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TRANSFORMATION OF *ESCHERICHIA COLI* WITH pET21A$^+$ AND EXPRESSION OF THE NOVEL ANTIMICROBIAL PEPTIDE INDOLICIDIN

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ABSTRACT

Indolicidin is an antimicrobial cationic peptide naturally present in the bovine neutrophils. In this study, transformed *Escherichia coli* cells were employed for greater and cost effective production of indolicidin. Four strains of *E. coli* [C41 (DE3), C43 (DE3), C41 (DE3) pLysS & C43 (DE3) pLysS] were transformed with recombinant plasmid pET21A$^+$ carrying the indolicidin gene and were successfully expressed for indolicidin in the presence of the inducer isopropyl β-D-1-thiogalactopyranoside (IPTG). Further, the production of indolicidin was confirmed by detecting the 2 KDa indolicidin band on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

KEYWORDS: **Indolicidin, *E. coli*, Transformation, Antimicrobial activity and pET21A$^+$**

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INTRODUCTION

Cationic antimicrobial peptides play a key role in the host defense system in many higher organisms such as plants, insects, amphibians and mammals[1]. One such peptides, indolicidin performs a remarkable role in defense and inhibits the invading gram-negative and gram-positive bacteria[2, 3], viruses[4] and fungi[5, 6, 7] more effectively. With the development of multi drug resistance against commonly used antimicrobial drugs in the last few years amongst microbial pathogens, fatal infections and difficult to treat diseases are emerging out frequently which pose a real-time challenge to the health industries and clinicians. Therefore, search for a novel, broad spectrum antimicrobial agent like indolicidin becomes an utmost priority and an urgent need. Indolicidin being a more promising alternative is recently in focus and being researched extensively. As a preliminary attempt, the cells of *E. coli* were transformed with plasmids (pET21A$^+$) carrying indolicidin gene and were allowed to express indolicidin. The peptide was subsequently confirmed using SDS-PAGE.

MATERIALS AND METHODS

(i) ***Bacterial strain and plasmid***

The plasmid pET21A$^+$ was used as the expression vector. Chemically competent *E. coli* cells were used for the propagation / maintenance of these recombinant plasmids and *E. coli* C41 (DE3), C43 (DE3), C41 (DE3) pLysS & C43 (DE3) pLysS were employed as indolicidin expression hosts. All the strains were grown in Luria- Bertani (LB) broth at 37°C.

(ii) E. coli competent cell preparation

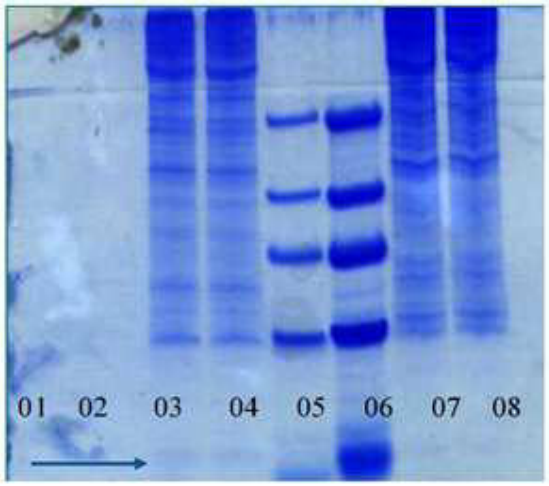
A single colony of *E. coli* from fresh LB agar plate was inoculated in 100 ml of LB broth medium and was incubated in an incubator shaker at 37°C for 4 - 6 h. After incubation, the cells were harvested by centrifugation at 4000 rpm for 10 min at 4°C. The collected cells thus obtained were resuspended in 10 ml of ice cold 0.1M $CaCl_2$ for 20 min. The cells were again centrifuged at 6000 rpm for10 min. The cells were resuspended in 5ml of 0.1M $CaCl_2$ and 15% of glycerol. The content was dispersed in microtubes and stored at - 80°C.

(iii)*Transformation of *E. coli

To 50 µl of *E. coli* competent cells, 2 µl of vector was introduced and incubated in ice for 30 min. After incubation the cells were kept in water bath at 42°C for 45 s initially and further incubated for 2 min in ice. Followed by, 950 µl of LB broth medium was added and incubated in a shaker incubator at 250 rpm for 1 minute. The culture thus obtained was then inoculated into the LB ampicillin medium and incubated for 37° C for overnight.

RESULTS

The transformation of *E. coli* culture was confirmed by the growth of the cells on LB agar containing ampicillin. Further, upon inoculation on LB broth and subsequent analysis, it was found that, the transformed culture could produce a total mean protein concentration as high as 6.4 mg/liter against 4.5 mg/liter by non-transformed cells. Thus, confirming transformed culture expressing indolicidin. Further, after 1 h of incubation, it was noted that the optical absorbance density (600nm) of transformed culture was higher (1.2 OD) than by non-transformed (0.8 OD) culture. This could be attributed to the expression of the antimicrobial peptide - indolicidin, which hindered the growth of bacterial cells during incubation. In order to have a concrete evidence of indolicidin production, the 2 KDa indolicidin peptide was confirmed on SDS – PAGE using samples from the transformed culture (Figure 1).



Lane no	Sample Details
1	Sample Buffer
2	Sample Buffer
3	IND 00110 after induction -1 h
4	IND 00110 After induction- 2 h
5	Ladder
6	Ladder
7	IND 00110 before induction
8	IND 00110 before induction
9	Sample Buffer

Figure 1
Extra cellular Protein profiles of E. coli [C41 (DE3)] transformants with the 2KDa Indolicidin band (indicated by an arrow)

DISCUSSION

Although, in the present study, *E. coli* C41 (DE3), C43 (DE3), C41 (DE3) pLysS & C43 (DE3) pLysS strains were used, it was found that, *E. coli* C41 (DE3) was more suitable for indolicidin expression. Morin *et al.* (2005) employed *E. coli* BL21DE3 transformed cells for indolicidin expression using the same vector. However, the reported yield by them was remarkably lower (150 µg/l) compared to the present study. Similarly, various authors have reported expression of other antimicrobial peptides within the range of 0.1–310 mg/l [8 -12]. For expression of indolicidin, apart from pET21A$^+$, use of pET28A$^+$ as a vector was also reported[13]. Even though, indolicidin is on intense research for antimicrobial activity, indolicidin analogue such as CP-11 with greater activity against gram negative bacteria and *Candida* spp. has been reported[14, 15]. Further, it is emphasized that indolicidin be tested for antimicrobial activity against regional pathogenic micro-organisms since the susceptibility pattern may vary.

CONCLUSION

Thus the cells of *E. coli* transformed with plasmids pET21A$^+$ carrying indolicidin gene is most suitable for the expression of the antimicrobial peptide indolicidin. In conclusion, research work can further be initiated towards validating and standardizing the expressed indolicidin for antimicrobial activity.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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