Journal of Applied Pharmaceutical Science Vol. 4 (07), pp. 047-049, July, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.40708
ISSN 2231-3354

Isolation of colicinogenic *E. coli*, optimization of colicin production and transformation of Col plasmid

Arumugam Suresh[1], Manikkam Radhakrishnan[1], Gopal Suresh[2], Nainangu Prasannabalaji[3], Sivaraj Anbarasu[1], Jerrine Joseph[1]

[1] Centre for Drug Discovery and Development, Sathyabama University, Chennai – 600 119, Tamil Nadu, India. [2] Department of Microbiology, Sri Sankara Arts and Science College, Kanchipuram, Tamil Nadu, India. [3] Department of Microbiology, Bharathidasan University, Trichy, Tamil Nadu, India.

ARTICLE INFO

Article history:
Received on: 02/02/2014
Revised on: 16/02/2014
Accepted on: 18/03/2014
Available online: 28/07/2014

Key words:
E. coli, colicin, transformation, *E. coli* O157:H7.

ABSTRACT

An attempt was made for the isolation of colicinogenic *E. coli* from cow and sheep dung samples. Total of 112 *E. coli* isolates were collected from sheep and cow dung samples in which 63 isolates are from cow dung and 49 isolates are from sheep dung samples. In the screening for colicin production, two *E. coli* isolates C22 and C51 from cow dung sample and one isolate S39 from sheep dung sample showed activity against the pathogenic strain *E. coli* O157:H7. Colicin production by all the three *E. coli* isolates was found to be enhanced by 0.25mg/l of mitomycin-c and three hours of incubation at 37^0C. Plasmid DNA was isolated from colicinogenic *E. coli* strains by alkali lysis method and their molecular weight was found to be 6.5 kb. The transformation experiment carried out with the standard strain *E. coli* DH5α confirmed that the plasmids were col-plasmids. The three colicinogenic *E. coli* strains isolated in this study will be a candidate to develop as probiotic for veterinary applications.

INTRODUCTION

Colicins are bactericidal macromolecules produced by certain strains of *Escherichia coli* to kill *E. coli* strains that lack the respective colicin-encoding plasmid. Approximately half of the natural *E. coli* isolates produce one or more of these toxins. Colicin production is encoded extra chromosomal DNA called col plasmid. Colicins either specifically cleave DNA, rRNA, tRNA or form pores in the cytoplasmic membrane, thereby dissipating the electrochemical potential (Zeth *et al.,* 2008). Colicin is a three-domain protein and their molecular ranges are 12 kDa to 90kDa. Each col plasmid confers immunity to the particular type of colicin which it encodes Colicin are divided into two groups according to their cross-resistance patterns, numbering over 20, the pore former and nuclease colicins are differentiated Group A (A, E1, E2, E3, K, L, N, S4, and X) and Group B (B, D, G, H, I, Ia, Ib, M, Q, S and V) (Imren, 2001). Pore-former toxins kill by creating channels in the cytoplasmic membrane. Nuclease colicins kill by non-specific degradation of DNA or specific cleavage of rRNA. Structurally, each colicin shows the same type of organization that comprises three domains responsible for recognition of a specific outer-membrane receptor, translocation, and lethal activity (Riley, 2000).

About 30-50% of colicinogenic *E. coli* found in the environment and animal specimens. They have highly-conserved organization of colicinogenic genes and amino acid sequences, suggests that they could play a positive role in the ecology of coliform bacteria (Gordon *et al.,* 2006; Lin, *et al.,* 2004). The Col plasmid contains an operon of three genes that are essential for colicin cytotoxicity.

Entero-hemorrhagic *Escherichia coli* (EHEC) O157:H7 cause severe enteric disease in cattle and death in humans, Human infection and outbreaks from EHEC O157:H7 have been attributed to the consumption of under cooked beef food products as well as various other foods. Colicin has inhibited the growth of pathogenic Shiga toxin producing *E. coli* (STEC) from cows. The STEC strains tested carried virulence attributes such as Shiga toxins (DebRoy *et al.,* 2003, Schamberger *et al.,* 2006). The present study is focused on detection of colicin producing *E. coli* from cow and sheep dung for veterinary applications as probiotic.

* *Corresponding Author*
Email: kpm_suresh@yahoo.com

MATERIALS AND METHODS

Sample collection, isolation and identification of *E. coli*

The cow dung and sheep dung samples were collected from Government Veterinary hospital, Kanchipuram. All the collected samples were transported to the laboratory using nutrient broth as transport medium. All the collected samples were inoculated into nutrient agar and MacConkey agar plates. Morphologically distinct colonies were selected, purified and subcultured on nutrient agar slants. Phenotypic characteristics such as micromorphology, motility, cultural characteristics on different media and biochemical properties were studied (Koneman *et al.*, (2006). *Escherichia coli* strains were identified and selected for further colicin production studies.

Screening of *E. coli* strains for colicin activity

All the *E. coli* strains were grown on LB broth at 37^0C. At mid log phase of growth, mitomycin-C (0.25µg/ml) was added to induce colicin production. Incubation was continued for another 3 hours at 37 ^{o}C so as to reach the final concentration of $2x10^5$CFU/ml. Then the cells were transferred into LB agar plates and grown overnight at 37^oC. The putative colicin producing *E. coli* strains were killed with chloroform dispensed in what man filter paper disc (DebRoy *et al.*, 2003; Lindeberg *et al.*, 2001). The crude colicin impregnated disc was placed over the Muller Hinton agar plates inculated with *E. coli* O157:H7. All the plates were incubated at 37 °C for 24 hours. The clear zone indicates the activity of colicin produced by *E. coli* strains. Potential colicinogenic *E. coli* strain was selected for further studies and stored in glycerol broth at -20 ^{o}C.

Optimization of colicin production

Effect of Mitomycin-C concentration:

The putative colicin producing *E. coli* strains were grown in LB broth. At mid log phase of growth mitomycin C was added atthe concentration of 0.10 µg/ml, 0.15 µg/ml, 0.20 µg/ml, 0.25 µg/ml, 0.30 µg/ml, 0.40 µg/ml, and 0.50 µg/ml to induce the cultures for colicin production. All the tubes were incubated for another 3 hours at 37 ^{o}C so as to reach $2x10^5$ CFU/ml, these was transferred into LB agar plates and grown overnight at 37 ^{o}C (Cavard, 1991).

Effect of incubation period

The colicin producing *E. coli* strains were grown in LB broth. At mid log phase of growth mitomycin C was added at optimum concentration 0.25 µg/ml, and 0.30 µg/ml in different aliquots for induction of colicin production, it was further incubated at 37 ^{o}C for 2 hour, 2.5 hours, 3.0 hours, and 3.5 hours, these were transferred in LB plates and grown overnight at 37 ^{o}C.

Transformation study

Plasmid isolation from colicinogenic E. coli

Colicinogenic *E. coli* strain was inoculated in LB broth containing chloramphenicol (0.25 mg/l), for plasmid amplification. Plasmid was isolated by alkaline lysis method and it was separated by agarose gel electrophoresis with standard marker DNA (Sambrook, 2001, Inoue *et al.*, 1991).

Competent cell preparation

Freshly grown colony of *E. coli* DH5α was inoculated into 2 ml of LB medium and incubated at 37 ^{o}C for 24 hours. About 0.5 ml of inoculum was transferred into 50 ml of LB medium and incubated in rotary shaker at 37 ^{o}C. Once the culture broth reached the 0.5 optical density the whole culture broth was collected and centrifuged at 4100 rpm for 10 minutes. The cells were suspended in 25 ml of 100 mM $CaCl_2$ solution gently and once again centrifuged at 5000 rpm for 10 minutes at 4 ^{o}C. The cells were re-suspended in 2.0 ml of pre-cooled CaCl2 solution and kept in ice for 30 minutes.

Transformation

The plasmid DNA isolated from colicinogenic *E. coli* strain was added into 200 µl of CaCl2 treated cells of *E. coli* DH5α taken in sterile tubes. The content was mixed gently by swirling and the tube was kept in ice for 30 minutes. Then the tube was kept at 42 °C for 90 seconds and rapidly shifted to ice box and allowed to stand for 1-2 minutes. Once again the tube was transferred to ice box. The whole content was added into 800 µl of SOC broth and incubated at 37 °C for 45 minutes. Appropriate volume of transformed competent cells was transferred onto SOB agar medium containing 20 mM $MgSO_4$ and appropriate plasmid encoded antibiotic, ampicillin. The plate was incubated at 37 °C for 24 hours. The presence of col plasmid of colicinogenic *E. coli* in the cells of *E. coli* DH5α was confirmed by isolating the DNA by alkali lysis method and agarose gel electrophoresis (Sambrook, 2006).

RESULTS AND DISCUSSION

Total of 112 *E. coli* isolates were collected from sheep and cow dung samples. Among 112 isolates, 63 isolates are from cow dung and 49 isolates are from sheep dung samples. In the screening for colicin production, two *E. coli* isolates from cow dung sample and one from sheep dung sample showed activity against the indicator strain *E. coli* O157:H7. The *E. coli* strains C51 and C22 showed 22 mm and 21 mm zone of inhibition, respectively against the indicator strain *E. coli* O157:H7 zone size measures 22 mm for C51 and 21 mm for C22. Debroy *et al.*, (2003), reported that about 2.5 – 15% of 496 *E. coli* strains isolated from humans, horses, pigs and sheep samples were found to inhibit the growth of pathogenic shiga toxin producing *E. coli* (STEC) isolated from cow.

The induced colicin production and activity were notified with the addition of 0.25 mg/l of Mitomycin C to the LB broth medium. The colicinogenic *E. coli* strains C51, C22 and S39 was showed maximum of 22 mm, 21 mm and 20 mm inhibition respectively against the pathogenic *E. coli* O157:H7. In addition with mitomycin induction, time duration of induction was also

played a vital role in production of colicin. The activity was increased at 3.5 hours of time duration at 37 °C. Strain C51, C22 and S39 was produced 22 mm, 22 and 21 mm zone of inhibition, respectively. On further incubation no increase in zone of inhibition by all the three colicinogenic *E. coli* strains (Table-1). In a previous studies by Cavard (2002), and Debroy (2003), addition of 300 nm/ml of mitomycin C was found to increase the colicin production.

The agarose gel electrophoretic separation of plasmids extracted from the three *E. coli* isolates was evidenced that the molecular weight of isolated plasmids was found to be 6 kb. The genetic basis of colicin production was determined by the transformation experiment using E. *coli* DH5α. The plasmids isolated from the tree *E. coli* strains were successfully transformed and the plasmids were identified as Col- plasmids (Figure 1). In a previous study, Tomizawa (2002) and Inou *et al.*, (1991) isolated col E1 plasmid from *E. coli* with 6.6. kb size.

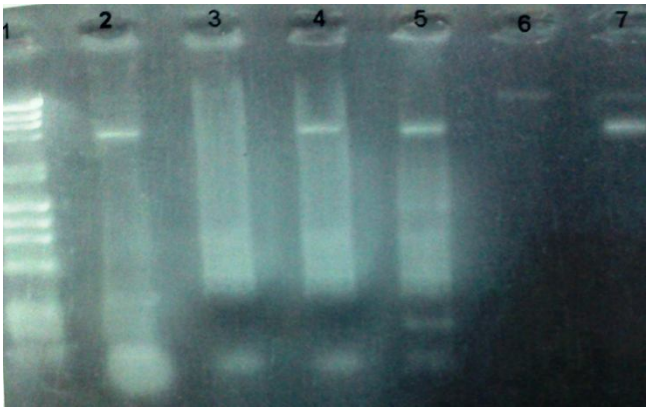

Fig. 1: Agarose gel electrophoresis showing Col plasmid DNA. Lane 2, 4 and 5 represent plasmid DNA from Colicinogenic E. coli strains. Lane 7 represent plasmid DNA extracted from transformant E. coli DH5α.).

CONCLUSION

Colicinogenic *E. coli* may prevent colonization of pathogenic *E. coli* by bacteriocin production. Colicinogenic *E. coli* strains isolated in this study may act as a candidate to develop as a probiotic for veterinary applications.

ACKNOWLEDGEMENT

Authors thank the Principal and the management of Sri Sankara Arts & Science College, Kanchipuram, for the laboratory facilities provided.

REFERENCES

Cavard D. Assembly of colicin A in the outer membrane of producing Escherichia coli cells requires both phospholipase A and one porin, but phospholipase A is sufficient for secretion. Journal of Bacteriology. 2002; 184(13):3723-33.

Cavard D. Synthesis and functioning of the colicin E1 lysis protein: comparison with the colicin A lysis protein. Journal of Bacteriology. 1991; 173(1):191-6

DebRoy C, and Jayarao B, Competitive Exclusion of *E. coli* O157:H7 using non-pathogenic bacteriocins producing Escherichia coli strains. The Pennsylvania State University, University Park, PA 16802 July 2002

Gordon DM, O'Brien CL. Bacteriocin diversity and the frequency of multiple bacteriocin production in *Escherichia coli*. Microbiology (Reading, England). 2006; 152(Pt 11):3239-44.

Imren A, Crikci G, Cosar, Colicin production and colicin typing of Uropathogenic Escherichia Coli, Turk J. Med. Sci, 2001; 31: 483-485

Inoue N, Uchida H. Transcription and initiation of ColE1 DNA replication in Escherichia coli K-12. Journal of bacteriology. 1991; 173(3):1208-14.

Lin YH, Liao CC, Liang PH, Yuan HS, Chak KF. Involvement of colicin in the limited protection of the colicin producing cells against bacteriophage. Biochemical and biophysical research communications. 2004; 318(1):81-7.

Lindeberg M, Cramer WA. Identification of specific residues in colicin E1 involved in immunity protein recognition. Journal of Bacteriology. 2001; 183(6):2132-6.

Riley MA, Cadavid L, Collett MS, et al. The newly characterized colicin Y provides evidence of positive selection in pore-former colicin diversification. Microbiology (Reading, England). 2000; 146 (Pt 7):1671-7.

Sambrook J, Russell DW. Preparation and Transformation of Competent *E. coli* Using Calcium Chloride. Cold Spring Harbor Protocols. 2006(1):pdb.prot3932.

Schamberger GP, Diez-Gonzalez F. Assessment of resistance to colicinogenic *Escherichia coli* by *E. coli* O157:H7 strains. Journal of Applied Microbiology. 2005; 98(1):245-52.

Winn. W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P and Wood G. 2005. In: Koneman's Color Atlas & Textbook of Diagnostic Microbiology, Lippincott Williams & Wilkins; 6th edition.

Zeth KC, Romer SI, Patzer and Braun V. Crystal Structure of Colicin M, a Novel Phosphatase Specifically Imported by *Escherichia coli*. The Journal of Biological Chemistry 2008; 283(37): 25324–25331.

How to cite this article:

Arumugam Suresh, Manikkam Radhakrishnan, Gopal Suresh, Nainangu Prasannabalaji, Sivaraj Anbarasu, Jerrine Joseph. Isolation of colicinogenic E. coli, optimization of colicin production and transformation of Col plasmid. J App Pharm Sci, 2014; 4 (07): 047-049.