

Short Communication

Isolation and Identification of a *Brevibacterium linens* strain degrading p-nitrophenol

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A bacterium was isolated from garden soil in basal salts medium containing p-nitrophenol (PNP). Subsequent subcultures in basal salts agar, nutrient agar plates and agar slants by streaking led to isolation of pure colonies. The pure culture could degrade up to 300 mg/L PNP in presence of yeast extract. It was Gram positive rods, mostly single, catalase-positive, hydrolyzing starch and casein but not urea. Gelatin liquefaction was positive whereas acid production from carbohydrates was negative. It showed tyrosine clearing and had meso-DAP as the characteristic cell wall amino acid. On the basis of the morphological, physiological, and biochemical tests the organism was identified as *Brevibacterium linens*. To our knowledge, this is the first report of any *Brevibacterium* strain able to degrade PNP.

Key words: p-Nitrophenol, nitroaromatics, (bio) degradation, isolation, *Brevibacterium*.

INTRODUCTION

Nitroaromatic compounds are raw materials for synthesis of pesticides, pharmaceuticals, plastics, azo dyes, explosives and solvents (Bruhn et al., 1987; Spain and Gibson, 1991). They are produced in the order of several thousands of tons annually (Marvin-Sikkema and de Bont, 1994). Nitro groups reduce electron intensity of the nitroaromatic ring, thereby impeding electrophilic attack by oxygenases and oxidative degradation of nitroaromatic compounds (Bruhn et al., 1987). Several of them pose serious health and environmental risks, as majority of the nitroaromatics are highly toxic to plants, animals, and microorganisms.

In spite of their toxicity, several microorganisms are capable of mineralizing or transforming nitroaromatics. p-Nitrophenol (PNP) is widely used in the manufacture of pesticides, pharmaceuticals and dyes. It is also the major metabolite resulting from the microbial degradation of parathion and methyl parathion, organophosphate pesticides widely used as agricultural insecticides in several third world countries including India. There are several reports on the biodegradation of PNP (Hanne et al., 1993). Microbial degradation of PNP has been reported for several bacteria including *Flavobacterium*, *Pseudomonas*, *Moraxella*, *Arthrobacter* and *Bacillus*

(Hanne et al., 1993; Dagley and Patel, 1957; Spain, 1995).

The aim of the present investigation was to study the biodegradation of PNP by soil bacteria and possibly isolate and identify the degrading organism. PNP was chosen as it can accumulate significantly in the environment and pose severe risks. Moreover the US EPA has listed o-nitrophenol, p-nitrophenol and 2,4-dinitrophenol as priority pollutants and recommended restricting their concentrations in natural waters to < 10 mg/L (Uberoi and Bhattacharya, 1997). Several aspects of PNP degradation have been reviewed by Spain & Kadiyala, 1998; Zablutowicz, 1999; Heiss, 2003; and Zhao & Ward, 1999

EXPERIMENTAL

Aerobic shake flask cultures were set up in basal salts medium containing PNP. The medium was adapted from that recommended by OECD for biodegradation testing and contained 1.0 mg FeCl₃.6H₂O, 27.5 mg CaCl₂, 22.5 mg MgSO₄.7H₂O, 40.0 mg (NH₄)₂SO₄, 42.5 mg KH₂PO₄, 108.75 mg K₂HPO₄, 166.0 mg Na₂HPO₄.7H₂O and 1.7 mg NH₄Cl. It was adjusted to pH 7.0-7.5 and inoculated with filtered soil suspension (10%, v/v initially and

1%, v/v in subsequent subcultures), derived from 50 g garden soil mixed with 100 ml basal salts medium and mixed in shaker (150 rpm) for 30 min. Incubation of the aerobic batch cultures was done at ambient temperature, usually 30-35°C. Degradation was monitored by visible turbidity and/or disappearance of the characteristic yellow colour of the nitrophenol. When growth was observed, subcultures were set up under the same conditions.

PNP-acclimated (20-100 mg/L) cultures were plated on basal salts agar (2%) and nutrient agar (3.5%) plates by streaking after serial dilutions. Plates were incubated at 37°C for 24 to 48 h. Isolated colonies were subcultured on agar slants containing PNP. Gram staining and other tests were performed to characterize the isolate.

RESULTS AND DISCUSSION

The colonies were round with entire margins, showing smooth, flat translucent surfaces with pale yellow pigmentation. The organism was Gram positive rods, with mostly single arrangement and was non-motile and non-spore forming. Growth was observed between 10 to 37°C. No growth was observed at 4 or 42°C. Optimum pH for growth was 7.0 to 7.5. However growth could be observed at pH 5.2 and 8.0, but not beyond these ranges. The strain could tolerate up to 2.5% NaCl, but not higher.

Starch and casein hydrolysis gave positive results whereas urea hydrolysis was negative. Catalase test was positive as was also gelatin liquefaction test. Acid production from a variety of carbohydrates gave negative results. Tyrosine clearing gave positive result and meso-DAP was found to be the characteristic cell wall amino acid. Growth on nutrient broth was membranous with mucous formation. Many of these tests were performed with the help of the Institute of Microbial Technology, (IMTECH) Chandigarh, India.

On the basis of these tests, the organism was identified as *Brevibacterium linens* and has been deposited at IMTECH, India (MTCC no. 3064). To our knowledge, this is the first report of any *Brevibacterium* strain degrading PNP. It will be interesting to carry out further work on the metabolic profiles, substrate range, toxicity limits and degradative pathways of this organism and compare them with those of other bacteria already reported as agents of PNP biodegradation.

REFERENCES

- Bruhn et al (1987). Nitrosubstituted aromatic compounds as nitrogen sources for bacteria. *Appl. Environ. Microbiol.* 53: 208-210.
- Gesche H, Natalie T, Yoshikatsu A, Masahiro T, Hans-Joachim K (2003). Homologous npdGI Genes in 2,4-Dinitrophenol- and 4-Nitrophenol-Degrading *Rhodococcus* spp.
- Hanne LF et al (1993). Degradation and induction specificity in actinomycetes that degrade p-nitrophenol. *Appl. Environ. Microbiol.* 59: 3505.
- Higson FK (1992). Microbial degradation of nitroaromatic compounds. *Adv. Appl. Microbiol.* 37: 1-19.
- Jain RK et al (1994). Biodegradation of p-nitrophenol via 1,2,4-benzenetriol by an Arthobacter. *Appl. Environ. Microbiol.* 60: 3030.
- Marvin-Sikkema FD, de Bont JAM (1994). Degradation of nitroaromatic compounds by microorganisms. *Appl. Microbiol. Biotechnol.* 42: 499.
- Simpson JR, Evans WC (1953). The metabolism of nitrophenols by certain bacteria. *Biochem. J.* 55 : XXIV.
- Spain JC (1995). Biodegradation of nitroaromatic compounds. *Annu. Rev. Microbiol.* 49: 523-555.
- Spain JC (1995). Biodegradation of nitroaromatic compounds. Plenum, New York.
- Spain JC et al (1979). Enzymatic oxidation of p-nitrophenol. *Biochem. Biophys. Res. Commun.* 88: 634-641.
- Spain JC, Gibson DT (1991). Pathway for biodegradation of p-nitrophenol in a *Moraxella* species. *Appl. Environ. Microbiol.* 57: 812-819.
- Uberoi V, Bhattacharya SK (1997). Toxicity and degradability of nitrophenols in anaerobic systems. *Wat. Environ. Res.* 69:146.
- Venkateswarlu K, Spain JC (1998). A Two-Component Monooxygenase Catalyzes Both the Hydroxylation of p-Nitrophenol and the Oxidative Release of Nitrite from 4-Nitrocatechol in *Bacillus sphaericus* JS905. *Appl. Environ. Microbiol.* 64: 2479-2484.
- Zablotowicz RM, Leung KT, Alber T, Cassidy MB, Trevors JT, Lee H, Veldhuis L, Hall JC (1999). Degradation of 2,4-dinitrophenol and selected nitroaromatic compounds by *Sphingomonas* sp. UG30. *Can. J. Microbiol.* 45(10): 840-8.
- Zhao JS, Wand OP (1999). Microbial degradation of nitrobenzene and mono-nitrophenol by bacteria enriched from municipal activated sludge. *Can. J. Microbiol.* 45: 427-432.