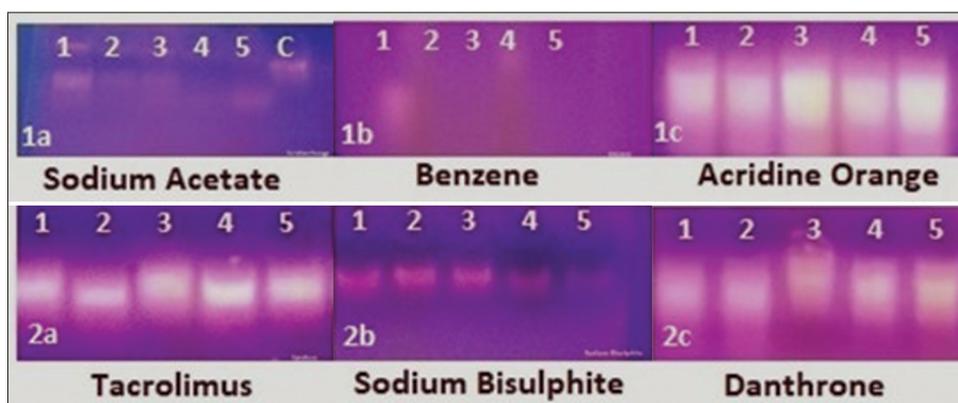
Plasmid DNA was isolated from 0.5 to 5 mL of overnight *E. coli* culture grown in LB or rich growth medium

(OD<sub>600</sub> = 3–5). Plasmid DNA was isolated usually by alkaline-lysis method, quantity and quality of isolated DNA were evaluated spectrophotometrically and by agarose gel electrophoresis, respectively. The isolated plasmid DNA has shown an A<sub>260</sub>/A<sub>280</sub> ratio of  $1.8 \pm 0.2$ , indicating relative purity. 10  $\mu$ L of the plasmid DNA is used for the detection on ethidium bromide stained agarose gels.

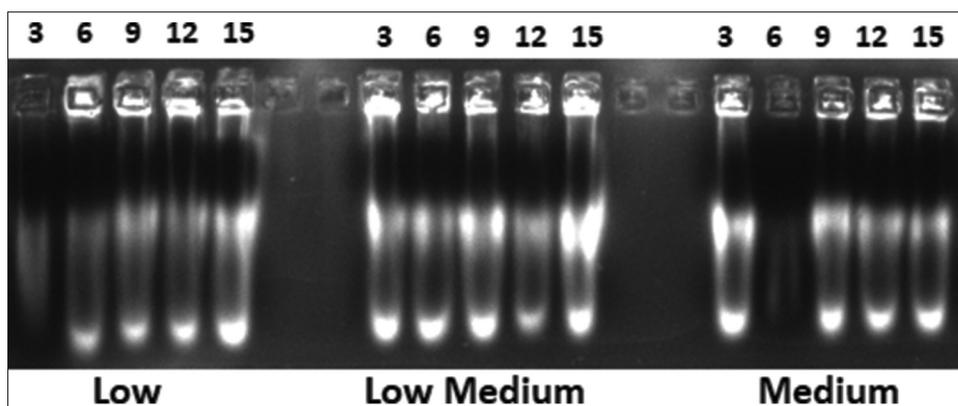
In addition, we were carried out further studies to increase the yield of the plasmid concentration. Varying proportions of chemicals and drugs with concentrations from 1mg/ml to 5mg/ml were used. Maximum yield was observed for cells treated with sodium acetate at 5 mg/ml and [Figure 1] tacrolimus at 3 mg/ml [Figure 2]. 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 s, [Figure 3] 1GHZ radio wave frequency for 15 min [Figure 4], and UV-A radiation for 3 min [Figure 5] were kept, respectively.

### Plasmid stability studies

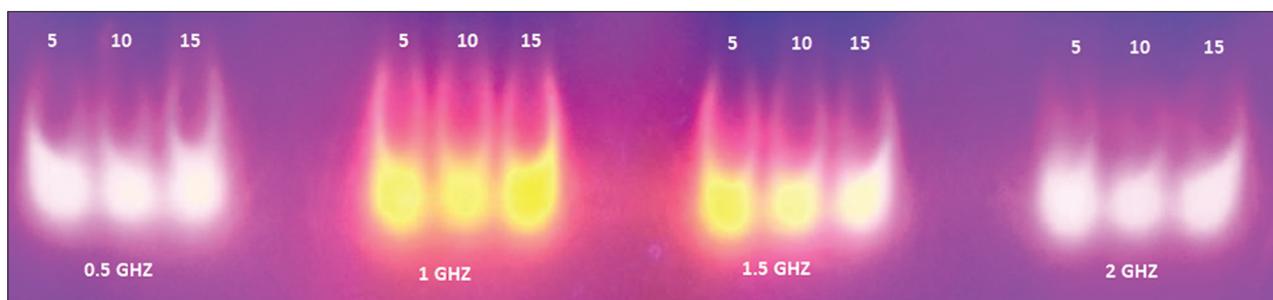
Plasmid stability has been problematic in bacterial studies, and historically antibiotics have been used to ensure plasmid



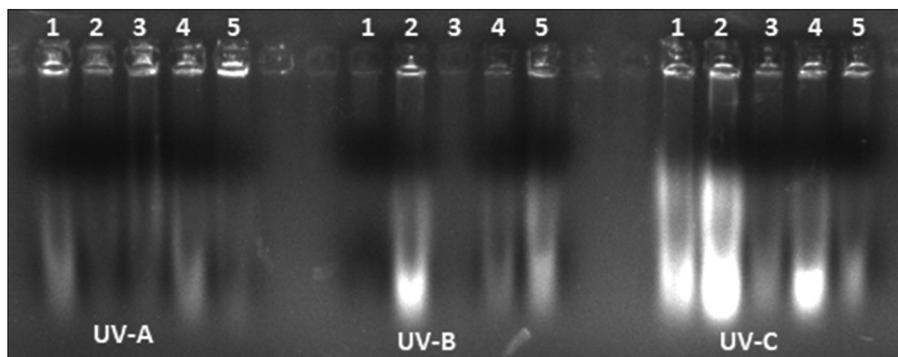
**Figure 6:** High copy number plasmid DNA was isolated from overnight bacterial culture (1a) treated with sodium acetate (1–5 mg/ml) (1b) treated with benzene (1–5 mg/ml) (1c) treated with acridine orange (1–5 mg/ml) (2a) treated with tacrolimus (1–5 mg/ml) (2b) treated with sodium bisulphite (1–5 mg/ml)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



**Figure 7:** 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



**Figure 8:** 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 9:** 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

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 [Figure 6] drugs [Figure 7] and exposed with various radiations [Figures 8 and 9] 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  $\mu\text{g}/\text{ml}$ .

## CONCLUSION

Although the molecular details of plasmid constructs and biology of host cell are of interest, biochemical engineers are concerned primarily with maintaining plasmid stability with environmental manipulations including media composition and selection 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 improved understanding, plasmid stability may be enhanced by manipulating plasmid composition and structure, modifying tile genetic, and physiological properties of host.

## CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest exist among them regarding the publication of this paper.

## AUTHORS CONTRIBUTION

Maheswara Reddy Mallu and Rajeswara Reddy Erva have designed and directed through the research work; Anupama Ammulu Manne, D. S. N. B. K. Prasanth and T. C. Venkateswarlu performed the experiment part and all together has analysed the results obtained. Supriya Chadalavada, Arun Kumar Kurpati reverified the results and rectified the mistakes. Nikhitha Chava prepared references and revised the complete manuscript. All authors read and approved the final version of the manuscript.

## REFERENCES

1. Kumar PK, Maschke HE, Friehs K, Schügerl K. Strategies for improving plasmid stability in genetically modified bacteria in bioreactors. *Trends Biotechnol* 1991;9:279-84.
2. Brabcová KP, Jamborová Z, Michaelidesová A, Davídková M, Kodaira S, Šeřl M, *et al.* Radiation-induced plasmid DNA damage: Effect of concentration and length. *Radiat Prot Dosimetry* 2019;186:168-71.
3. Ma JK, Drake PM, Christou P. The production of recombinant pharmaceutical proteins in plants. *Nat Rev Genet* 2003;4:794-805.
4. Seo JH, Bailey JE. Effects of recombinant plasmid content on growth properties and cloned gene product formation in *Escherichia coli*. *Biotechnol Bioeng* 1985;27:1668-74.
5. Zhang Z, Moo-Young M, Chisti Y. Plasmid stability in recombinant *Saccharomyces cerevisiae*. *Biotechnol Adv*

- 1996;14:401-35.
6. Jana S, Deb JK. Retracted article: Strategies for efficient production of heterologous proteins in *Escherichia coli*. *Appl Microbiol Biotechnol* 2005;67:289-98.
  7. Popov M, Petrov S, Nacheva G, Ivanov I, Reichl U. Effects of a recombinant gene expression on ColE1-like plasmid segregation in *Escherichia coli*. *BMC Biotechnol* 2011;11:18.
  8. Kumar VP, Prasanthi S, Reddy AC, Raj ND, Anudeep L. Characterization studies of thermostable alkaline phosphatase from various plant seeds. *J Appl Biosci* 2010;36:2403-8.
  9. Tyo KE, Ajikumar PK, Stephanopoulos G. Stabilized gene duplication enables long-term selection-free heterologous pathway expression. *Nat Biotechnol* 2009;27:760-5.
  10. Morales VM, Bäckman A, Bagdasarian M. A series of wide-host-range low-copy-number vectors that allow direct screening for recombinants. *Gene* 1991;97:39-47.
  11. Bennett PM. Plasmid encoded antibiotic resistance: Acquisition and transfer of antibiotic resistance genes in bacteria. *Br J Pharmacol* 2008;153:S347-57.
  12. Russell AD. Bacterial resistance to disinfectants: Present knowledge and future problems. *J Hosp Infect* 1999;43:S57-68.
  13. Huang YY, Zhang XY, Zhu P, Ji L. Development of clustered regularly interspaced short palindromic repeats/CRISPR-associated technology for potential clinical applications. *World J Clin Cases* 2022;10:5934-45.
  14. Grimm D. Production methods for gene transfer vectors based on adeno-associated virus serotypes. *Methods* 2002;28:146-57.
  15. Liu B, Eydallin G, Maharjan RP, Feng L, Wang L, Ferenci T. Natural *Escherichia coli* isolates rapidly acquire genetic changes upon laboratory domestication. *Microbiology (Reading)* 2017;163:22-30.
  16. Engebrecht J, Brent R, Kaderbhai MA. Minipreps of plasmid DNA. *Curr Protoc Mol Biol* 1991;15:1-6.

**Source of Support:** Nil. **Conflicts of Interest:** None declared.