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PCR Amplification of DNA Isolated from a Pectinolytic Fungus *Aspergillus foetidus* MTCC 10367

K. Mehraj Pasha[1]*, P. Anuradha[2] and D. Subba Rao[3]

[1]Department of Biotechnology, JNTUA, Anantapur - 515 002, India.
[2]Director, Scintilla Bio-Marc Pvt. Ltd., Bangalore - 560 056, India.
[3]Department of Chemical Engineering, JNTUA, Anantapur - 515 002, India.

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The present study was undertaken with the primary objective, PCR amplification of DNA isolated from a Pectinolytic fungus *Aspergillus foetidus,* which was identified and characterized by IMTECH, Chandigarh as MTCC 10367. Genomic DNA was extracted by using phenol: chloroform method. The quality of DNA was assessed by UV spectrophotometry. PCR was carried out in a final reaction volume of 25 µl reaction mixture by using 18S rDNA fungal primers. Increase in the stringency during initial cycles of PCR reaction increased the sharpness and brightness of the bands. Approximately an amplified product of 350 bp size was obtained for Pectinase enzyme with the genomic DNA of *Aspergillus foetidus* MTCC 10367.

Key words: *Aspergillus foetidus,* Pectinases, PCR (Polymerase Chain Reaction), Agarose Gel Electrophoresis and DNA (Deoxyribonucleic Acid).

The enzymes that hydrolyze pectic substances are broadly known as pectinolytic enzymes or pectinases, these include- Polygalacturonase, pectin esterase, pectin lyase and pectate lyase on the basis of their mode of action[1]. Polygalacturonases are widely distributed among fungi, bacteria and yeasts [2]. Numerous Polygalacturonase genes, and the enzymes they encode, have been isolated and characterized from a number of prokaryotic and eukaryotic species[3]. The ascomycete fungal species *Aspergillus niger*, has as many as eleven Polygalacturonase genes[4]. The genome of *Aspergillus niger* contains four potential exo-polygalacturonase: PgaX, PgxA, PgxB, and PgxC[5]. The structures of two *Aspergillus niger* endo-PGs, PG I and PG II, have been solved[6, 7]. The isolation of pure DNA is a pre-requisite for molecular biology techniques, like polymerase chain reaction (PCR) as it helps for the study of gene expression in filamentous fungi[8]. In this study we report PCR amplification of genomic DNA isolated from a pectinolytic fungus *Aspergillus foetidus* MTCC 10367 by using 18S rDNA fungal primers.

MATERIALS AND METHODS

Aspergillus foetidus MTCC 10367 was cultivated on Potato Dextrose Broth in a 250 ml Erlenmeyer flask incubated at 28 ± 2°C on a rotary shaker at 150 rpm for 3 days. The genomic DNA was extracted from *Aspergillus foetidus* MTCC 10367 by using phenol: chloroform extraction

* To whom all correspondence should be addressed.
Tel.: +91-9703398983;
E-mail: k.mehraj@gmail.com

protocol with suitable modification[9]. Quality and quantity of the extracted DNA were analysed using Agarose gel electrophoresis and UV spectrophotometry at A_{260}/A_{280} nm[10]. The samples having optical density (OD) ratio between 1.8 -2.0 were considered good and quality samples were used for further study i.e. PCR amplification.

The sequences of the forward and reverse primers are given below:

Forward primer

5' GTAACCCGTTGAACCCCATT - 3'

Reverse primer

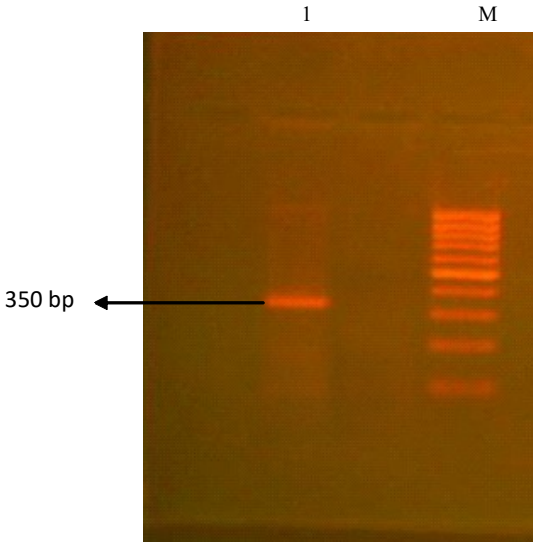
5' CCATCCAATCGGTAGTAGCG - 3'

The PCR reaction contained 100 ng/µl DNA, 10 picomoles of each primer, 1.5 mM $MgCl_2$, 100µM of dNTPs, PCR buffer (10 mM KCl , 10 mM $(NH_4)_2 S0_4$, 20 mM Tris-HCl , 2 mM $MgS0_4$, 0.1% Triton X – 100, pH 8.8) and 3 U / 1µl of Taq DNA Polymerase , 1.5 µl Tris (20 mM) were placed in a PCR tube. Amplification was performed in a thermocycler (Research Corbett, Australia). The cycling program was (i) 1 cycle of 94 °C for 3 minutes (ii) 30 cycles of 94°C for 30 seconds for final denaturation, 48 °C for 1 minute for annealing primer and 72 °C for 1 minute 30 seconds for extension and (iii) final extension at 72 °C for 7 minutes. The amplification products were resolved by electrophoresis in 1.5 % Agarose using 1 x TAE buffer. 100-1000 bp DNA Marker was used as to determine molecular size to confirm the amplification of target region. After staining the gel with ethidium bromide, PCR products were visualized by gel documentation system and photographed[11].

RESULTS AND DISCUSSION

Genomic DNA was isolated from *Aspergillus foetidus* MTCC 10367 and purity was checked in 0.7% Agarose gels. In the present study, the gene responsible for Pectinase enzyme from *Aspergillus foetidus* MTCC 10367 was amplified with a set of Primers. PCR amplified product of *Aspergillus foetidus* MTCC 10367 was run in 1.5% Agarose gel. Approximately an amplified product of 350 bp size was obtained for Pectinase enzyme with the genomic DNA of *Aspergillus foetidus* MTCC 10367.

PCR involves multiple cycles of template denaturation, primer annealing and primer elongation to amplify a specific gene that is present in the template DNA. Optimized parameters that produced the expected amplicon, that carries the target gene. It is largely dependent on the annealing of primers designed for *Aspergillus sp.* DNA based method such as PCR is based on the detection of particular gene (Pectinase gene) coding for the target enzyme (Pectinase). Most of the better characterized fungal pectinolytic systems were first studied at a biochemical level and are now being studied at the molecular level. It is to be hoped that with the pectinase gene identified, more information will soon become available on the regulation of these enzymes in *Aspergillus foetidus* MTCC 10367.



M: 100-1000 bp DNA Marker
1: PCR Amplified Product

Fig. 1. Agarose gel electrophoresis of PCR amplified product

REFERENCES

1. Mohamed, S.A., Abdulrahman, L., Al-Malki, and Kumosani, T.A. Characterization of a Polygalacturonase from *Trichoderma harzianum* Grown on Citrus Peel with Application for Apple Juice. *Australian Journal of Basic and Applied Sciences.*, 2009; **3**(3): 2770-7.
2. Yadav, P. K., Singh, V. K., Yadav, S., Yadav, K. D. S., and Yadav, D. *In silico* Analysis of Pectin Lyase and Pectinase Sequences. *Biochemistry* (Moscow), 2009; **74**(9): 1049-55.

3. Markovic, O., and Janacek, Š. Pectin degrading glycoside hydrolases of family 28: sequence-structural features specificities and evolution. *Prot. Eng*., 2001; **14**: 615–31.
4. De Vries, R.P., and Visser, J. *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. *Microbiol. Mol. Biol. Rev*., 2001; **65**: 497–522.
5. Martens-Uzunova, E.S., Zandleven, J.S., Benen, J.A.E., Awad, H., Kools, H.J., Beldman, G., Voragen, A.G.J., Van Den Berg, J.A., and Schaap., P.J. A new group of exo-acting family 28 glycoside hydrolases of *Aspergillus niger* that are involved in pectin degradation. *Biochem. J.,* 2006; **400**: 43–52.
6. Van Pouderoyen, G., Snijder, H.J., Benen, A.E., and Dijkstra, B.W. Structural insights into the processivity of endopolygalacturonase I from *Aspergillus niger*. *FEBS Lett.,* 2003; **554**: 462–66.
7. Van Santen, Y., Benen, J. A. E., Schroter, K.-H., Kalk, K. H., Armand, S., Visser, J., and Dijkstra, B.W. 1.68-A crystal structure of endopoly-galacturonase II from *Aspergillus niger* and identification of active site residues by site-directed mutagenesis. *J. Biol. Chem*., 1999; **274**: 30474–80.
8. González-Mendoza, D., Argumedo-Delira, R., Morales-Trejo, A., Pulido-Herrera, A., Cervantes-Díaz, L., Grimaldo-Juarez, O., and Alarcón, A. A rapid method for isolation of total DNA from pathogenic filamentous plant fungi. *Genetics and Molecular Research.,* 2010; **9**(1): 162-66.
9. Sambrook, J., and Russell, D.W. Commonly Used Techniques in Molecular Cloning. In: *Molecular cloning -A laboratory manual* (3rd edition). New York: Cold Spring Laboratory Press, 2001; Appendix 8.
10. Mann C.K., Goliniewski, and J. R., Fismanidis C.A. Spectrophotometric analysis by cross-co-relation. *Applied Spectroscopy.,* 1982; **36**: 223-29.
11. Medeiros, L.V., Maciel, D.B., Medeiros, V.V., Houllou Kido, L.M., and Oliveira, N.T. *pel*B gene in isolates of *Colletotrichum gloeosporioides* from several hosts. *Genetics and Molecular Research*., 2010; **9**(2): 661-73.