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Isolation And Characterization Of PNP Degrading Bacteria From Pharma Industry

S.Anbuselvi*,

Department of Industrial Biotechnology, Bharath University, Chennai-73.India.

***Corres.author: anbuselvichennai@yahoo.com**

Abstract: In industrial areas both metal and organic pollution is of vital concern, interest in bacterial resistance to toxic pollutant chemicals like PNP and salts, especially when associated with degradative activities, Para nitro phenols are used as a intermediates for the synthesis of number of organophosphate pesticides, azo dyes and some medical products as well as their corresponding amino phenols by reduction. In the present study, soil was collected from the pharma company of chennnai found to be degrading Paranitrophenol it was identified as *Bacillus sp* and its genomic DNA, plasmid DNA and its degradating genes were characterized. The maximum activity of PNP degrading bacteria bacillus was observed at 300mg/l.
Keywords: PNP degrading Bacteria, Pharma Industry, Isolation characterization.

Introduction

Biodegradation can reduce wastes and clean up some types of environmental contaminants. through composting, we accelerate natural "Biodegradation" and convert organic wastes to a valuable resource. Different strains of *Pseudomonas*, that are capable of detoxifying more than 100 organic compounds, have been identified. The examples of organic compounds are several hydro carbons, phenols, Organophosphates, Polychlorinated biphenyls (PCBS) and polycyclic aromatics and naphthalene. Microbial degradation of aromatic compounds occurs through aerobic and anaerobic processes Debomanda Ningthoujam., (2005) reported that *Brevibacteria* strain able to degrade PNP. This bacteria was isolated from garden soil in basal salts medium containing Paranitrophenol. This bacterium pure culture could degrade upto 300-mg/l. PNP presence of yeast extract[1]. Microbial degradation of *PNP* has been reported for several bacterial including *Arthrobacter, Bacillus, Flavobacterium, Moraxella* and *Pseudomonas* [2,3,4]. *Pseudomonas* strain YTK 17 isolated using the medium supplemented with mineral salt medium with 10 mg PNP. The isolated bacteria were characterized by determining their partial 16S r DNA sequences (696 – 702 bp)[5].

Paranitophenol is a strong oxidizing bases, acid chlorides, combustible material, organics and reducing agents. Inhalation of paranitrophenol causes irritation to the respiratory tract, coughing and shortness of breath. Ingestion of paranitrophenol cause formation of methemoglobin, resulting in cyanosis (Blue lips, finger nails, ears) headaches, dizziness and other symptoms may include upset stomach weakness, confusion, nausea, cough, sore throat, bronchitis, vomiting, thirsty, rapid heartbeat, ringing in the ears unconsciousness, abdominal pain and fever[6].

When PNP released into the soil, this material is expected to readily biodegrade and is expected to have a half – life of less than one day. . When released into water this material has a half –life between one and ten days and is significantly bio accumulated. When released in to the air this material is moderately biodegraded by photolysis. Para nitro phenols are used as a intermediates for the synthesis of number of organophosphate pesticides, azo dyes and some medical products as well their corresponding amino phenols by reduction[7]. This compound is used on a large scale in the synthesis of the aspirin substitute acetaminophen and in the manufacture of pesticides such as parathion and methyl parathion[8].

Some end products derived from paranitrophenols include dyes and pigments acetaminophen (from P-Amino phenol), carboefuran, (from O-nitro phenol), parathion, Parathion-methyl flurodifen (insecticides from paranitrophenols), nitrofen, bifenox (Herbicides form paranitrophenols) fungicides and rubber chemicals([9]. Nitro phenols are used as pH range indicators (color less at 5 and Yellow at 7) Nitrophenol derivates are used as intermediates for synthesis of number of target products. Paranitrophenol has been used as a substrate for cytochrome P450 2E1 although the major urinary metabolite is its glucoromide conjugate[10] . Para nitro phenol is both a breakdown product and a metabolite of parathion and derivates. Its measurement in urine is used as a one of the markers for exposure to these pesticides, as well as to paranitrophenols itself, which can be absorbed through the skin.

Highly contaminated lake formation was done by pouring the waste water streams of the surrounding industries that include pharmacy, chemical process units, and also many process units, electrical and heavy machinery. The residential streams are also discharged into the lake. The wastes that are discharging into the lake contain many non-degradable organic pollutants and toxic metals.The aim of the present investigation is to study the biodegradation of PNP by soil bacteria from pharma company and possibly isolate and identify the degrading organisms, characterization of metabolites and detection of genes for PNP degradation

Materials and methods

Soil samples are collected from different places in and around the pharma company and are immediately transferred to the laboratory and stored at –20º C freezer. Serial dilution of 1 gram of soil sediments upto 10^{-9} concentration was carried out under laminar airflow. Glassware used for the serial dilution was autoclaved at 15 lbs for 15 minutes. Aliquots of $10^{-1,}$ $10^{-3,}$ $10^{-5,}$ $10^{-7,}$ 10^{-9} dilutions are inoculated into the minimal salt media containing 0.1 mg. of PNP. Soil sample was inoculated into 100 ml. Of minimal salt medium and incubated at 37’ C. with aeration speed 200 rpm for 48 hours. Growth was observed by the turbidity in the conical flask. 1 ml. of growth was transfer to the minimal salt medium with glucose concentration at 1 gm and PNP concentration at 100 mg. and for the incubated for 48 hours[11].

Characterization of PNP

Preliminary characterization of PNP degrading bacterial isolates obtained by enrichment technique was done by morphological identification through gram’s staining, endospores staining, capsule staining, motility test by hanging drop preparation and colony morphology studies.

Analysis of PNP resistance

To determine the tolerance of the isolated bacteria obtained by enrichment technique, isolated organism was incubated at various PNP concentrations ranging from 0.8 mg. to 10 mg. Viability of resistant bacteria isolates is checked by screening on mineral salt medium supplemented with PNP[12].

Biodegradation of metabolites by pnp degradating bacteria

Fermentation degradation of various carbohydrates such as glucose (a monosaccharide), sucrose (disaccharide), cellulose (polysaccharide) by microbes, under anaerobic condition is carried out in a fermentation tube. A fermentation tube is a culture tube that contains a Durham tube (i.e. a small tube placed in an inverted position in the culture tube) for the detection of gas production, as an end product of metabolism. The fermentation broth contains ingredients of nutrient broth, a specific carbohydrate (glucose, lactose, maltose, sucrose, or mannitol) and a P^H indicator (phenol red), which is red at a neutral. The biochemical activity of PNP degradating bacteria was confirmed by catalase,hydrogen sulphide formation, methyl red voges-proskauer and citrate utilization,and urease activity. Hydroxylated derivatives were found to be intermediates of *PNP* degradation pathway which was confirmed by spectrometer at 600nm[13].

Molecular characterization of PNP degrading bacteria

Genomic DNA was isolated from PNP degrading bacteria and the size and purity of DNA isolated were determined by 1% gel electrophoresis[14]. To detect whether PNP degraded genes are Plasmid borne or main chromosomal borne. Curing of the plasmid DNA is done to remove the plasmid from the cells. To remove the plasmid, curing agents like Sodium lauryl sulphate, Sodium dodecyl sulphate, Acridine orange, Mitomycin-C, paranitrophenols can be used at various concentrations. For this study Sodium lauryl sulphte, was used for curing plasmid from the bacterium and to get multiple copies of DNA by PCR[15].

Results and discussion

This *PNP* depredating bacterium was grown on Nutrient Agar medium by Streak plate technique Yellow colour were observed and it was identified as gram positive rod shaped capsulated bacterium and green color spores were observed by endospore staining. After 48 hrs of incubation it was observed that PNP degradating bacteria cannot be utilized sugars that were glucose and lactose. The biochemical characteristics of PNP degrading bacteria were confirmed as *bacillus* by various tests shown in Table 1. The isolate was efficient in metabolizing 0.2% w/v *PNP* added to 100ml of degradation mineral salt medium. Bacillus *sp* could degrade PNP upto a maximum level 300mg/l.The PNP degradating activity of bacillus was found to be maximum at37ºC and pH 5-6.Hydroxylated derivatives were found to be intermediates of *PNP* degradation pathway which was confirmed by using spectrometer at 600nm The Genomic DNA was isolated and can be confirmed by the 1% running agarose gel electrophoresis and visualized the strong bands under UV Trans illuminator. (Fig.1).

Curing experiment indicated that PNP degrading genes are found to be location on genomic DNA of *Bacillus sp.*Curing concentration was determined by observing turbidity in test tubes after treating the isolates at different concentrations. A concentration less than lethal concentration was selected as curing concentration. An SDS concentration above 750µg/ml was lethal to the isolates. Hence, 750µg/ml was used for plasmid curing studies. Absences of plasmid from cured isolates are confirmed by 1% Agarose gel electrophoresis. Two primers (CDO primer, TOD primer) were used for *bacillus* DNA amplification. These primers produced various bands (both monomorphic and polymorphic).The primer CDO and TOD gave the bands in *bacillus.* No. of bands amplified was compared with the standard DNA molecular Marker (Fig**2).**

Table 1: Biochemcal tests for identification of *Bacillus sp*

S.No	Biochemical tests	Inference
1	Catalase Test	+ve
2	Methyl red Test	+ve
3	Indole production Test	-ve
4	Voges-Proskauer Test	-ve
5	Glucose-lactose utilization Test	-ve
6	Citrate utilization Test	+ve
7	Urease	+ve

Conclusion

In the present study isolate obtained from soil from the Hussain Sagar Lake was found to be degrading Paranitrophenol. By morphological and biochemical studies it was identified as *Bacillus sp.* Bacillus *sp* could degrade PNP upto a maximum level 300mg/l. Curing experiment indicated that PNP degrading genes are found to be location on genomic DNA of *Bacillus sp.* Thus the *bacillus* present in the soil of pharma industry which degrade PNP to less toxic components which protects our environment pollution.

Marker Genomic DNA

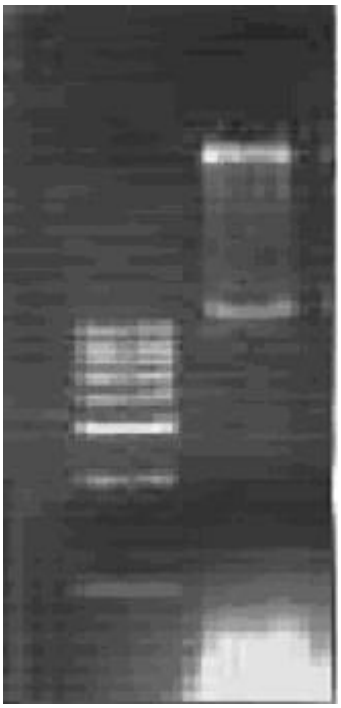

Figure 1:Genomic DNA

Marker PNP degrading genes

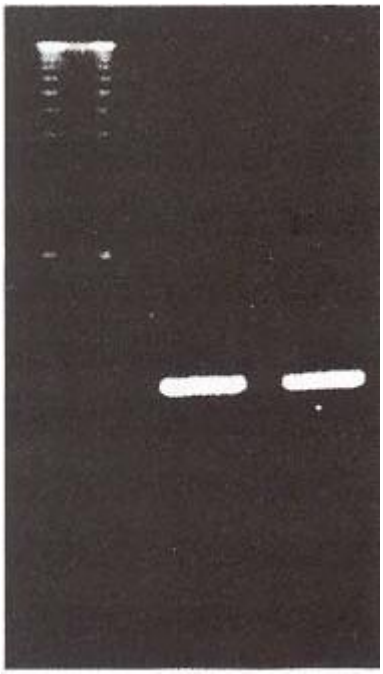

Figure2:PNP degrading genes

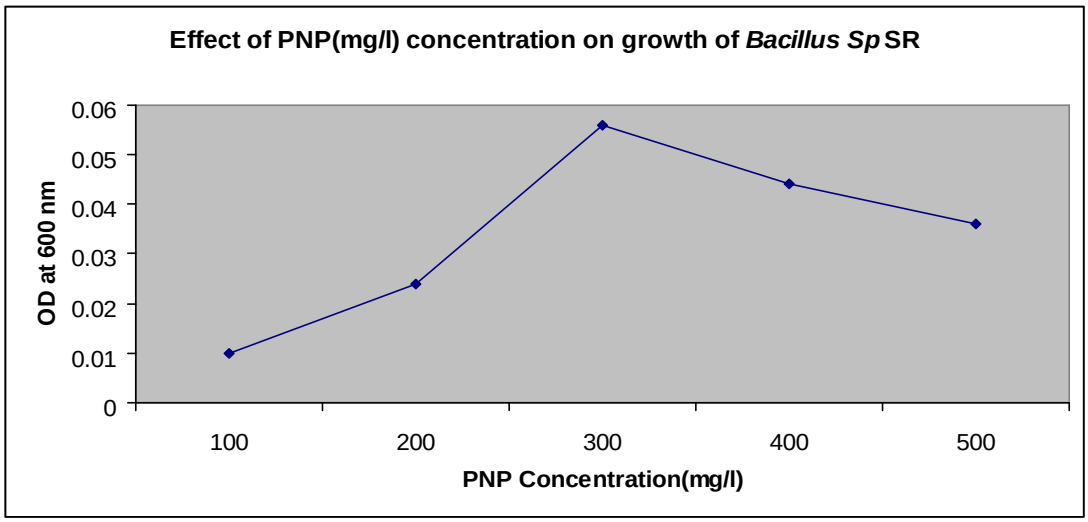

Figure 3:Effect of PNP on groeth of Bacillus

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