ISSN No:-2456-2165

Isolation and Identification of Pseudomonas SP. Degrading P-Nitrophenol

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Abstract:- Nitroaromatic compounds are used as raw materials for synthesis of not only plastics, pesticides, pharmaceuticals, but also for explosives, solvents, paper etc. *p*-Nitrophenol (PNP) is one of the nitroaromatic chemical that has a hydroxyl group and a nitro group which is used as transitional for the synthesis of number of organophosphate pesticides, azo dyes, etc. According to the U.S. EPA PNP is a priority pollutant. Enzymes that can degrade the PNP are secreted by soil micro-organisms, of which oxygenases are the key enzymes for aerobic biodegradation of aromatic compounds for the complete mineralization of these compounds. *Pseudomonas sp.*, isolated from farm soil is one of the many micro-organisms that have the ability to produce such enzyme.

Keywords:- *P-Nitrophenol; biodegradation; oxygenases; isolation; Pseudomonas sp.*

I. INTRODUCTION

Due to extensive use of nitro-aromatics, in the synthesis of pharmaceuticals, pigments, azo dyes, pesticides and fungicides, explosives and industrial solvents, paper etc. groundwater and surface waters, have become contaminated with this compound [8]. *p*-Nitrophenol (PNP) is a chemical compound that has a hydroxyl group and a nitro group attached to a benzene ring in para position [1], [4]. The nitrophenolic compounds accumulate in the soil and may enter the natural water resources causing deleterious effects to the biological systems due to their acute toxicity. However, the half-live period of nitrophenolic molecules may be up to 10 days [2]. Biodegradation can reduce environmental contaminants. Different strains of Pseudomonas have been identified that are capable of degrading more than 100 toxic organic compounds. The examples of organic compounds are several phenols, hydro carbons, Organophosphates, Polychlorinated biphenyls (PCBS) and polycyclic aromatics and naphthalene [7]. From the last few decades, extensive research has focused on isolation of microorganisms with the ability to degrade nitroaromatic compounds not only by aerobic but also anaerobic pathways. Anaerobic pathways lead to the formation of aromatic amines, while aerobic pathways lead to formation of hydroquinone and nitrates. However, bacteria utilizing nitro-aromatics as a sole source of C and/or N are very rare [4], [3]. However, due to the high toxicity of PNP to microorganisms, biodegradation of PNP was mostly studied at lower concentrations. Some aerobic microorganisms that have an ability to utilize PNP and degrade them been found to exist in environment. Many microbial enzymes such as oxygenases, reductases, dehalogenases, hydroxylases, dehydrogenases, etc. are involved in the degradation of these pollutants. Because of their involvement in the ring cleavage, oxygenases are the important enzymes for aerobic degradation by microorganisms of aromatic compound [4]. Two pathways have been characterized among PNP degrading bacteria; one degradation process leads to the formation of 4-Nitrocatechol (4-NC) and other leads to formation of hydroquinone (HQ) [4], [6].

The objective of the present investigation is to isolate and identify the potential PNP degrading microorganism from soil.

II. MATERIALS AND METHODS

The enrichment media (Na_2HPO_4-5.8g, KH_2PO_4-3g, NaCl-0.5g, NH_4Cl-1 g, PNP- 20mg, Distilled water- 1000 ml) with PNP inoculated with 10% filtered soil suspension derived from garden soil form the department campus and farm soil. The inoculated broth was incubated at 30-35°C temperature, at 100 rpm. 100µl of diluted cultures were spread on Nutrient agar containing PNP plates using glass spreader. The plates were incubated at 37ºC for 24-48 hrs. After incubation plates were observed for bacterial colonies [4], [5]. Each bacterial colony showing promising zone of clearance was selected and pure culture was prepared. The colony from garden soil was named C1p and colony from farm soil was named C2p. The bacteria from C2p were used for their morphological and biochemical characterization as they showed more zone of clearance.

III. RESULT AND DISCUSSION

The colony C2p showed promising zone of clearance (fig. 1). The colonies were round with entire margins, showing smooth, raised surfaces with yellowish pigmentation and the organism was Gram negative short thin rods (Table I). Growth was observed at 37^0C. Starch hydrolysis gave negative results while lipid hydrolysis gave positive results where urea hydrolysis was negative. Catalase test was negative but gelatin liquefaction test was positive. Acid production from a variety of carbohydrates gave positive results. The H_2S production test was positive so was oxidase and phenylalanine deaminase test. The indole and Voges- Proskauer tests were negative while methyl red and citrate utilization tests were positive (Table II). On the basis of these tests, the organism was identified as *pseudomonas* sp. Thus, isolates may be potential agents for biodegradation of nitroaromatic xenobiotics, PNP to less toxic compounds environment.

The production and detailed characterization of Nitrophenol Oxygenase from *Pseudomonas* sp. and detailed

metabolic pathways involved in PNP degradation by bacteria will be the target of further studies, towards the aim of exploiting it for bioremediation of contaminated sites.

IV. FIGURES AND TABLES

Fig 1:- Pure culture on NA + PNP plate showing clear zone

Characteristics	C_{2P}
Size	3mm
Shape	Circular
Colour	Yellowish
Margin	Entire
Opacity	Translucent
Consistency	Sticky
Elevation	Raised
Gram nature	Gram –ve Short thin Rods

Table 1. Colony Characteristics

Tests	C_{2P}
Catalase test	-
Galatin hydrolysis	+
H_2S production and motility	+
IMViC test	
Indole test	-
Methyl red test	+
Voges-Proskauer test	-
Citrate utilization test	+
Lipid hydrolysis	+
Oxidase test	+
Phenylalanine deaminase test	+
Starch hydrolysis	-
Urease test	-

Table 2. Biochemical Tests

V. ACKNOWLEDGMENT

We would like to extend my gratitude and sincere thanks to Principal, HOD, Department of Biotechnology, Rajarshi Shahu Collage (Autonomous), Latur for giving permission to undertake the work at Department of Biotechnology, R. S. College (Autonomous), Latur.

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