ISSN (Online) 2456-1304

International Journal of ,Science Engineering and Management(IJSEM)
Vol 1, Issue 2, June 2016

Biodegradation of Polycyclic Aromatic Hydrocarbon by Alternaria Alterna

[1] Jyoti Verma,[2] Deeksha Sharma,[3] Pavan Kumar Agarwal
[1][2][3] Department of Biotechnology, G.B. Pant Engneering College
[1]Jyoti.verma40174@gmail.com, [2]deekshasharma314@gmail.com, [3]P_K_agarwal@rediffmail.com

Abstract- **The use of fossil fuels for energy and raw material in the past century has led to a wide spread environmental pollution. Among these pollutants are polycyclic aromatic hydrocarbons (PAHs), which are considered a potential health risk because of their possible carcinogenic and mutagenic activities. Bioremediation by microorganisms (Algae, Bacteria, Yeast and Fungi) is a promising option for the complete removal and destruction of PAHs contaminants in industrial waste. The purpose of our research was to enlarge the scope of PAH-degrading fungi and explore the endophytic fungi resource for bioremediation of PAHs. In view of biodegrading capability of various microorganisms, endophytic fungi were isolated from Cupressus torulosa D.Don. In this study, naphthalene, anthracene and phenanthrne was used as a model PAHs compound. Six strains of endophytic fungi isolated from C.torulosa D.Don were screened for degradation of these PAH (Napthalene, Phenanthrene and Anthracene). All these fungal endophytes show variation in their growth at various concentration of hydrocarbon. The endophytic fungus strain PCTS21 showed good degradation efficiency for PAHs. Strain PCTS21 were identified as Alternaria alternate on the basis of their morphotypic characteristics on PDA and LCB staining techniques as well as their molecular characterization by using 18S rDNA sequencing. A. alternate have shown a good growth on a mineral salt medium containing hydrocarbon as a carbon source at concentration of 200 ppm. Alternaria alternata could degrade the hydrocarbon by producing manganese dependent peroxide and polyphenol oxidase enzyme. This study suggests that endophytic fungi might be a novel and important resource for microorganisms that have PAH-degrading capabilities.**

Keywords: **Biodegradation, Endophytic fungi, *Alternaria alternata,* Polycyclic aromatic hydrocarbon**

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are the major culprits in urban area and can be introduced in the by various such as oil spills, urban runoff, domestic and industrial wastewater discharge, incomplete combustion offossil fuel and organic matter. Polycyclic aromatic hydrocarbons (PAHs) are refers to a group of chemicals that consists of carbon and hydrogen,made out of two or more intertwined fragrant rings arranged in a linear, angular or cluster form. That are generated from both characteristic and anthropogenic procedures and it makes a serious concern to the health of aquatic life and humans through bioaccumulation [1,2]. Polycyclic aromatic hydrocarbon (PAHs) are one of the main kind of ubiquitous environmental poisons which as a rule happen amid fuel burning, and are widely distributed in our surroundings.

PAHs are hydrophobic in nature and it gets promptly adsorbed onto particulate matter and thus, coastal and marine sediments turned into a definitive sinks for such mixes [3]. The marine organisms such as benthic, demersal and pelagic fishes, scavangers and shellfishes are thoroughly influenced due to the carcinogenic nature of PAH'S [4].

PAHs are pervasive poisons and are produced from anthropogenic activities such as the burning of fossil fuels, the use of wood additives, for example, creosote and the generation of wastes from coal gasification plants [5]. PAHs have been distinguished as hazardous chemicals by different State and Central Pollution Control Boards due to their dangerous, cancer-causing and tetragenic impacts on living body [6]. PAHs, is a persistent and toxic soil contaminant. Pollution by PAHs is usually found on the sites of gas factories and wood preservation plants.

There are numerous physical and compound evacuation strategies for PAHs pollutants. But, these techniques have significant burdens, for example, their mechanical multifaceted nature, high cost; they produce another source of pollution and also build the oil recuperation cost .Biodegradation by microorganisms (Algae, Bacteria, Yeast and Fungi) on the contrast, is a promising choice for the complete evacuation and destruction of PAHs contaminants [7].

Investigations into the microbial bioconversion of PAHs have demonstrated that organisms are effective degraders of these organic pollutants.These fungi have additionally been appeared to drain and detoxify PAHs in debased soil [8]. The US EPA has identified 16 PAHs as main concerned toxins and perhaps some of these PAHs are considered to cause human carcinogens, and hence their distributions in

ISSN (Online) 2456-1304

International Journal of ,Science Engineering and Management(IJSEM)
Vol 1, Issue 2, June 2016

the earth and conceivable presentation to people have been of concerns [9].

Now a day, biological agent replaces the use of harmful chemicals, because of eco-friendly nature. These microbes can expel or decrease them to at any rate non-lethal level. Therefore, in current era research is increasingly being concentrated on organic techniques for the corruption and disposal of these pollutants.The biodegradation of Polycyclic aromatic hydrocarbons in nature is a perplexing procedure, who's quantitative and qualitative aspects depend on the nature also, measure of the oil or hydrocarbons introduce, the surrounding and seasonal environmental conditions, and the composition of the autochthonous microbial community.

Because of refractory nature the high sub-atomic weight PAHs are paid particular attention, although PAHs are relatively steady and obstinate in soils and less simple to debase than many other organic compounds. The abiotic and biotic procedures, for example, volatilization, photograph oxidation, chemical oxidation, bioaccumulation and microbial transformation are in charge of the result of PAHs into the earth.Microbial activity has been considered to play a significant role in cause of PAH removal [10–13].

II. MATERIALS AND METHODS

1.) Materials and fungal inoculums preparation

The endophytic fungus was previously isolated from *Cupressus torulosa* L. of Govind Ballabh Pant Engineering College, Ghurdauri, Pauri, Garhwal. It was stored at 4°C on potato dextrose agar (PDA, containing 200 g/ L potato extract, 20 g /L glucose and 20 g /L agar, pH 7.0). These endophytic fungus are identified on the basis of their morphotypic characterstics on PDA and LCB stanning techniques as well as their molecular charaterization.They are characterized as *Penicillium oxalicum* (PCTS 13), *Pestallotiopsis neglecta* (KCTS 14), *Alternaria alternata* (KCTS 15), *Penicillium oxalicum* (PCTS 25), *Alternaria alternata* (PCTS 21), *Daldini sp.* (KCTS 34) .

2.) Mineral Salts Medium used in this study

The carbon free mineral salts medium (MSM) contained (1 g $L^{-1}K_2HPO4$, 0.5 g L^{-1}KCl, 0.5 g L^{-1} MgSO4, 0.01 g L^{-1} FeSO4, 0.05 mg L^{-1} CuSO4, 0.05mg L^{-1} MnSO4, 1.0 mgL^{-1} ZnSO4,0.05mg $L^{-1}NaMoO_4$) was prepared. The final pH of the medium was adjusted to 4.5 with 0.1N HCL and 0.1N NaOH solution and the medium was autoclaved (121 °C for 15 min) and stored [14].

3.) Preparation of Stock Solution

Stock solution of each hydrocarbon of different concentration– Add. 001gm, .0015gm, .002gm, .004gm, .006gm of hydrocarbon (naphthalene, anthracene and phenanthrene) in 10 ml of methanolto make concentration of 100, 150, 200,400, 600ppm and dissolve it until the crystal disappeared.

4.) Screening of PAHs degrading ability of endophytic fungi

All fungal endophytes were tested for their ability to degrade PAHs (naphthalene, phenanthrene, and anthracene) by using as a sole source of carbon in solid mineral salts medium. Stock solution of each PAHs were made in the concentration of 100, 150, 200, 400, and 600 ppm. 0.1 ml from PAHs solution was spreaded on solid medium MSM agar plates then methanol evaporated by left plates for 1 hr. Inside sterile hood, white thin layer formed, then the plates were inoculated with fungal disk 6mm from7 days old culture of fungal isolates. The plates were incubated at 28°C for 7 days to allow growth of test fungi. They were then examined for their growth formation around the fungal test. The growth diameter was measured in each case[15].

5.) Quantitative analysis of the PAHs degradation by Alternaria alternata

On the basis of primary screening, Fungus *Alternaria alternata (*PCTS 21) were choosen for further work as its show a good growth on solid MSM agar by utilizing all tested PAHS. The fungal isolates were grown in 100 ml Erlenmeyer flasks containing 30 ml liquid mineral salts medium with 200ppm naphthalene, phenanthrene, and anthracene, pH was adjusted to 4.5, then autoclaved at 121°C for 15 min, the sterilized media were inoculated with two fungal disk (6mm) from7 days old culture of fungal isolates. Duplicate for each isolate and control, then flasks were incubated in shaker incubator 120rpm for 7days at 27°C [16].

Biodegradation Efficiency (%)={(Co-Ce)/Co}×100

Where, Co initial concentration of PAHS (PPM), and Ce final concentration of PAHS (PPM).

III. RESULTS AND DISSCUSSIONS

1.) Identification of Fungal Isolates

The initial examination of the fungal colonies developing were done by using a dissecting microscope, and

ISSN (Online) 2456-1304

International Journal of ,Science Engineering and Management(IJSEM)
Vol 1, Issue 2, June 2016

attended the slides for these colonies for the purpose of study their characteristics under an optical microscope compound. The results are shown in fig1. The most active isolates are *Penicillium oxalicum* (PCTS 13), *Pestallotiopsis neglecta* (KCTS 14), *Alternaria alternata* (KCTS 15), *Alternaria alternata* (PCTS 21), *Penicillium oxalicum* (PCTS 25,) *Daldini sp* (KCTS 34).

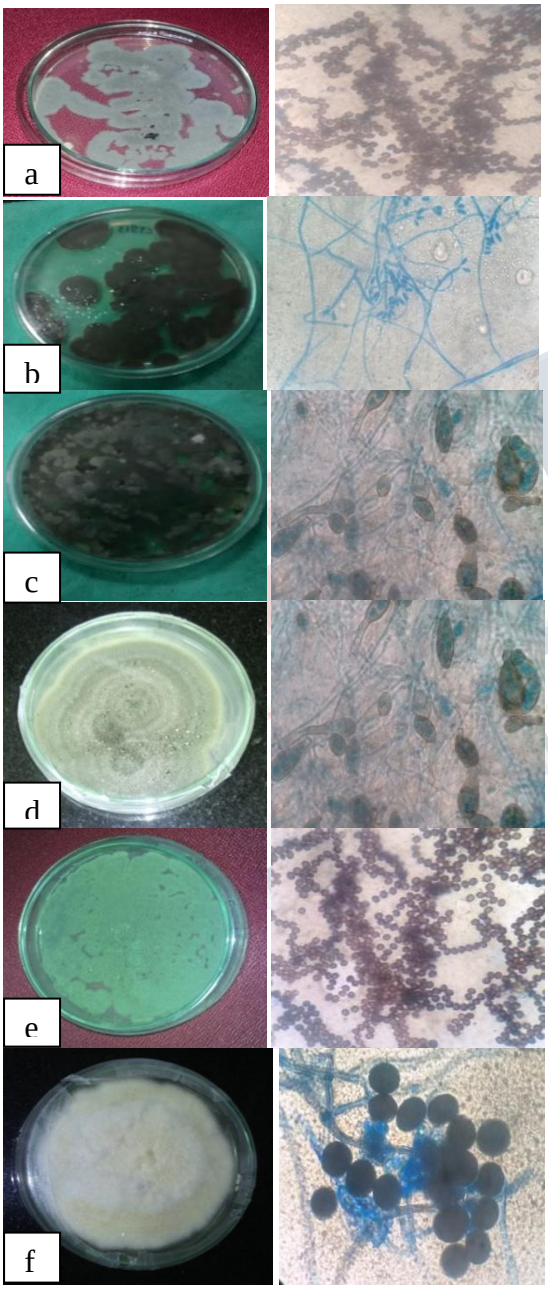

Fig. 1. Macroscopic and microscopic feature of
(a) *Penicillium oxalicum* (PCTS 13),
(b) *Pestallotiopsis neglecta* (KCTS 14),
(c) *Alternaria alternata* (KCTS 15),
(d) *Alternaria alternata* (PCTS 21),
(e) *Penicillium oxalicum* (PCTS 25),
(f) *Daldini sp.* (KCTS 34)

2.) Screening of the isolates for PAHS biodegradation

A total of six fungal endophytes were tested for its ability to degrade PAHs such as naphthalene, phenanthrene and anthracene. The results shows difference in growth of fungus in different concentration of hydrocarbon (Table 2, 3 ,4). Diameter of fungus are measure as Good growth (60-80mm), Moderate growth (10-50mm), weak growth (Spreaded dots of fungus) and poor growth(no growth) on solid media of MSM containing PAHS (Table 1), (Fig. 2). This may be attributed to the reason that fungal colonies are not growing on this medium because they do not possess the ability to degrade these compounds as a result of the lack of enzymatic system specialist, or due to low solubility of this compound which reduce availability to microorganisms [17]. Identical study done by [18] found failure of fungal isolates to grow on solid MSM with phenanthrene which attribute the degradation of compound containing more than one cycle is more resistant to oxidative enzymes.

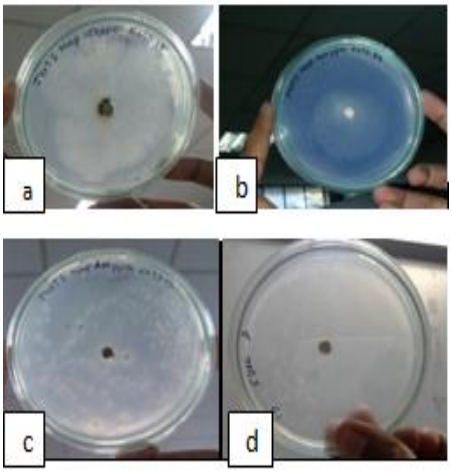

Fig. 2. .a, b, c, d- Fungal growth on solid media containing PAHS after 7 days at 28^0C

ISSN (Online) 2456-1304

International Journal of ,Science Engineering and Management(IJSEM)
Vol 1, Issue 2, June 2016

Table 1. Growth development of isolate on media containing PAHS

S.no	Growth Development of Isolate	Diameter of growth (mm)
1.	+ + + Good Growth	60-80
2.	+ + Moderate Growth	10-50
3.	+ WeakGrowth	Spreaded dots
4.	- Poor Growth	No growth

Table 2. Preliminary screening of fungal endophytes on Solid Media with Naphthalene at different concentration (ppm)

Fungus	Code	100	150	200	400	600
Penicillium oxalicum	PCTS13	+	+	+	+	+
Pestallotiop sisneglecta	KCTS14	++	+++	+++	+++	+++
Alternaria alternata	KCTS15	+	+	+	+	+
Alternaria alternata	PCTS21	+++	+++	++	++	++
Penicillium oxalicum	PCTS25	+	+	+	+	+
Daldinisp	KCTS34	+++	+++	++	++	++

Table 3. Preliminary screening of fungal isolates on Solid Media with Phenanthrene at different concentration (ppm)

Fungus	Code	100	150	200	400	600
Penicillium oxalicum	PCTS13	+	+	+	+	+
Pestallotiop sisneglecta	KCTS14	++	++	++	++	++
Alternaria alternata	KCTS15	+	+	+	+	+
Alternaria alternata	PCTS21	++	++	++	+++	+++
Penicillium oxalicum	PCTS25	+	+	+	+	+
Daldinisp	KCTS34	+	+	+	+	+

Table 4. Preliminary screening of fungal isolates on Solid Media with Anthracene at different concentration (ppm)

3.) Quantitative analysis of the PAHs degradation by endophytic fungi Alternaria alternata (PCTS 21)

The selected fungal isolates that displayed "good" growth on solid media containing PAHS test and its ability

Fungus	Code	100	150	200	400	600
Penicillium oxalicum	PCTS13	+	+	+	+	+
Pestallotiopsisneglecta	KCTS14	++	++	++	++	++
Alternaria alternata	KCTS15	+	+	+	+	+
Alternaria alternata	PCTS21	++	++	+	+	+
Penicillium oxalicum	PCTS25	++	++	-	-	-
Daldinisp	KCTS34	-	-	-	-	-

to degrade hydrocarbons of conc. 200ppm after a period lap for 7 days, the results showed the ability of fungal isolate to degrade napthalene, phenanthrene, anthracene. The degradation ability of *Alternaria alternata* for naphthalene as a carbon source is high as compare to phenanthrene following anthracene. Napthalene degrades upto 39% followed by phenanthrene 36.8% then by anthracene 32.2%. Similar results is found in [19] phenanthrene degradation is more than anthracene.

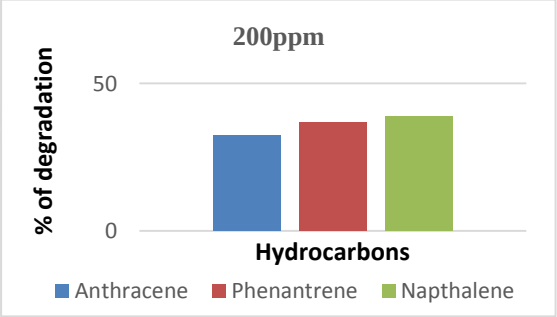

Fig. 3. Quantitative analysis of the PAHs degradation by Alternaria alternata at 200ppm

IV. CONCLUSION:

Microbial activities are very important for the renewal of our environment and maintenance of the global carbon cycle.This study showed that aendophytic fungus *Alternaria alternata* (PCTS21) isolated from leaves of C. torulosa D.Don had the ability to degrade the PAHS such as naphthalene, phenanthrene and anthracene which are common environmental pollutant with toxic, genotoxic, mutagenic and carcinogenic properties. Biodegradation of

these hydrocarbons by endophytic *A.alternta* on the basis of their growth on mineral salt medium was investigated. Hydrocarbons were metabolized by fungus to form several oxidized product.Out of all six endophytic fungus *A.alternata* showed maximum level of PAHS degradation. The degradation level of naphthalene is maximum among all considering hydrocarbons, It is 36.8%. These results indicate a possible application of the novel endophytic fungus *A.alternata from C. torulosa D.Don* in the bioremediation of these harmful contaminate environment.

ACKNOWLEDGEMENT

I gratefully acknowledge TEQIP-II and G. B. Pant Engineering College, Pauri, Garhwal for financial support and providing instrumentation facilities.

REFERENCES

[1] E.C. Okafor, K. Opuene, Int. J. Environ. Sci. Technol., 4(2), 233–240, 2007,

[2] E.O. Fagbote, E.O. Olanipekun, Int. J. Environ. Sci. Technol., 7(3), 561–570, 2010,

[3] S.H.Yu, L. Ke, Y.S. Wong, N.F.Y. Tam, Environ. Int., 31(2), 149–154, 2005,

[4] P. Arulazhagan, N. Vasudevan, I.T. Yeom, Int. J. Environ. Sci. Technol., 7(4), 639–652, 2010.

[5] Ni.Chadhain, S.M, Norman RS, Pesce K.V, Kukor J.J and Zylstra G.J. "Microbial dioxygenase gene population shifts during polycyclic aromatic hydrocarbon biodegradation. Appl," Environ. Microbiol., 72: 4078-4087, 2006.

[6] Ruma R, Ray R, Chowdhury R, Bhattacharya P., "Degradation of polyaromatic hydrocarbons by mixed culture isolated from oil contaminated soil—A bioprocess engineering study," Indian Journal of Biotechnology, 6: 107-113, 2007.

[7] Bamforth, S. M. and Singleton, I. "Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions," Journal of Chemical Technology and Biotechnology, 80(7), pp: 723-736, 2005.

[8] Natalia N.P., "Involvement of the ligninolytic system of white-rot and litter-decomposing fungi in thedegradation of polycyclic aromatic hydrocarbons," Biotech Res Inter, 20, pp:1-21. 2, 2012.

[9]Cerniglia, C. E., "Biodegradation of polycyclic aromatic hydrocarbons, Springer. Advance Applied Microbiology 3, pp: 351-368, 1992.

[10] C.O. Nwuche, E.O. Ugoji, Int. J. Environ. Sci. Technol., 5(3), 409–414, 2008.

[11] S. Haghighat, A. AkhavanSepahy, M. MazaheriAssadi, H. Pasdar, Int. J. Environ. Sci. Technol., 5(3), 385–390, 2008.

[12] I.E. Agbozu, K. Opuene, Int. J. Environ. Res., 3(1), 117–120, 2009.

[13] R. Atlas, J. Bragg, Microbiol. Biotechnol., 2(2), 213–221, 2009.

[14] Yan Chen, Xing-GuangXie, Cheng-Gang Ren, Chuan-Chao Dai, "Degradation of N-heterocyclic indole by a novel endophytic fungus Phomopsis liquidambari," Bioresource Technology, 129 568–574, 2013.

[15] Duha B.Mohammed, Saad Husein Khudeir, MunaH.Al-Jubouri, "The Optimum Conditions for Naphthalene Biodegradation by Filamentous Fungi," Iraqi Journal of Science, Vol 55, No.4B, pp:1780-1791, 2014.

[16] Bartha, R., "Biotechnology of petroleum pollutant biodegradation," Microbial Ecology, 12, pp:155-172, 1986.

[17] Mollea, C., Bosco, F., and Ruggeri, B.," Fungal biodegradation of naphthalene: Microcosms studies," Chemosphere, 60, pp: 636–643, 2005.

[18] Toledo, F. L., Calvo, C., Rodelas, B. and Lopez, L.G., "Selection and identification of bacteria-fungi isolated from waste crude oil with polycyclic aromatic hydrocarbons removal capacities, "Systematic and Appli. Microbial, 29, pp: 244-252, 2006.

[19] Kiran Bishnoi, Rajender Kumar and Narsi R. Bishnoi, "Biodegradation of Polycyclic Aromatic Hydrocarbon by white rot fungus Phanerochaete Chryososporium in sterile and unsterile soil," Journal of Scientific and Industrial Research, vol 67,pp 538-542, july, 2008.