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Biodegradation of P-Nitro Phenol by an Actinomycete

KEYWORDS: Biodegradation, p-nitrophenol, Actinomycetes.

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ABSTRACT *p-Nitrophenol (PNP), a major nitroaromatic xenobiotic is released into the environment as a result of its widespread use and as a breakdown product of organophosphate (OP) agricultural pesticides such as parathion and methyl parathion. It is highly toxic to soil microflora and other non-target organisms. Two strains of actinomycetes A1 and A5 isolated from premises of pesticide industries were found to be promising PNP biodegraders, These srains could degrade up to 300 and 400 mg/L PNP respectively,remarkably high concentrations as PNP is quite toxic even at low concentrations. As strain A5 seem promising, it was subjected to phenotypic and genotypic characterization. The phenotypic characteristics of this strain was typical of actinomycetes. 16S recombinant deoxyribonucleic acid (rDNA) sequence analyses indicated strain A5 to have the highest level of sequence similarity with Streptomyces coeruleorubidus strain HBUM174910, EU841625.1(99%).Further studies on optimization, kinetics and pathways of biodegradation can improve the biodegrading capacities of these actinomycete strains.*

Introduction

Nitroaromatic compounds, including nitrophenols, are widely distributed in environment. p-nitrophenol or 4-nitrophenol (also called para nitrophenol and abbreviated as PNP) is an important member of the nitrophenol group.This chemical is a manufactured item that does not occur naturally in the environment (1).p-nitrophenol has applications in agriculture, dyes/pigments, engineering polymers and pharmaceuticals. P-nitrophenol is used as fungicide for leather, production of parathion and organic synthesis (6).As fungicide, it is used to control fungal mold on leather. p-nitrophenol is used in specialty industry products used by the military (16).PNP is also used in the manufacture of acetaminophen, a non-aspirin pain reliever and as a raw material in the manufacture of certain dyes and pesticides.p-nitrophenol is also a major urinary metabolite of parathion and can be used as a biomarker of human exposure since it is a breakdown product of pesticides including parathion and fluoridifen(10).

Most nitrophenols, including p-nitrophenol, enter the environment during manufacturing and processing. It readily breaks down in surface waters but takes a long time in deep soil and in groundwater. p-nitrophenol is toxic to plant, animal and human health. Animal studies suggest that p-nitrophenol may cause a blood disorder (1).Acute exposure of p-nitrophenol may lead to methemoglobin formation, liver and kidney damage, anemia, skin and eye irritation, and systemic poisoning. According to the Standards for Chemical Products in UK, the use of p-nitrophenol is no longer recommended(17).

In view of its toxic effects, efforts have been made to examine the removal of p-nitrophenol from effluents. Though studies have been conducted to investigate the p-nitrophenol degradation. For this study we have collected different soil samples surrounding pesticide, pharmaceutical dye and polymer industries, as well as contaminated agricultural land soil, specific effluents. Isolation on selective media to obtained Micro organisms belonging to groups of bacteria, fungi, actinomycetes and yeasts (Fig.1). Screening is based on maximum growth in presence of xenobiotics like P-nitrophenol.The efficient cultures were screened out from different groups of micro organisms as mentioned above. Depending upon degradation rate of PNP total 23 actinomycetes cultures have been isolated. On the basis of biotransformation efficiency A1 and A5 were screened based on maximum degradation of PNP. These strains were used further to study growth parameters like optimization of pH, Temperature, Growth profile activity, substrate concentration, and carbon and nitrogen source (9).

The further degradation studies were carried out using the A5 isolate. Strain A5 was identified by 16srDNA sequence analysis and morphological and biochemical characterization. The enzymes involved in the biodegradation of PNP are under study.

Material and Methods

Enrichment and Isolation

Enrichment was performed by successive sub-culturing of soil samples from premises of pesticide industry. One gram of soil sample was used as the inoculum suspended in mineral salt glucose (MSG) medium (pH 7.3) 100 ml in 250 ml Erlenmeyer flask. PNP 100 ppm was added aseptically to sterilized and cooled medium. The flask was incubated at 30°C on rotary shaker. After 7 days 2ml of this culture was transferred to 100ml of fresh medium. The process was repeated for a total four transfers step by step. After one month of acclimatization, the last enrichment culture flask was used to isolate microorganisms on mineral salt glucose agar containing PNP. The morphological characterization and gram staining of Actinomycete culture were carried out. The pure cultures were maintained on mineral salt glucose agar. The isolates which grew fastest and rapidly turned the culture from yellow to colorless were selected for further investigation.

Culture Medium and Condition for Propagation of Actinomycete

MSG medium (pH 7.3) containing glucose, 4; K_2HPO_4, 0.65; KH_2PO_4, 0.2; $MgSO_4.7H_2O$, 0.09 and $FeSO_4$, 0.01gL^{-1} was supplemented with filter sterilized (0.45 µm membrane filter) PNP at an effective concentration of 100 ppm. Actinomycete strain was propagated aerobically on a rotary shaker (100 rpm,30°C)

Screening

The primary screening was carried on the basis of ability of the culture to grow in presence of PNP (100 ppm).A total of 23 PNP degraders were selected on the basis of change in

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the colour of the medium from yellow to colourless. Secondary screening was carried out on the basis of rate of degradation of PNP (100 ppm) in terms of nitrite production. Two Actinomycete isolates A1 and A5 were selected on the basis of their ability to degrade PNP.

Intact Cell Preparation

MSG broth containing 100 ppm of PNP was prepared and was inoculated with selected Actinomycete strains A1 and A5. These flasks were incubated on rotary shaker at 100 rpm for 48 hrs. After 48 hours cells were harvested by centrifugation at 1000 rpm for 10 min. The cell pellet was washed with saline to remove the traces of growth medium. These intact cells were suspended in saline and used further to study growth parameters.

Phenotypic characterization

Various morphological, physiological and biochemical characterization tests were carried out using the standard procedures (Cappuccino and Sherman, 2004; Bergey's Manual of Systematic Bacteriology edition IV). Phenotypic characteristics of the biodegrading strains are listed in Table 1.

Phylogenetic analysis

16S rDNA sequencing of the biodegrading strains were performed with the help of Xcelris Labs Ltd.,Gujrat. The complete 16S rDNA sequence was submitted to EzTaxon server (3) which contains manually curated databases of type strains of prokaryotes. Related strains were selected for alignment by CLUSTAL W program and phylogenetic analyses were done according to the neighbor-joining method (11) using the MEGA version 4.0 (14). To determine the support of each clade, bootstrap analysis was performed with 500 replications (4). The phylogenetic tree of this strain are shown in Fig.3.

p-Nitrophenol biodegradation studies

MSG broth containing various concentrations of PNP, viz. 100 ppm to 500 ppm was prepared, adjusted to optimum pH and inoculated with 1% intact cells of the respective culture. All flasks were incubated on rotary shaker at 100 rpm for 48 hours at respective optimum temperature and the rate of PNP degradation and estimation of nitrite was measured by standard method as described (15). The results are as shown in Fig.2.

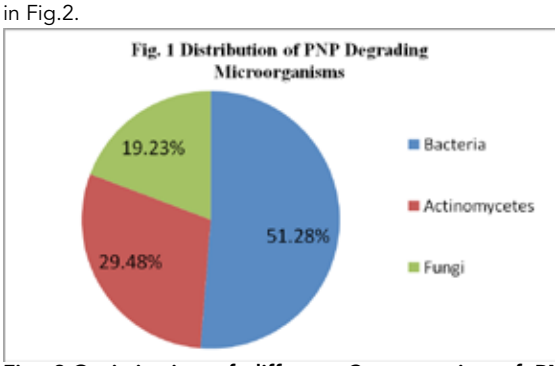

Fig. 2:Optimization of different Concentration of PNP degradation

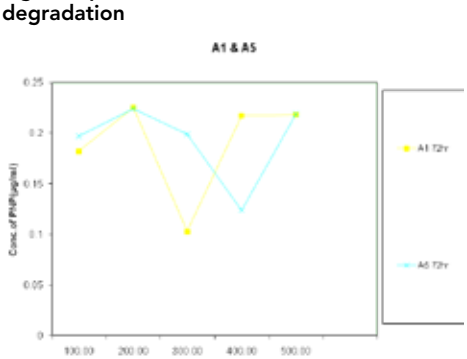

Table-1
Phenotypic characterization of the strain A5

Characteristics	Reaction
Colony morphology	
Shape	Round
Entire	Margin
Elevation	Convex
Gram's staining	+
Production of diffusible pigment	-
Growth at	
4°c	-
20°c	+
25°c	+
30°c	++
37°c	+++
42°c	+++
50°c	-
60°c	-
Growth at (pH)	
4.0	++
5.0	+++
6.0	++++
7.0	++++
8.0	+++
9.0	+++
10.0	++
Growth in the presence of NaCl	
0.2% NaCl	++++
0.5% NaCl	++++
1% NaCl	++++
2% NaCl	+++
5% Nacl	++
7% Nacl	++
10 % Nacl	-
Hydrolysis of	
Casein	+
Starch	+
Urea	-
Catalase	+
Oxidase	-
Methyl Red (MR)	-
Voges Proskauer (VP)	
Citrate utilization	-
Indole production	-
Nitrate reduction	-
H2S production	-
Utilization of C sources	
D-Glucose	++++
D-Fructose	+++
Maltose	++
Glycerol	++++
Manitol	+
Lactose	++++
Sucrose	++
Starch	++++
Utilization of N sources	
Arginine	++
Cysteine	-
Histidine	-
Alanine	+++
Potassium nitrate	++++
Arginine	++
Valine	-
Asparagine	+++
Glycine	+++
Glutamicacid	-
Proline	+++
Tryptophan	++
Lysine	+
Cystine	-

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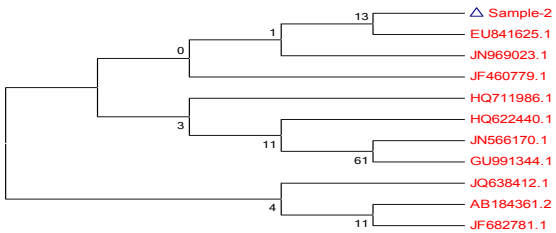

Fig.3.Phylogenetic relashionship of strain A5 with its closest homologos.

Result and Discussion

23 Actinomycetes cultures were isolated from soil capable of growing on mineral salt glucose (MSG) medium containing p-nitrophenol as sole source of carbon and nitrogen .As shown in Fig.1. Out of these 23 isolates 2 strains viz. A1 and A5 were screened based on degradation rate of PNP in terms of nitrite production . The isolates A1, A5 exhibited 52% and 62% degradation in 48 hrs.The toxicity of high concentrations of PNP inhibits its degradation by most microorganisms (5). The further degradation studies were carried out using the A5 isolate.Phenotypic and biochemical characterization of the efficient isolate reveals that the Streptomyses sp.has optimum temperature between 37°c to 42°c.The optimum pH was found to be 7.0 and can tolerate NaCl upto 2%,can hydrolyze casein & starch.The biochemical characters & the 16s rDNA sequence analysis identified the isolate as Streptomyses coeruleorubidus.

There are only a few reports on PNP degradation by Rhodococcus species (12). Only one report exist in the literature for degradation of PNP by Brevibacterium (7). Our findings show this strain to be promising additions to the repertoire of PNP degrading microbial isolates. A1 and A5 could degrade up to 300 and 400 mg/L respectively; quite high concentrations considering that PNP can be highly toxic even at much lower concentrations (8). Complete degradation of PNP was observed in 48 h.

Further studies are now being undertaken to ascertain optimization, kinetics and pathway(s) of PNP biodegradation.

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