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MITIGATION OF THE TOXIC EFFECTS OF XENOBIOTICS IN MUNG SEEDLINGS BY PLANT SYNERGISTIC BACTERIA

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ABSTRACT

In today's world, the problems due to the rising pollution are inevitable. A major contributor to this pollution is due to the effluents released by textile industries, chemical factories and others. They adversely affect the agricultural lands nearby leading to the accumulation of xenobiotics and heavy metals in the soil followed by barrenness. A great amount of effort had directed towards the exploration of remedial approach by various workers. Employing the indigenous microbes with degradative capabilities *in situ* had been a solution to it. Engineering of microbes is the latest trend for degrading a vast spectrum of compounds. The present study aims to isolate bacteria capable of degrading Phenol, Hexane, Toluene and Xylene to test their synergism with the test plant, *Vigna radiata*. We collected our soil sample from petroleum station. Three-gram negative bacteria (*Proteus* sp., *Pseudomonas* sp and *Aeromonas* sp) and two-gram positive bacteria (*Enterococcus* sp and *Streptococcus* sp) were isolated and identified with test compound degradative potential. They were further used to assess their synergism with the study plant by employing hydroponics technique at 100 ppm concentration of test compound. *Streptococcus*spand*Proteus*spgave a promising result followed by *Enterococcus* sp. The seedlings experienced a negative inhibitory effect with *Aeromonas* sp and *Pseudomonas* sp.

Keywords: Xenobiotics, Hydroponics, Synergism

INTRODUCTION

A country's major economic backbone is its natural resources, human resources and the agricultural empire it possesses. It was reported that over 70% of the rural homes depends on the agriculture as prime means of livelihood. Economically speaking this sector accounts for almost one-third of the country's GDP and is its single largest contributor (India Brand Equity Foundation). It makes it clear that anything that affects the mentioned sector would directly result in a major downfall of Indian economy **(Bhat, 2015).** Over the decades due to the increasing human intervention and for a race of development it has in many ways disrupted the ecological balance. Degradation in the quality of soil, low agricultural output and rapid spreading of the barren land belt is a reflection of this anthropogenic activities. Worldwide, soil is being seriously degraded as a result of increasing industrial, agricultural and civil activities. Soil contamination, both diffuse and localised, can lead to damage to several soil functions and contamination of surface- and groundwater **(Vamerali *et al.* 2010)**. Pali, a district of Rajasthan, is the largest erstwhile hand processing clusters, now gradually moving to power processing machines. The effluents discharged from these units cause environmental pollution. Textile effluents discharged from various textile processing units of Pali, flow about 55 kilometres downstream, making the groundwater in several riverbank villages unfit for drinking and irrigation and also cause an adverse effect on crops productivity and health of people residing in those areas **(Srivastava *et al.* 2014).** Pollution of soils by radionuclides may be of different kinds. An important type of radionuclides originates from the emission to the atmosphere, e.g. nuclear explosions (^{3}H) or reactor operations (^{85}Kr).The subsequent fallout of radionuclides with precipitation and infiltration causes pollution of different aqueous and terrestrial ecosystems **(Groudev *et al.* 2001).** The consumption of this radionuclides and heavy metal-contaminated food can seriously deplete some essential nutrients in the body that are further responsible for decreasing immunological defences, intrauterine growth retardation, impaired physio-social faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates **(Khan *et al.* 2008).** Researchers worldwide are finding ways to cope up with this problem, and a significant amount of work has been done. Phytoremediation, the use of plants for environmental restoration, is an emerging cleanup technology. It uses plants to reduce, remove, degrade or immobilise environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition usable for private or public applications **(Mukhopadhyay and Maiti, 2010).** Research on natural plants is mainly focused on detecting heavy metal hyper-accu3mulator plants and the mechanism of absorption by analysing heavy metal content in the dominant plant **(Wang *et al.* 2004).** Oil spillage or oil pollution on soil has adverse effects on bodies of surface water used by drinking household, industrial purposes aquatic life and the vast tract of agricultural lands. **Emuh 2010** reported that mushroom inoculated in locally sourced substrates showed promise in bioremediation of crude oil polluted the soil. Current evidence suggests that in aquatic and terrestrial environments microorganisms are the chief agents for the biodegradation of molecules of environmental concern, including petroleum hydrocarbons. Hydrocarbon-degrading bacteria, yeast and fungi are widely distributed in marine, freshwater and soil habitats **(Balba *et al.* 1998).**

In this study, we aim to isolate those xenobiotic-degrading bacterial species which shows synergism with our test plant, *Vigna radiata*, so that the physiological ill effects observed due to stress induction by xenobiotic compounds in seedlings can reduce to a greater extent for better productivity. Up to the best of our knowledge, it is the first report on the use of bacteria to reduce the abiotic stress on *Vigna radiata* due to xenobiotic (Phenol, Toluene, Xylene and Hexane) stress.

MATERIAL AND METHODS

Collection and processing of sample

The soil sample collected from a petroleum station expecting to heavily contaminated with various hydrocarbons and xenobiotics. Soil suspension was prepared in saline and used for the screening of xenobiotic-degrading bacteria.

Enrichment of the bacteria

The enrichment of the bacteria was carried out by using Trypticase Soy Broth. The media was supplemented with crystal violet-phenol red to selectively enrich gram-negative bacteria and sodium azide was added to enrich gram-positive ones. Supplemented with 100 and 500 ppm of xenobiotic (phenol, toluene, xylene, and hexane) with respect to control.

Identification of the isolates

The isolates were identified through the biochemical test, and morphological characterization and the results were validated by Bergey's manual of determinative bacteriology.

Hydroponics analysis of the test plant

After two leaf stages of mungbean plants, culturing media were established which consisted 100ppm of xenobiotic supplementation (test group) along with biotic (bacteria+media+plant) and abiotic control (xenobiotic+media+plant) group respectively. The culture bottles were divided into four groups wherein each group 5different bacteria along with biotic control and abiotic control. The germinated seedlings were then transplanted in these culture bottles in the hydroponic system. The mouth of the bottles is wrapped with aluminium foil. The seedlings are placed on the foil by piercing it and inserting the root ensuring it touches the medium. All the culture bottles were then incubated at 26°± 2° C, 2000 lux illumination in tissue culture rack for two weeks. The root shoot lengths of the seedlings are noted, and the physical health of the seedlings is also periodically monitored.

RESULTS AND DISCUSSION

The present study aims to isolate and identify those bacteria possessing the potent degrading capacity of the xenobiotics (test compounds) *i.e.;* Phenol, Toluene, Xylene and Hexane. Bacteria possessing degradative capability may affect the physiological health of the plant and hence were tested for the synergism of the bacterial isolates with study plant through the hydroponic system.

A total of 20 isolates were obtained. Among them, nine were gram positive, and eleven were gram-negative bacteria based onGram staining. Morphological and biochemical characterization test was performed, and the isolates were identified by Bergey's manual of determinative bacteriology (Table 1). The isolates identified are *Proteus*sp., *Pseudomonas* sp.and*Aeromonas*sp. (gram negative) and *Enterococcus* sp and *Streptococcus* sp (gram positive) (Table 2). These isolates were tested for their ability to survive and degrade our test compounds at 100 ppm of xenobiotic concentration. *Pseudomonas* sp was found to tolerate and degrade xylene, phenol under *in vitro* conditions.A similar evidence of*Pseudomonas* degrading phenol and monochlorophenols from the soil samples adjacent to textile, pharmaceuticals, industries and automobile workshops has been reported **(Jame *et al.* 2010; Buitron and Gonzalez 1996; Razika 2010).** In another study, *Pseudomonas* was able to degrade toluene and xylene, but benzene was not metabolised **(Otenio *et al.* 2005; Worsey and Williams 1975; Hemalatha 2011; Nahar 2000).** *Pseudomonas* possess XYL, a Nonconjugative Xylene-Degradative Plasmid which helps it to degrade xylene **(Friello *et al.*, 1976).** *Aeromonas*sp. Degraded hexane, toluene and xylene. *Aeromonas*is capable of good growth on toluene in the range of 6.25 to 386 mg/l **(Nahar, 1999).***Proteus* sp was able to grow on hexane and toluene. It is also able to degrade Benzene, Toluene, Ethylbenzene and Xylene (BTEX) from heavily polluted sites. *Streptococcus* sp was able to grow on toluene, xylene and phenol. It is used in the degradation of phenol from oil contaminated soil **(Bhavna *et al.* 2010).** It is also used for the efficient biotransformation of phenol and its derivative by Catechol 2,3-Dioxygenase metabolism **(Mohite, 2010 and 2015).** *Enterococcus* sp was able to grow on hexane and xylene. It can degrade C.I. Reactive Red 195, an azodye that is extensively used in textile dyeing, paper, printing, colour photography, pharmaceutics, cosmetics and other industries **(Mate and Pathade 2012).**

The test of synergism between the isolates and the study plant was performed by Hydroponics. *Proteus*, *Enterococcus* and *Streptococcus* were being found to be more synergistic with the seedlings helped the plant improved the phenotype in the presence of the xenobiotic [Fig 1].The effect of the isolated bacteria on the root-shoot length of the seedlings was also evaluated [Fig 2].*Streptococcus* sp. was found to be performing maximum degradation followed by *Proteus* sp. and *Enterococcus* sp. respectively[Fig 3].

Table 1 Morphological and Biochemical Characterization Test for the identification of bacterial isolates during the study conduct

Isolate No.	Shape	Microscopic		Biochemical							TSIA				Identification
		Wet Mount	Gram Staining	Indole	Catalase	MR	VP	Urease	Oxidase	Citrate	Slant	Butt	Gas	H_2S	
A	Rod	P/N	N	N	P	N	N	D	P	P	P/AL	Y/A	N	N	*Pseudomonas*
B	Rod	P	N	N	P	N	N	P	N	P	P/AL	Y/A	P	P	*Proteus*
C	Rod	P	N	P	P				p						*Aeromonas*
D	Rod	P/N	N	N	P	N	N	D	P	P	P/AL	Y/A	N	N	*Pseudomonas*
E	Rod	P	N	N	P	N	N	P	N	P	P/AL	Y/A	P	P	*Proteus*
F	Rod	P	N	P	P				p						*Aeromonas*
G	Cocci	N	P		N				N						*Enterococcus*
H	Cocci	N	P	N	N	N	P			P					*Streptococcus*
I	Rod	P	N	N	P	N	N	P	N	P	P/AL	Y/A	P	P	*Proteus*
J	Rod	P/N	N	N	P	N	N	D	P	P	P/AL	Y/A	N	N	*Pseudomonas*
K	Cocci	N	P	N	N	N	P			P					*Streptococcus*
L	Cocci	N	P		N				N						*Enterococcus*
M	Cocci	N	P	N	N	N	P			P					*Streptococcus*
N	Cocci	N	P	N	N	N	P			P					*Streptococcus*
O	Rod	P/N	N	N	P	N	N	D	P	P	P/AL	Y/A	N	N	*Pseudomonas*
P	Cocci	N	P		N				N						*Enterococcus*
Q	Rod	P	N	N	P	N	N	P	N	P	P/AL	Y/A	P	P	*Proteus*
R	Cocci	N	P	N	N	N	P			P					*Streptococcus*
S	Cocci	N	P		N				N						*Enterococcus*
T	Rod	P/N	N	N	P	N	N	D	P	P	P/AL	Y/A	N	N	*Pseudomonas*

Table 2 Microbial isolates obtained at various concentrations of xenobiotic

Xenobiotic	Concentration	Gram Positive	Gram Negative
Hexane	100 ppm	*Enterococcus* sp	*Aeromonas* sp
	500 ppm	*Enterococcus* sp	*Proteus* sp
Toluene	100 ppm	*Streptococcus* sp	*Aeromonas sp, Proteus sp*
	500 ppm	*Streptococcus* sp	–
Xylene	100 ppm	*Streptococcus* sp	*Aeromonas* sp
	500 ppm	*Enterococcus* sp	*Pseudomonas* sp
Phenol	100 ppm	*Streptococcus* sp	*Pseudomonas* sp
	500 ppm	–	–

Figure 1 Hydroponically raising seedlings in the presence of xenobiotic and isolated bacteria (test group) a- control (plant & media), b- *Enterococcus*, c- *Proteus*, d- *Streptococcus*

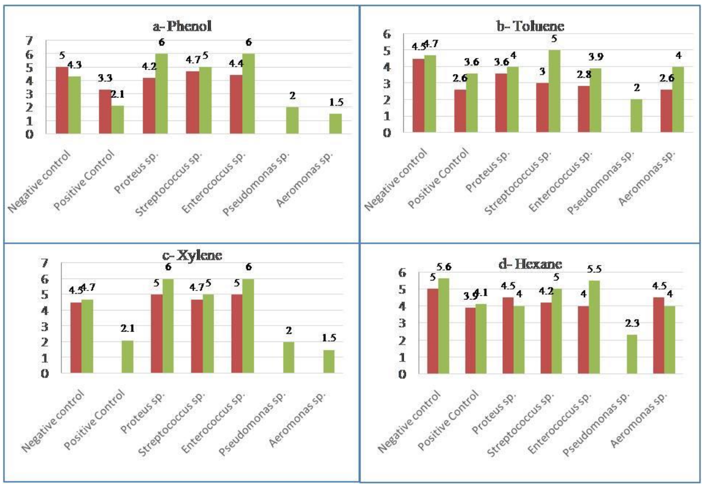
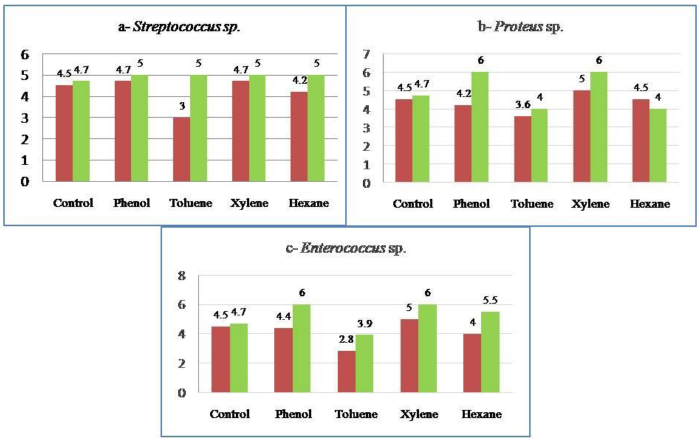


Figure 2 Comparative analysis on xenobiotics from isolated bacteria under hydroponically raised seedling's root shoot length due to: (a) phenol, (b) toluene, (c) xylene and(d) hexane (Red bars- root length (in cms) and Green bars- shoot length (in cms) Negative control: Plant+Medium, Positive control: Plant+Medium+Xenobiotic



Fiureg 3 A glimpse of the efficacy of plant synergistic bacteria (a- *Streptococcus*sp., b- *Proteus*sp., c- *Enterococcus*sp.) on the root-shoot length of the hydroponically grown seedlings(Red bars- root length (in cms) and Green bars- shoot length (in cms)

CONCLUSION

There is a rising demand for the increase in agricultural output to feed the exponentially growing population in India. This comes by compromising with the good agriculture practices such as using organic manure and plant derived pesticides. Due to the increased use of chemical pesticide and fertilisers for an instant benefit, we are disrupting the homoeostasis of the soil biome. The effluents from the industry also directly contribute to this process which in turn elevate the level of xenobiotics and heavy elements in the soil. The worst happens when the land becomes barren due to the persistent use of the chemicals leading to its accumulation and bio magnification in the food chain. The remedial approach for bioremediation, which offers a great promise in future to deal with this problem. Subsequent exploration and designing of microbes capable of degrading spectrum of compounds can be a wise approach to regain the loss of agricultural resources we are incurring now.

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REFERENCES

Balba, M. T., Al-Awadhi, N., & Al-Daher, R. (1998). Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation. *Journal of Microbiological Methods*, *32*(2), 155–164. http://doi.org/10.1016/S0167-7012(98)00020-7

Bhat, R. R. (2015). Industrial Effluents A Major Threat to India. *International Journal of Research*,*2*(4), 115-119.

Buitrón, G., & González, A. (1996). Characterization of the microorganisms from an acclimated activated sludge degrading phenolic compounds. *Water Science and Technology*, *34*(5-6), 289-294. http://doi.org/10.1016/0273-1223(96)00657-9

Emuh, F. N. (2010). Mushroom as a purifier of crude oil polluted soil. *Inter. J. Sci. Nat*, *1*(2), 127-132.

Groudev, S. N., Georgiev, P. S., Spasova, I. I., & Komnitsas, K. (2001). Bioremediation of a soil contaminated with radioactive elements.*Hydrometallurgy*, *59*(2), 311-318. http://doi.org/10.1016/S1572-4409(99)80152-7

Hemalatha, S. (2011). Characterization of Aromatic Hydrocarbon Rading Bacteria from Petroleum Contaminated Sites. *Journal of Environmental Protection*, *02*(03), 243–254. http://doi.org/10.4236/jep.2011.23028

Jame, S. a, Alam, a K. M., Alam, M. K., & Fakhruddin, a N. M. (2010). Isolation and Identification of Phenol and Monochlorophenols-Degrading Bacteria: Pseudomonas and Aeromonas Species. *Bangladesh Journal of Microbiology*, *25*(1), 41–44.http://dx.doi.org/10.3329/bjm.v25i1.4854

Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z., & Zhu, Y. G. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution*, *152*(3), 686–692. http://doi.org/10.1016/j.envpol.2007.06.056

Mate, M. S., & Pathade, G. (2012). Biodegradation of C.I. Reactive Red 195 by Enterococcus faecalis strain YZ66. *World Journal of Microbiology and Biotechnology*, *28*(3), 815–826. http://doi.org/10.1007/s11274-011-0874-4

Mohite, B. V. (2015). Efficient biotransformation of phenol and its derivatives using Streptococcus epidermis by CATECHOL 2, 3-Dioxygenase metabolism. *Environmental Engineering & Management Journal (EEMJ)*, *14*(4).ISSN 1843-3707

Mohite, B. V., Jalgaonwala, R. E., Pawar, S., & Morankar, A. (2010). Isolation and characterization of phenol degrading bacteria from oil contaminated soil.*Innovative Romanian Food Biotechnology*, *7*, 61-65.http://www.bioaliment.ugal.ro/revista/7/paper%2078.pdf

Mukhopadhyay, S., & Maiti, S. (2010). Phytoremediation of metal enriched mine waste. A review. *Global Journal of Environmental Research*, *4*(3), 135–150.

Nahar, N., & Quilty, B. (1999). Cultural conditions for the Growth of Pseudomonas and Aeromonas Spp on Toluene. *Journal of Scientific and Industrial Research*, *58*, 586-590.

Nahar, N., Alauddin, M. & Quilty, B (2000). Toxic effects of toluene on the growth of activated sludge bacteria.*World Journal of Microbiology and Biotechnology*, 16: 307. doi:10.1023/A:1008986823590

Otenio, M. H., Lopes Da Silva, M. T., Marques, M. L. O., Roseiro, J. C., & Bidoia, E. D. (2005). Benzene, toluene and xylene biodegradation by Pseudomonas putida CCMI 852. *Brazilian Journal of Microbiology*, *36*(3), 258–261. http://doi.org/10.1590/S1517-83822005000300010

Friello, D. A., Mylroie, J. R., Gibson, D. T., Rogers, J. E., & Chakrabarty, A. M. (1976). XYL, a nonconjugative xylene-degradative plasmid in Pseudomonas Pxy. *Journal of bacteriology*, *127*(3), 1217-1224.

Razika, B. (2010). Phenol and Benzoic Acid Degradation by Pseudomonas aeruginosa. *Journal of Water Resource and Protection*, *02*(09), 788–791. http://doi.org/10.4236/jwarp.2010.29092

Srivastava, Meenu and Koka, Vinita (2014). Assessment of textile effluent dischage on soil quality in Pali district of Rajasthan. Asian J. Home Sci., 9 (1) : 232-236

Vamerali, T., Bandiera, M., & Mosca, G. (2010). Field crops for phytoremediation of metal-contaminated land. A review. *Environmental Chemistry Letters*, *8*(1), 1–17. http://doi.org/10.1007/s10311-009-0268-0

Wang, Y. B., Liu, D. Y., Zhang, L., Li, Y., & Chu, L. (2004). Patterns of vegetation succession in the process of ecological restoration on the deserted land of Shizishan copper tailings in Tongling city. *Acta Botanica Sinica*, *46*(7), 780–

787.
Worsey, M. J., & Williams, P. a. (1975). Metabolism of toluene and xylenes by Pseudomonas (putida (arvilla) mt-2: evidence for a new function of the TOL plasmid. *Journal of Bacteriology*, *124*(1), 7–13.