ISSN: 2278-778X
Research Article
www.ijbio.com

EXPRESSION AND PURIFICATION OF LECTIN A FROM *PSEUDOMONAS AERUGINOSA* IN *E. COLI*

B Veeresh, G Adarsh, T Ramesh, M Ramesh*
Department of Biotechnology, JNTU Kakinada-UCEV, Andhra Pradesh, India

***Corresponding Author:** Dr. Ramesh Malothu, Assistant Professor and Head, Department of Biotechnology, JNTU, Kakinada-UCEV, Vizianagaram, Andhra Pradesh, India.

Received for publication: October 11, 2012; **Accepted**: October 28, 2012.

Abstract: *Pseudomonas aeruginosa* is a pathogenic micro organism which infects cystic fibrosis patients. The mode of infection is by attaching to the host cell using lectin proteins present in cell wall of *Pseudomonas aeruginosa*. The genomic DNA has been isolated from *Pseudomonas aeruginosa.* The Lec A gene coding for lectin protein has been isolated from genomic DNA of *Pseudomonas aeruginosa* using specific primers by specific gene amplification using PCR. The isolated Lec A gene is cloned by T/A cloning. The Lec A gene is inserted into expression vector pET-32a. The recombinant vector is transformed into *E. coli* BL-21. The protein is over expressed by adding inducer IPTG. The expressed protein is purified by using affinity chromatography and confirmed by SDS PAGE.

Keywords: Lec A, *Pseudomonas aeruginosa*, PCR, SDS PAGE, Affinity chromatography.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen, which is involved in acute infections, as well as chronic infections, especially in cystic fibrosis patients (King EO *et al.,*). The therapeutic options for these infections remain limited because this pathogen exhibits increasing resistance to many antibiotics (D'Argenio DA *et al.,*). Currently, antibiotic research and development are at an all-time low, and few new anti *Pseudomonal* compounds are in the pipeline. Therefore, there is a need for therapeutic approaches other than antibiotics.

For *P. aeruginosa*, as for other pathogenic microorganisms, the ability to adhere to host tissues is essential for initiating infection. Adhesion is often mediated by host cell surface glycoconjugates, which are a specific target for bacterial receptors (Williams HD *et al.,*). Such oligosaccharide-mediated bacterium-cell recognition and adhesion have been shown to be crucial in the early steps of *P. aeruginosa* pathogenesis. *P. aeruginosa* adhesion is mediated by a glycanic recognition pattern involving several adhesins, including lectins. Only a limited number of the carbohydrate-binding proteins of *P. aeruginosa* have been studied, and their role in recognition and adhesion is far from being elucidated. Two soluble lectins, LecA (PA-IL) and LecB (PA-IIL), specifically binding galactose and fucose, respectively, were initially identified and characterized in the cytoplasm of *P. aeruginosa* (Vander Wauven C *et al.,*). However, large quantities of both of these lectins are present on the outer membrane of the bacteria, suggesting that lectins may play a role in adhesion (Hachem RY *et al.,*). These two lectins, which are produced by the bacteria, are also associated with virulence factors and regulated by both quorum sensing and the alternative sigma factor RpoS (Forestier C *et al.,*), suggesting that they are also parts of the numerous systems involved in *P. aeruginosa* virulence.

The *P. aeruginosa* Lec A gene can be expressed in *E.coli* to produce in bulk amount and then it can be used in the preparation of vaccine to treat *P. aeruginosa* infections.

MATERIALS AND METHODS

Isolation of genomic DNA from *Pseudomonas aeruginosa* by potassium acetate method:

The genomic DNA from *Pseudomonas aeruginosa* has been isolated using the following protocol. Overnight grown culture of *Pseudomonas aeruginosa* in 2% LB broth at 37°C is harvested till good pellet is formed by centrifuging at 5000 rpm for 5 min in a 1.5ml microfuge tube. The pellet is re suspended in 575µl of 1X TE buffer. 150µl of 10% SDS is added, mixed well and incubated for 1hour at 37°C. 150µl of 5M potassium acetate is added, mixed well by inverting and it is incubated on ice for 15 min. It is centrifuged at 10,000 rpm for 10 min, and the supernatant was transferred in to a fresh vial. 1µl of RNase A is added to it and incubated for 30 min at 37°C. 0.6ml of isopropanol is added to it, mixed gently and allowed it precipitate at -20°C for 15-30 min. It was centrifuged at 12,000 rpm for 8 min at 4°C and the supernatant is discarded. 400µl of 70% ethanol is added and it was centrifuged at 12,000 rpm for 5 min at 4°C.

The supernatant is discarded; the pellet is dried by inverting the vial on the tissue paper towel. The pellet is dissolved in 40µl of TE buffer and stored at 4°C. DNA is electrophoresed on 0.8% agarose gel to observe the bands.

Amplification of Lec A gene by PCR:

The nucleotide sequence of *Pseudomonas aeruginosa* Lec A gene was downloaded from NCBI website. The primers are designed to amplify full length 369bp of *P.aeruginosa* Lec A using primer designing tools.

For PCR experiment, about 1.0 µl of genomic DNA was added to 25 µl of PCR mixture containing 1 U of Taq polymerase, 2.5 mM each of dATP, dTTP, dGTP and dCTP, 10X assay buffer, 10 Pico moles of Forward Primer with Bam HI site 5'ggatccATGGCTTGGAAAGGTGAG 3' and Reverse primer with Eco RI site 5'TATTGGAAAGGATCAGTCCTGATAAgaattc 3'. The reaction mixture was cycled through the following temperature profile: 94°C for 2minutes, 93°C for 1minute, 65°C for 1minute, 72°C for 1minute, the PCR was run for 20 cycles and finally terminated at 72°C for 3minutes and cooled at 4°C. PCR fragment was analyzed by applying 10 µl of sample to 1.2% agarose gel and run at 50 V for one hour in 1X TAE buffer and subsequently gel was documented with gel doc.

T/A cloning:

The PCR amplified DNA fragments are ligated to pTZ57 R/T cloning vector (2886 bp) as described in instructions T/A clone™ PCR product cloning kit (MBI, Fermentas, USA) and transformed to *E. coli* DH5α following standard molecular biology procedures.

Expression of Lec A gene in *E. coli* using prokaryotic expression vector:

For sub cloning of the gene, prokaryotic expression vector pET 32-a (Novagen) was used as expression vector that provide his fusion tags to the expressed protein and thus facilitate the recombinant protein purification much easier using nickel based columns. The vector and PCR amplified DNA fragment was digested with Bam HI and *Eco RI* (sites included in primers) restriction enzyme. The complete restriction was confirmed by electrophoresis. The ligation reaction was carried out with molar ratio of 1:3 (ends of vector: insert). The components of the ligation mixture was taken in a 25 µl micro centrifuge tube and incubated at 16°C for 12 hrs and transformed to *E. coli* BL 21 and plated on Luria agar containing Amp (100 mg/ml) and incubated at 37°C for 12 hrs and the recombinants were confirmed by Colony PCR.

Induction and purification of Lectin A protein:

The Lectin A protein is induced by adding inducer IPTG to the LB broth containing transformed cells. After induction, the cells are lysed to extract the intracellular protein using the procedure outlined in Sambrook and Russell and the recombinant protein was purified using His-Tag Fusion Protein Purification Kit, Bangalore Genei Private Ltd., Bangalore. The purified protein is confirmed by using SDS PAGE.

RESULTS AND DISCUSSION

The genomic DNA from *Pseudomonas aeruginosa* is isolated. The forward and reverse primers for amplification of Lec A gene are designed. The full length Lec A gene coding for Lectin protein was successfully amplified by PCR from *Pseudomonas aeruginosa*, giving a product of expected size (369bp). This is confirmed by electrophoresing the PCR amplified DNA fragments in 1.2% agarose gel. All samples has shown band at 369bp corresponding to the size of Lec A gene (fig.1). Which will confirm that Lec A gene is amplified.

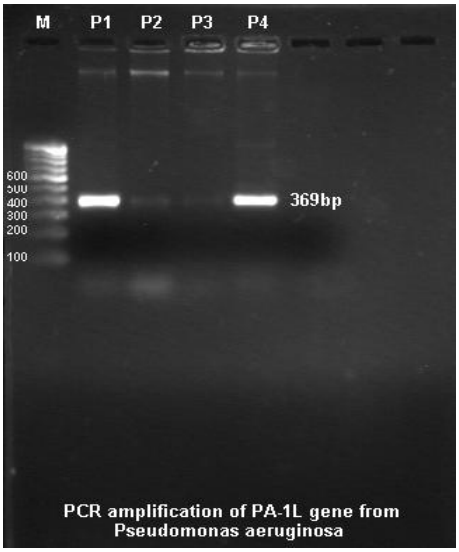

Fig.1: Gel doc of PCR amplified product of Lec A gene
Where M is Marker DNA, P1 to P4 are PCR amplified products

The Lec A gene is successfully cloned in *E. coli* by T/A cloning with the help of pTZ57 R/T cloning vector. This was confirmed by Colony PCR. The Lec A gene is successfully inserted into pET 32a vector by restriction digestion and ligation. The recombinant pET 32a vector containing Lec A gene is successfully transformed into *E. coli* BL21. After transformation, the cells are grown in ampicillin containing LB agar medium to confirm transformation. The results are obtained as shown in following table:

Table 1: Result for transformation

Medium	Cell type	Volume of inoculums	Rate of growth
LB agar	Competent cell	100µl	Growth
LB agar + Ampicillin	Competent cell	100µl	No growth
LB agar + Ampicillin	Transformed cell	50µl	Growth
LB agar + Ampicillin	Transformed cell	100µl	Growth

This was confirmed by subjecting selected colonies to Colony PCR (fig.2)

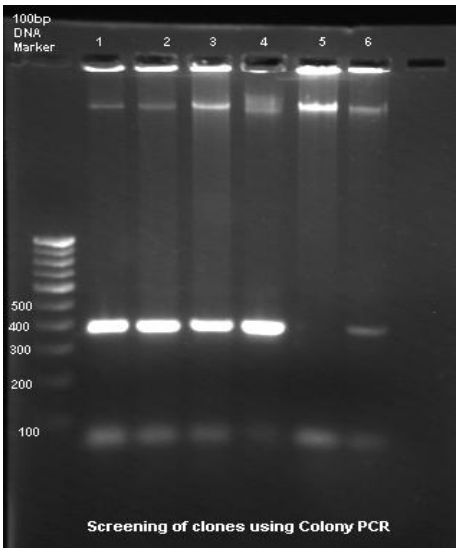

Fig.2: Gel doc of Colony PCR product

The Lec A gene is over expressed to produce Lectin A protein, by adding inducer IPTG. The crude protein extract from IPTG-induced clones along with *E. coli* BL21 having pET 32a (+) were isolated. The protein was purified by using affinity chromatography containing nickel column. The isolated protein was subjected to SDS-PAGE. The gels stained with Coomassie brilliant blue showed differential banding pattern in comparison with controls and a single band ~13.5 KDa was observed in nickel column purified protein sample. The protein band corresponding to approximately ~13.5 KDa indicated the expression of *Lec A* gene in the recombinants.

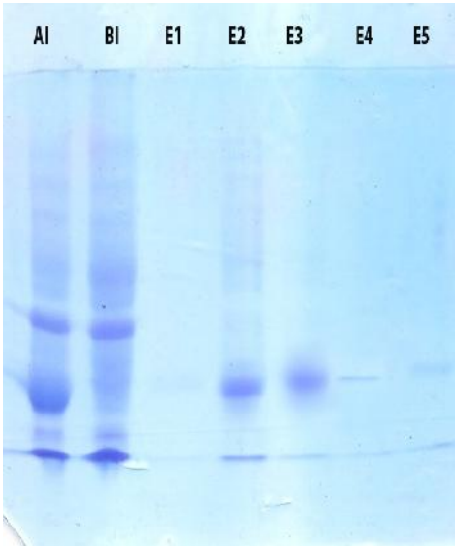

Fig.3: SDS PAGE gel 1

In figure, 'AI' represents the cell lysate after induction, 'BI' represents the cell lysate before induction and E1 to E5 represents the eluted samples.

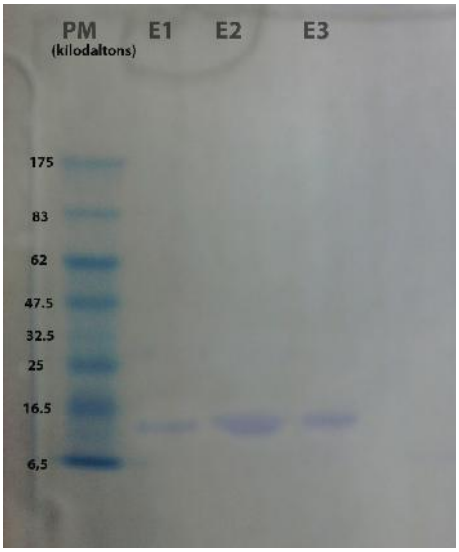

Fig.4: SDS PAGE gel 2

In figure, 'PM' represents protein marker and E1 to E3 represents the eluted samples.

CONCLUSION

Pseudomonas aeruginosa is an opportunistic pathogen involved in acute infections, as well as chronic infections, especially in cystic fibrosis patients. *P. aeruginosa*, as for other pathogenic microorganisms, the ability to adhere to host tissues is essential for initiating infection. Adhesion is often mediated by lectins, which are present on the cell wall of *Pseudomonas*. The main Lectin which is involved in adhesion of *Pseudomonas aeruginosa* is Lectin A protein.

The present study is aimed at expressing Lectin A protein from *Pseudomonas aeruginosa* in *E. coli*. In the current project we have isolated the Lec A gene which is responsible for expression of Lectin A protein. This Lec A gene has been expressed in *E. coli* using expression vector pET-32a. The recombinant *E. coli* cells which contain the Lec A gene has been cultured in L.B media. The production of Lectin A protein can be induced by adding inducer IPTG. The expressed protein has been separated and purified by using affinity chromatography. The expressed Lectin A protein has been confirmed by using SDS PAGE by matching with standard marker.

Further, more Lectin A protein can be produced by adding different concentrations of inducer IPTG. The concentration of IPTG which gives the maximum production of Lectin A protein is the standard concentration of IPTG. After that the rate of RPM, pH, temperature can be standardized to get maximum

production of Lectin A protein. Lectin A protein can further be studied to determine its structure, physical and biochemical properties. By knowing the structure of Lectin A, we can design the vaccine using Lectin A protein, which can be used to design vaccine against *Pseudomonas* infections.

REFERENCES

1. Cornelis P, Pseudomonas: Genomics and Molecular Biology (1st ed.). Caister Academic Press, 2008, ISBN 1-904455-19-0.

2. Forestier C, Guelon D, Cluytens V, Gillart T, Sirot J, de Champs C, Oral probiotic and prevention of Pseudomonas aeruginosa infections: a randomized, double-blind, placebo-controlled pilot study in ICU-patients, Crit. Care., 2008, 12 (3): R69. Doi:10.1186/cc6907.PMC 2481460.PMID 18489775.

3. Hachem RY, Chemaly RF, Ahmar CA, Jiang Y, Boktour MR, Rjaili GA, Bodey GP, Raad II, Colistin is effective in treatment of infections caused by multidrug-resistant Pseudomonas aeruginosa in cancer patients". Antimicrob. Agents Chemother., 2007, 51 (6): 1905–1911. doi:10.1128/AAC.01015-06. PMC 1891378. PMID 17387153.

4. Novagen, Protein Expression: Prokaryotic Expression: pETBlue and pET System Overview. Novagen 2002-2003 Catalog. p 84-91.

5. Poole, K, Efflux-mediated multiresistance in Gram-negative bacteria". Clinical Microbiology and Infection, 2004, 10 (1): 12–26. doi:10.1111/j.1469-0691.2004.00763.x. PMID 14706082.

6. Sambrook J and DW Russel, 2001, Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor New York.

7. TA Cloning Kit Manual. Invitrogen. 7 April 2004. pp. 31. http://stanxterm.aecom.yu.edu/wiki/data/Product_manuals_attach/ta.pdf. Retrieved 2009-10-17

8. Thomas Bjarnsholt, Peter Østrup Jensen, Thomas B. Rasmussen, Lars Christophersen, Henrik Calum, Morten Hentzer, Hans-Petter Hougen, Jørgen Rygaard, Claus Moser, Leo Eberl, Niels Høiby and Michael Givskov1 (2005). Garlic blocks quorum sensing and promotes rapid clearing of pulmonary Pseudomonas aeruginosa infections. Microbiology, 151 (4): 3873–3880. doi:10.1099/mic.0.27955-0. PMID 16339933.

9. Vander Wauven C, Piérard A, Kley-Raymann M, Haas D (1984). "Pseudomonas aeruginosa mutants affected in anaerobic growth on arginine: evidence for a four-gene cluster encoding the arginine deiminase pathway". J. Bacteriol. 160 (3): 928–34. PMC 215798.PMID 6438064. http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=215798

10. Williams HD, Zlosnik JE, Ryall B, Oxygen, cyanide and energy generation in the cystic fibrosis pathogen Pseudomonas aeruginosa. Adv. Microb. Physiol. 2007, 52, 1–71. doi:10.1016/S0065-2911(06)52001-6. PMID 17027370.

11. Bajolet-Laudinat O, S Girod-de Bentzmann, JM Tournier, C Madoulet, MC Plotkowski, C Chippaux and E Puchelle, 1994. *Cytotoxicity of Pseudomonas aeruginosa internal lectin PA-I to respiratory epithelial cells in primary culture.* Infect. Immun. 62:4481-4487.

12. Labaer J, Q Qiu, A Anumanthan, W Mar, D Zuo, TV Murthy, H Taycher, A Halleck, E Hainsworth, S Lory and L Brizuela, 2004, *The Pseudomonas aeruginosa PA01 gene collection.* Genome Res. 14:2190-2200.

13. Lanne B, J Ciopraga, J Bergstrom, C Motas and KA Karlsson, 1994. *Binding of the galactose-specific Pseudomonas aeruginosa lectin, PA-I, to glycosphingolipids and other glycoconjugates.* Glycoconj. J. 11:292-298.

14. Chen CP, SC Song N, Gilboa-Garber KS Chang and AM Wu, 1998. *Studies on the binding site of the galactose-specific agglutinin PA-IL from Pseudomonas aeruginosa.* Glycobiology 8:7-16.

15. Cioci G, EP Mitchell C, Gautier, M Wimmerova, D Sudakevitz, S Perez, N Gilboa-Garber and A Imberty. 2003. *Structural basis of calcium and galactose recognition by the lectin PA-IL of Pseudomonas aeruginosa.* FEBS Lett. 555:297-301.

16. Diggle SP, RE Stacey, C Dodd, M Camara, P Williams and K Winzer, 2006, *The galactophilic lectin, LecA, contributes to biofilm development in Pseudomonas aeruginosa.* Environ. Microbiol. 8:1095-1104.

17. Gilboa-Garber N, 1982. *Pseudomonas aeruginosa lectins.* Methods Enzymol. 83:378-385.

Source of support: Nil
Conflict of interest: None Declared