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Isolation and Identification of p-Nitrophenol Degrading Bacteria from Polluted Soil

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Abstract **Original Research Article**

Now-a-days due to an extensive industrialization, urbanization has leads concern towards organic pollution. Among the nitro-aromatic paranitrophenols are widely used as intermediates for the pesticides, dyes manufacturing processes. In the present study, a strain of Pseudomonas aeruginosa was isolated from the polluted soil especially in the vicinity of pesticide, chemical industry. This strain was able to utilize PNP as a sole source of carbon and energy by degrading it. The sub-culturing of these isolate on nutrient agar slants, growth on the Cetrimide agar formed well isolated colonies. The organism also gives bluish, greenish pigmentation on PIA (pseudomonas isolation agar). The organism is found to be gram-negative, exhibiting rods, motile. It is the catalase positive, MR positive, VP negative, Indole production is absent, it could utilize citrate as a sole source of carbon, it could rapidly liquefy gelatin and an oxidase negative. This studies have been showed that isolate NP1 have the ability to degrade the PNP. On the basis of the morphological, cultural and biochemical characteristics it was confirmed that the NP1 isolate have been identified as *Pseudomonas aeruginosa*.

Keywords: Bioremediation, Biodegradation, *Pseudomonas aeruginosa*, Paranitrophenol.

INTRODUCTION

The bioremediation technology is most widely used for the remediation of toxic compounds, pollutants from soil. The bioremediation technology is an alternative to all conventional methods as this is the most economic, efficient than other methods. (Liu *et al*., 2007; Labana, 2005; Pandey *et al*., 2006). There are various health consequences of human exposure to PNP like methemoglobin formation, damage to liver and kidney, irritation of skin and eye, poisoning etc. (Qiu, *et al*., 2007).

An extensive literature was showed that the different microorganisms are able to degrade p-Nitrophenol by utilizing it as a sole source of carbon and energy, these are e.g. *Arthrobacter* (Jain and Spain, 1994; Arora, 2012; Chauhan *et al*., 2000), *Bacillus* (Kadiyala and Spain, 1998), *Burkholderia* (Chauhan *et al*., 2000), *Pseudomonas* (Meenal and Ambalal,2006; Wei *et al*., 2010; Zheng *et al*., 2008; Zhang *et al*., 2009), *Alcaligenes* (Pirie *et al*., 2011) and *Rhodococcus* (Leilei *et al*., 2012).

In the present study, it has been reported that the biodegradation of paranitrophenol has been achieved by using the individual *Pseudomonas aeruginosa* strain which is isolated from polluted soil. This bacterium was also shown the biodegradation of PNP in previous studies.

MATERIALS AND METHODS

Chemicals

Paranitrophenol (PNP), Hydroquinone (HQ), MRVP broth, Nutrient agar, Grams Stain, were purchased from Hi-Media, Mumbai and other chemicals used were of the analytical grade (AR).

Enrichment

Soil samples were collected from the waste of pesticide industries. Serial dilution of the samples was done and was transferred into the MS medium containing 100 mg/L of PNP. The MSM (minimal salt medium) containing (g/L) of NaH_2PO_4, 0.75; $Na_2HPO_4.2H_2O$, 2.5; NH_4NO_3, 0.25; $MgSO_4.7H_2O$, 0.2; Ca $(NO)_3$ 0. 1. The pH was adjusted to 7, was autoclaved for 20 min. under 15 lbs pressure.

1 g of soil sample was added to 250 ml Erlenmeyer flask containing 100 ml of MS medium with filter sterilized PNP of 100 mg/L final concentration. The flask then incubated on the rotary shaker with 120 rpm at 28^0C. for 3-4 weeks, simultaneously 5 ml of culture broth was sub-cultured onto fresh MSM-PNP medium. Absorbance was measured at 600 nm.

Isolation

After incubation for 2 to 3 months 5 ml of the culture broth was taken and spread onto the nutrient agar plates and incubated for 48-72 h. The visible colonies formed are picked up and transferred onto nutrient agar plates.

Morphological, biochemical tests

Morphological characteristics were observed by light microscopy while the motility of the organism was performed by hanging drop technique (Gunasekaran, 2000). Starch hydrolysis was performed by standard methods (Gunasekaran, 2000). MR test, VP test, Indole test, Citrate utilization test, Gelatin liquefaction tests, nitrate reduction test were performed (Gunasekaran, 2000; Cappuccio and Sherman, 2004). Oxidase activity was determined by disc method (HiMedia, Mumbai). All the tests were performed by the standard procedures.

RESULTS AND DISCUSSION

The PNP degrading bacteria were isolated on nutrient agar by streaking. The nutrient agar has showed yellow coloration colonies and subjected to the further identification.

Microscopic Observations

The strain was formed bluish-green colonies on Cetrimide and PIA agar. The strain was found to be Gram-negative, with rods in mostly single arrangements, motile, non-spore formers, and best growth was observed at 42^0C and not at 4^0C.

Biochemical tests

Test	Results
Glucose Fermentation	-
Lactose broth	-
Indole test	
MR test	-
VP test	-
Citrate test	+
Nitrate reduction	+
Catalase test	+
Gelatin liquefaction	+
Starch hydrolysis	-

From above microscopic and biochemical tests it has been definitely found that the PNP degrading isolate NP1 is identified as *Pseudomonas aeruginosa.* Further confirmation was done by the growth on the specific media like Cetrimide agar, pseudomonas isolation agar.

CONCLUSION

This study has showed that the isolate obtained from the soil contaminated with pesticides primarily has been identified as *Pseudomonas aeruginosa*. It has capability of surviving in PNP polluted environment. Earlier studies were also confirmed the ability of bacterium *Pseudomonas aeruginosa* to degrade p-Nitrophenol.

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