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RESEARCH ARTICLE

EVALUATION OF BIOREMEDIATION POTENTIAL OF FUNGAL STRAINS FOR P-NITROPHENOL

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ABSTRACT

Bioremediation is modern technique in remediation of pollution of environment. Now a days this technique is widely used for remediation of soil and water. According to literature surveysvarious microorganisms have the potential to degrade nitro-aromatic compounds, xenobiotics etc. The white rot fungi are found to excellent organisms for mineralization processes. The purpose of the study is to determine bioremediation potential for PNP by fungal strains. The fungal stains used for degradation assay are (i) Aspergillus flavus NCIM 650, (ii) Aspergillus oryzae NCIM 1212, (iii) Aspergillus niger NCIM 1025, (iv) Fusarium oxysporium NCIM 1008, (v) Rhizopusoryzae NCIM 997, (vi) Scleratiumrolfssi NCIM 1048 and (vii) some agriculturally important microbes including Aspergillus awamori, Trichoderma sp. About 100 ml of sterile MSG medium was supplemented with filter sterilized PNP at 50 ppm concentration was taken in Erlenmeyer flask. This medium was inoculated separately with above fungal strain and incubated on a rotary shaker (120 rpm) at room temperature. The flasks were monitored for 8 days for the disappearance of yellow colour of PNP. After 8 days, the each fungal culture was renioculated in fresh sterile MSG medium with 50 ppm PNP. After five repeated enrichment for 5 weeks, each flask was observed for disappearance of yellow colour.

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INTRODUCTION

"Bioremediation" can be a preferred cost-effective method of removing contaminant hydrocarbons from oil-contaminated environments (Ivshina *et al*., 1998). Nitroaromatic compounds are major pollutants produced by industrial productions. Among nitroaromatics PNP is uses as a major raw material. PNP found to more toxic to soil microflora (Karin and Gupta, 2002) and Fungi feature among Nature's most vigorous agents for the decomposition of waste matter, and are an essential component of the soil food web (Rhodes, 2012), Bioremediation uses microorganisms that are yeast, fungi, and bacteria to remediate contaminated sites (Strong and Burges, 2008). Bioremediationtechnology is based on the growth of specific indigenous microflora that has activity of remediation (Agarwal, 1998). In bioremediation processes microbes uses the pollutant as nutritional sources and mineralizes it (Hess *et al*., 1997). For effective bioremediation processes microbes are able to attack pollutants to convert them to harmless products (Vidali, 2001). In the era of industrialization new inventions involving a variety of chemicals for agricultural and industrial use. Most of these chemicals were genuinely produced to serve a particular purpose; they often end up in the environment and mostly in the soil as contaminants (Valentín *et al*., 2013a). Soil contamination are mainly from the improper use of insecticides, pesticides and chemical fertilizers, spillage of oil and leakages of pollutants from landfills and waste treatment plants into the surrounding soil (Rao *et al*., 2010). Bioremediation, which is the use of microorganisms consortia or microbial processes to degrade and detoxify environmental contaminants, is also amongst these new technologies, which derives its scientific justification from the emerging concept of Green Chemistry and Green Engineering (Asha *et al*., 2010). The success of bioremediation of oil spill not only depends on the ability of the strains but also on the physical, chemical and biological conditions of the contaminated environment (Chandankere *et al*., 2014). Bioremediation is a very slow process. Only certain species of bacteria and fungi have found effective pollutant degraders. Some strains are effective as bioremediation agents but only under laboratory conditions. The limitation of bacterial growth is under the influence of pH, temperature, oxygen, soil structure, moisture and appropriate level of nutrients, poor bioavailability of contaminants, and presence of other toxic compounds. Although microorganisms can exist in extreme environment, most of them prefer optimal conditiona situation that is difficult to achieve outside thelaboratory (Bernhard-Reversat and Schwartz, 1997; Dana and Bauder, 2011).

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MATERIALS AND METHODS

Chemicals

p-nitrophenol (PNP) was obtained from Hi-Media, Mumbai. All other chemicals used during these studies were analytical grade (AR).

Culture Media and Cultivation Conditions

Mineral salt glucose (MSG) medium (*p*H 7.3), containing (gL^{-1}) glucose, 4.0; K_2HPO_4, 0.2; $MgSO_4.7H_2O$, 0.09 and $FeSO_4$, 0.01, was used as an enrichment medium. Mineral salt (MS) medium (*p*H 7.3), containing (gL^{-1}) K_2HPO_4, 0.65; KH_2PO_4, 0.2; $MgSO_4.7H_2O$, 0.09 and $FeSO_4$, 0.06, supplemented with filter sterilized (0.45 µm, Septrane membrane filter) PNP at various concentrations (20, 50,100), was used as an assay medium for degradation studies. Fungal cultures for PNP degradation were acclimatized to grow in MS medium containing respective concentration of PNP. MS-medium with yeast extract (5.0 gL^{-1}) was used as a supplement for obtaining fungal biomass.

Fungal Strains

The fungal stains used for degradation assay are (i) *Aspergillus flavus* NCIM 650, (ii) *Aspergillus oryzae* NCIM 1212, (iii) *Aspergillus niger* NCIM 1025, (iv) *Fusarium oxysporium* NCIM 1008, (v) *Rhizopusoryzae* NCIM 997, (vi) *Scleratiumrolfssi* NCIM 1048 and (vii) some agriculturally important microbes including *Aspergillus awamori*, *Trichoderma* sp.and screened for their degradation potential as per the methods described in literature. These strains were maintained on mineral salt glucose (MSG) medium fortified with 50 ppm concentration of PNP at 4^0C.

Experimental Setup

About 100 ml of sterile MSG medium was supplemented with filter sterilized (0.45µm membrane filter, Septrane) PNP at 50 ppm concentration was taken in Erlenmeyer flask. This medium was inoculated separately with above fungal strain and incubated on a rotary shaker (120 rpm) at room temperature. The flasks were monitored for 8 days for the disappearance of yellow colour of PNP. After 8 days, the each fungal culture was renioculated in fresh sterile MSG medium with 50 ppm PNP. After five repeated enrichment for 5 weeks, each flask was observed for disappearance of yellow colour.

Selection of potential fungal strain

An aliquot of 100 ml of MS medium (without glucose) supplemented with filter sterilized 50 ppm PNP was dispensed in 500 ml Erlenmeyer flasks. Each flask was inoculated with fungal strain capable of degrading PNP in previous experiment and kept on rotary shaker (120 rpm) at 30^0C. The samples were withdrawn intermittently at interval for analysis of PNP, nitrite and biomass as per standard protocol.

RESULTS AND DISCUSSION

Screening of potential fungal strain(s) for PNP degradation

Serial enrichment technique with successive transfers has proved to be effective in isolating microbes endowed with potential trait to degrade PNP (Herman and Costerton, 1993; Zaidi and Mehta, 1996; Zhao and Ward, 1999; Nishino *et al*., 2000). Mineral salt (MS) medium (*p*H 7.3) fortified with PNP (50 ppm) was used for screening of the fungal culture(s) with an ability to degrade PNP. It was supplemented with filter-sterilized (0.45 µm membrane filter, Septrane) 50 ppm PNP. About 100 ml of sterile MS-PNP medium was incubated on a rotary shaker (120 rpm) at room temperature. The flasks were monitored for 8 days for the disappearance of PNP (yellow color). After 8 days, each fungal cultures were re-inoculated in fresh sterile MS medium with 50 ppm PNP.

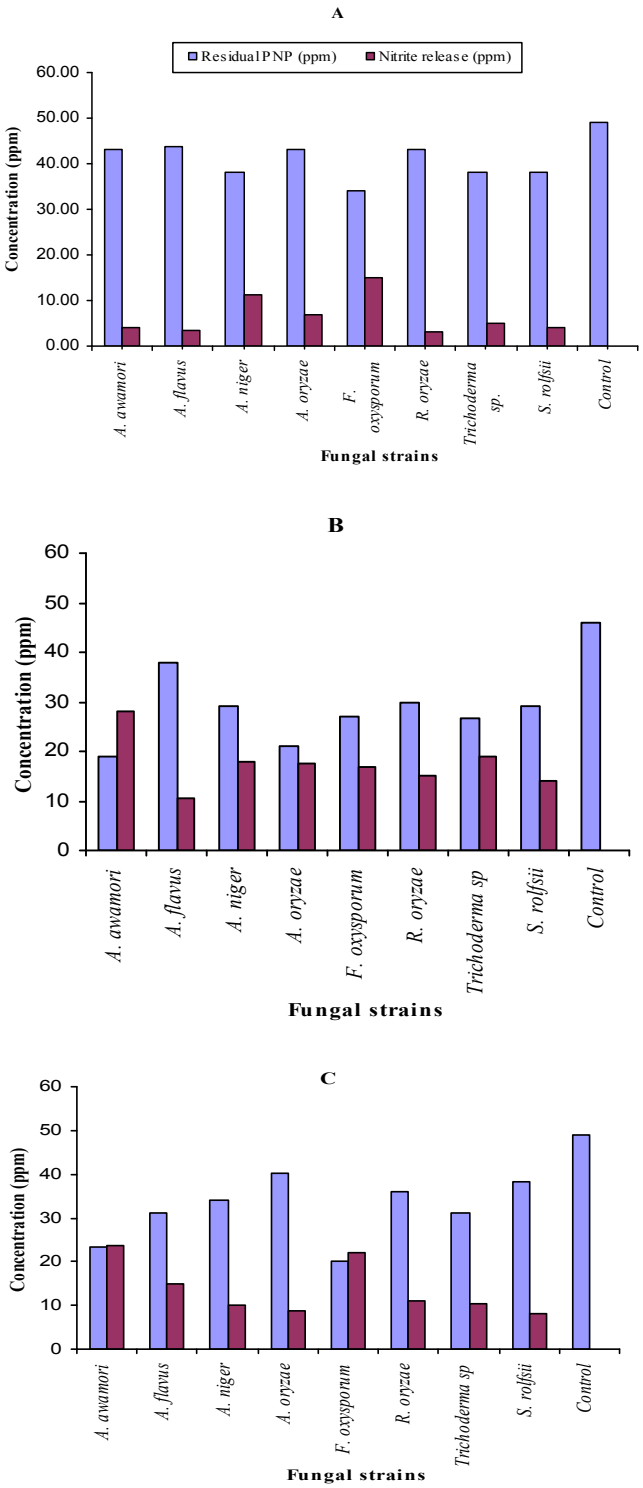

Fig. 1. (A, B and C) : Profile of PNP degradation by 24 h (A), 48 (h) (B) and 96 h (C) mycelial growth of various fungal cultures in MS medium containing PNP (50 ppm) at pH 7.0, 37^0C (120 rpm) after 24 h incubation

After repeated enrichment for 5 weeks, each flask containing respective fungal strain showed disappearance of yellow colour after 42 h, indicating their potential ability to degrade PNP. No disappearance of PNP occurred in the simultaneously run un-inoculated (control) flask. Disappearance of yellow colour (intrinsic of PNP) of medium along with concomitant release of nitrite, as confirmed spectrophotometrically, was used as a criterion to screen the potent PNP bio-degrader. A similar nitrophenol feeding approach has been employed by other researchers (Nishino and Spain, 1993; Zhao and Ward, 1999; Kulkarni, 2005). Among 8 fungal cultures, *Fusarium oxysporium* NCIM 1008 exhibited better performance and hence, selected on the basis of its ability, (i) to decrease the PNP concentration (50 to 34.4 ppm in 24 h) and (ii) increase in the amount of nitrite released (15.4 ppm) after 24 h and (iii) increase in growth with a concomitant disappearance of PNP (yellow colour) from MS medium containing PNP after every 18 - 20 h successive transfers at 35^0C indicated it's potential to degrade PNP.

Typical PNP biodegradation studies

Exponentially growing cells being metabolically most active, age of the inoculum used for the assay has a profound effect on the PNP degradation process. Fungal culture of each strain grown at different age (24 and 48 h) mycelial and resting (96 h grown) cultures were separately inoculated in MS medium containing 50 ppmPNP at 30^0C. After 24 h incubation (Fig. 1 A, B and c), it is revealed that

(i) 24 h pre-grown mycelial growth of *A. awamori, A. flavus, A. niger, A. oryzae, F. oxysporum, R. oryzae, Trichoderma sp. and S. rolfssi*degraded PNP (6.0, 5.2, 11.0, 14.8, 6.0, 11.0 and 11.0 ppm, respectively) with concomittent release of nitrite (4.0, 3.4, 11.25, 7.0, 15.0, 3.0 and 4.0 ppm), (ii) 48 h grown fungal inoculum of *A. awamori, A. flavus, A. niger, A. oryzae, F. oxysporum, R. oryzae, Trichoderma sp. and S. rolfssi* enhanced degradation of PNP (19.0, 17.0, 25.0, 8.0, 27.0, 16.0, 19.5, 17.0 ppm respectively) with concomittent increase in nitrite content of biotreated spent culture (17.0, 18.0, 17.5, 10.5, 28.0, 15.0, 19.0 and 4.0 ppm) and (iii) 96 h resting mycelial growth of *A. awamori, A. flavus, A. niger, A. oryzae, F. oxysporum, R. oryzae, Trichoderma sp. and S. rolfssi* degraded PNP (30.0, 16.0, 9.7, 19.0, 26.7, 14.0, 19.0 and 11.7 ppm, respectively with release of nitrite (22.0, 10.0, 8.8, 15.0, 23.8, 11.0, 10.3 and 8.2 ppm respectively) and (iv) almost all fungal strains utilize PNP as C and N source in the said medium. Among 8 fungal cultures, 48 h pre-grown mycelial growth of *F. oxysporum* showed maximum PNP degradation (22 ppm) with release of nitrite (17 ppm). From these, it is evident that mycelial growth (48 h) of *F. oxysporum* was metabolically better for PNP degradation.

Conclusion

The strains *Aspergillus flavus* NCIM 650, *Aspergillus oryzae* NCIM 1212, *Aspergillus niger* NCIM 1025*, Fusarium oxysporum* NCIM 1008, *Rhizopusoryzae* NCIM 997, *Scleratiumrolfssi* NCIM 1048 and some agriculturally important microbes including *Aspergillus awamori*, *Trichoderma sp*has potential to mineralize PNP whereas *Fusarium oxysporum* NCIM 1008 showed maximum PNP degradation (22 ppm) with release of nitrite (17 ppm). From these, it is evident that mycelial growth (48 h) of *F. oxysporum* was metabolically better for PNP degradation.

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