ISOLATION AND IDENTIFICATION OF BACILLUS SUBTILIS DEGRADING P-NITROPHENOL

Suraj Kadam
Dept. of Biotechnology
R.S.M. Latur
Latur, India
Suraj.dkadam21@gmail.com

Suryaji Patil
Dept. of Biotechnology
School of Life Sciences, S.R.T.M.U. Nanded
Nanded, India
psuryaji@gmail.com

Abstract **–** Nitroaromatic compounds are used as raw materials for synthesis of not only plastics, pesticides, pharmaceuticals, but also for explosives, solvents, 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 United States Environmental Protection agency (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. *Bacillus subtilis,* isolated from garden soil is one of the many micro-organisms that have the ability to produce such enzyme.

Keywords **–** p-Nitrophenol, biodegradation, oxygenases, *Bacillus subtilis*

I. INTRODUCTION

The buildup of nitrophenols in the soil has been the concerning fact and the reasons for this upsurge is due to extensive use of nitro-aromatics, as an intermediary in the synthesis of pharmaceuticals, pigments, azo dyes, pesticides and fungicides, explosives and industrial solvents, etc. *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]. 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]. 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 [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 like oxygenases, reductases, dehalogenases, hydroxylases and dehydrogenases 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) [5].

The aim of the present investigation was to isolate and identify the 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 culture was spread on Nutrient agar with PNP plates using glass spreader. The plates were incubated at 37°C for 24-48 hrs. After incubation plates were observed for bacterial colonies. The colonies showing colourless zone were selected from each plate namely C1 from farm soil and C2 from garden soil and pure culture was prepared using nutrient agar containing *p*- Nitrophenol. The colonies from C1 were used for their morphological and biochemical characterization because of their zone of clearance**.**

III. RESULT AND DISCUSSION

The colonies were round with entire margins, showing smooth, flat surfaces with whitish pigmentation. The organism was Gram positive rods and was spore forming. Growth was observed between 10 to 37°C. Starch and lipid hydrolysis gave positive results where urea hydrolysis was negative. Catalase test was positive so was gelatin liquefaction test. Acid production from a variety of carbohydrates gave positive results. The H_2S production test was negative so was the oxidase and phenylalanine deaminase test. On the basis of these tests, the organism was identified as *Bacillus subtilis.* Thus, isolates may be potential agents for biodegradation of nitroaromatic xenobiotics, PNP to less toxic compounds environment.

The production Nitrophenol Oxygenase from *Bacillus subtilis* and detailed metabolic pathways and genetic characterizations with isolation of plasmid and genomic DNA and identification of genes 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

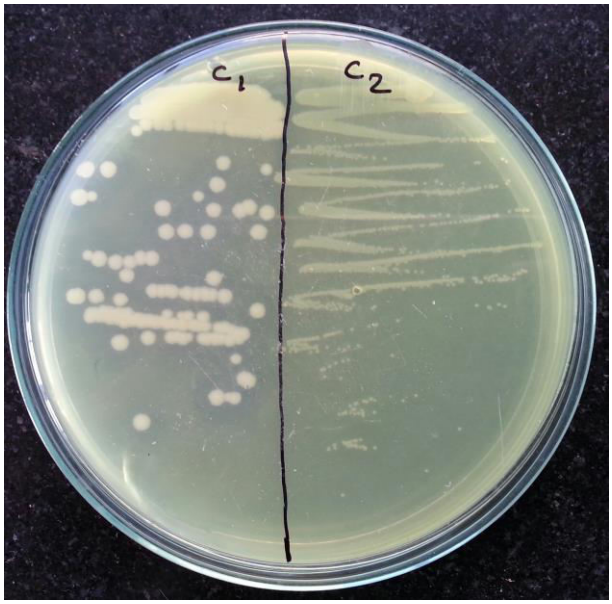

Fig.1. Pure culture on NA + PNP plate showing clear zone

Table I: Colony characteristics

Characteristics	**C_1**
Size	3.5mm
Shape	Circular
Colour	Whitish
Margin	Entire
Opacity	Opaque
Consistency	Sticky
Elevation	Flat
Gram Nature	Gram +ve Rods

Table II: Biochemical tests

Tests	**C_1**
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	-

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Author Profile

Suryaji C. Patil
Department of Biotechnology,
School of Life Sciences, S.R.T.M.U. Nanded,
Nanded, India
psuryaji@gmail.com