International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Research Article Open Access

Bioremediation of Picoline by Adapting *P.Aeruginosa* Isolated from Industrial Effluent Polluted Water

Joseph Anil Mundra*[1], Harika Boorsu[2], Satya Narayana PVV[1], Shabana Mulla[1]

[1]*Department of Biochemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India.*
[2]*Scientific and Applied Research Center, Hyderabad, Telangana, India*

A B S T R A C T

Environmental pollution states alteration in the surroundings but it is restricted in use especially to any Worsening in the physical, chemical, and biological quality of the environment. All types of pollution, directly or indirectly, affect human health. Present scheme of pollution calls for immediate attention towards the remediation and detoxification of these hazardous agents in order to have a healthy living environment. Present study will deal with the use of naturally occurring microbes having the ability of bi ore mediating the Pico line, which is a major environmental pollutant. The present work aimed at identifying the inhabitant bacteria in the highly polluted environments for their property to bio remediate Pico line. The present study concludes that naturally developing species of p. *aeruginosa* can consume Pico line and effectively eliminate it from its nearness and this capacity of this organism is encode by its plasmid.

Keywords: *P. aeruginosa,* Pico line, plasmid, bioremediation and environmental pollution.

A R T I C L E I N F O

CONTENTS

Article History: Received 18 June 2015, **Accepted** 29 July 2015, **Available Online** 15 September 2015

***Corresponding Author**
Joseph Anil Mundra
Department of Biochemistry,
Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur, A.P, India.
Manuscript ID: IJCTPR2704

PAPER-QR CODE

Citation: Joseph Anil Mundra, et al. Bioremediation of Picoline by Adapting *P.Aeruginosa* Isolated from Industrial Effluent Polluted Water. *Int. J. Curnt. Tren. Pharm, Res.*, 2015, 3(5): 1064-1068.

1. Introduction

The waste management technique that involves removal or neutralization of pollutants from a contaminated site using microorganisms is called bioremediation [1]. As day to day production of waste and pollutants being enhanced scientist's interest has grown in the microbial biodegradation procedures. To find feasible ways to clean up contaminated environments [2]. Microbial xenobiotic metabolism is the soul basis behind these bioremediation and biotransformation methods where, this naturally occurring ability of microbes .Transform, degrade, or gather together an extremely large range of compounds including, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), heterocyclic compounds (pyridine or quinolone), hydrocarbons (e.g. oil), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in recent years have deeply explored the microbial key biodegradative pathways and adaptation mechanisms to changing environmental conditions. Unprecedented insights into Proteomic, genomic, bioinformatics, metagenomic and other high-throughput analyses enabled scientists to survey into the details. To promote a feasible development of our society, elimination of varied types of pollutants and wastes with low environmental areimpact is an absolute requirement. Microbial catabolic processes are versatile and by this virtue, degradation or conversion of contaminants takes place.

Genome-based global studies open a new generation in the field of Environmental microbiology, provided unprecedented *in silica* views of metabolic and regulatory networks and clues to the evolution of degradation pathways and to the molecular adoption planning to changing environmental conditions. Development of bioremediation technologies and biotransformation processes requires understanding of close importance of different pathways and regulatory networks to carbon flux in specific environments and for particular compounds. Functional genomic and meta genomic approaches are increasing our understanding and they will certainly accelerate this technology.

Aromatic compounds that are present in petroleum oil contain toxins and they contribute to both episodic and chronic pollution of the environment causing major ecological perturbations. Since oil spills of coastal regions and the open sea are poorly containable, marine environments are especially vulnerable and moderation is difficult. It is estimated that both through human activities and natural seepage about 250 million liters of petroleum enter the marine environment every year [3]. Picoline is a pyridine derivative and has three different methyl pyridine isomers with a common chemical formula CsHyN. Picolines are slowly degraded than their carboxylic acid counterparts and exhibit greater volatility. Volatilization is much extensive in water than in soil, owing to the adsorption of compounds to organic matter and soil clay [4]. Actinobacteria is the major bacterial isolates that mediate picoline degradation. Among the three isomers 3-Methylpyridine is the slowest degrading due to the effect of resonance in heterocyclic ring. Degradation process of picoline generally excretes ammonium to the environment because picolines have nitrogen in excess to the required amount growth of microorganisms [5].

The present work aims at identifying the inhabitant bacteria in the highly polluted environments for their property to bioremediatepicoline.

2. Materials and Methods

Sample collection

Railway new colony is considered very near to the industrial area of Visakhapatnam and has effluent deposition point from various industries like cement, polythene, cosmetic and paint industries. This point is chosen as a potential point for the collection of water. Sample is collected according to the standard WHO guidelines. The collected sample is brought to the lab in 60 min and was transferred to nutrient broth to assist the growth of bacteria.

Preparation and inoculation

The nutrient broth was purchased from Hi Media chemicals, which is in the powdered form. Dissolved 8 g of powder in l000 mL of distilled water and the conical flask was cotton plugged and wrapped with tin foil. This broth was then autoclaved for 15 minutes at 121°C. After the sterilization procedure the broth was taken in 5 different conical flasks each containing 200mL and in these 5 conical flasks ImL of collected water sample was inoculated under sterile conditions and incubated at 37°C for 24 hrs.

Selective medium

Enzymatic Digest of Gelatin provides the nitrogen, vitamins, and carbon in Cetrimide Agar. Magnesium Chloride and Potassium Sulfate enhance the production of pyocyanin and fluorescein. Cetrimide (cetyltrimethyl ammonium bromide) is the selective agent. Cetrimide acts as a quaternary ammonium cationic detergent causing nitrogen and phosphorous to be released from bacterial cells other than Pseudomonas aeruginosa. Agar is the solidifying agent. Glycerol is supplemented as a source of carboa

Acclimation to picoline

The procedure of gradual acclimatization to picoline was performed to make the isolated pseudomonas aeruginosa adapted to the higher concentrations of picoline. This was done by first introducing the inoculums to the culture broth containing 1 part picoline and 9 parts glucose. After observing the growth in this broth, this would be further inoculated in to broth containing 2 parts picoline and 8 parts glucose and so on. This procedure would make the bacteria acclimatized, to gradually increasing picoline concentrations.

Plasmld curing

Add 50ul of SDS to 5ml of LB broth. Picoline resistant *P. aeruginosa* culture is inoculated to LB broth having different concentrations of SDS. Kept it for incubation (24 hrs) in a shaker incubator. After incubation, this culture is prepared for the plasmid isolation. Isolated plasmids are run on agarose gel electrophoresis.

Citrate utilization test

Inoculate Simmons Citrate Agar on the slant by touching the tip of a needle to a colony that is 18 to 24 hours old. Incubate at 35°C to 37°C for 18 to 24 hours. Few organisms require up to 7 days of incubation because of their limited rate of growth on citrate medium. Observe the development of blue color; denoting alkalinization.

Production of Catalasetest

Shift a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick. Place a drop of 3% H2O2 on to the slide and mix. A positive result is the evolution of oxygen (within 5-10 sec.) as evidenced by bubbling. A negative result is no bubbles or only a few scattered bubbles. Dispose of your slide in the biohazard glass disposal container.

Production of Oxidase test

Take a filter paper soaked with the substrate dihydro chloridetetramethyl-p-phenylenediamine. Moisten the paper with sterile water. Pick the colony to be tested with wooden or platinum loop and smear in the filter paper. Find inoculated areaof paper for a color change to deep blue or purple within 10-30 seconds.

3. Results and Discussion

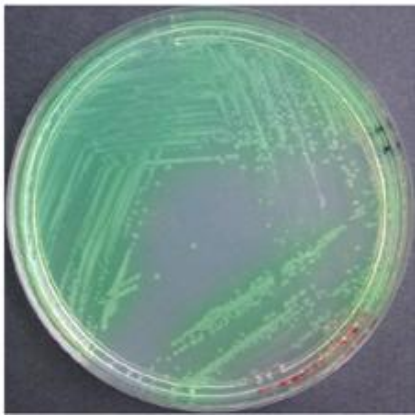

Figure 1: Growth of P. *aeruginosa* on selective medium, which is cetrimide agar medium

The role of bacteria in the biodegradation and detoxification of the toxicants is well documented [5, 6]. Matsumura et al. in 1976 first reported aerobic degradation of HCH, a persistent pesticide, by a Pseudomonas strain [7]. Later on, its degradation by a Pseudomonas paucimobilis was reported by Wada et al. in 1989 [8]. The role of Pseudomonas species in the biodegradation of y-HCH is also well established [6]. The bacteria involved in the metabolism of 2, 4-D and its other derivatives have been extensively studied by several investigators [9,10].

Biodegradations of carbamate pesticides by different bacteria were also demonstrated by several workers [11, 12, 13]. When the turbidity was observed in the inoculated broth, inoculum was prepared for the streak plate technique on selective cetrimide agar medium for *P. aeruginosa.* After inoculation the petriplate was incubated at 37°C for 48 hrs. Light green colonies were observed on the pertidish shown in figure 1. There were single colonies observed, which were carefully transferred to the sterile nutrient medium for further studies.

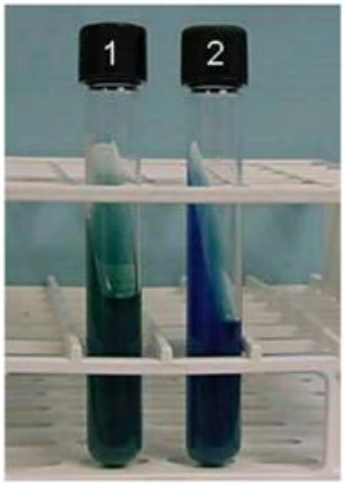

Figure 2: Citrate utilization test. 1) Negative. 2) Positive.

Simmons citrate agar was prepared and sterilized. This was used for the preparation of agar slants to conduct citrate utilization test. Citrate positive: Growth was visible on the slant surface and the medium was an intense Prussian blue. The alkaline carbonates and bicarbonates produced as by-products of citrate catabolism raised the pH of the medium to above 7.6, causing the bromothymol blue to change from the original green color to blue.

Citrate negative:

Trace or no growth was visible. No color change occurred; the medium remained the deep forest green color of the uninoculated agar. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from an uninoculated slant

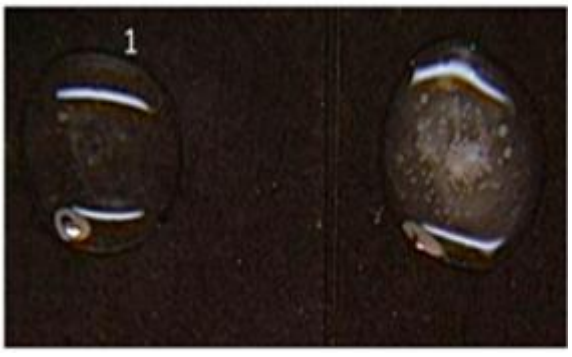

Figure 3: production of Catalase test. 1) Negative. 2) Positive.

Catalase mediates the breakdown of hydrogen peroxide $H_2C>2$ into oxygen and water. To find out if a particular bacterial isolate is able to produce catalase enzyme, small inoculums of bacterial isolate is mixed into hydrogen peroxide solution (3%) and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of

or weak bubble production. When compared to the control 3(1) the test has shown the positive result 3(2).

Figure 4: Production Oxidase test. 1) Negative. 2) Positive.

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenyl enediamine) to (indophenols) purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless.
Positive: Development of dark purple color (indophenols) within 10 seconds
Negative: Absence of color

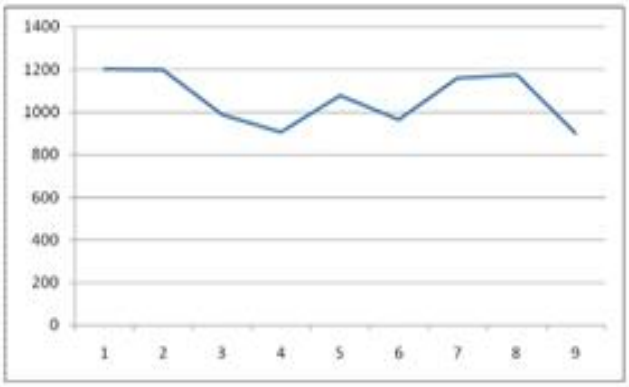

Figure 5: Graph showing the number of bacteria grown at different concentrations of picoline

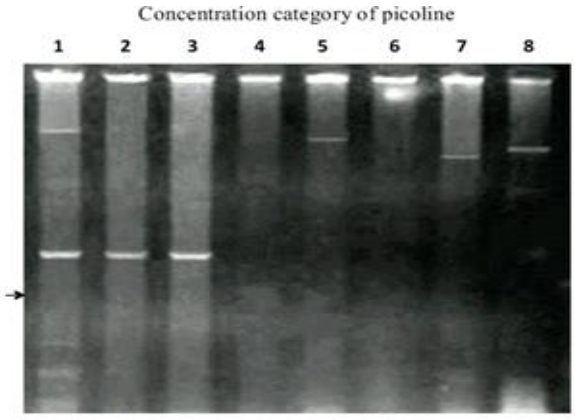

Figure 6: Agarose gel electro phoreslsphotograph showing bands corresponding to the plasmid isolated from the culture grown at different concentrations of SDS.

Acclimation is the procedure that is followed to prepare the bacterial culture adapt to the new environment. The concentration category above represents the concentration enhancement of picoline i.e. as the concentration category increases, picoline concentration increases. The successful adaptation is manifested by the number of bacteria grown in the relevant broth. Figure 5 show that the given bacterial species have developed the function of thriving in the increasing concentrations of picoline, which is otherwise toxic. At highest concentration bacteria failed to thrive, hence number decreased.

Plasmid curing is the procedure to remove the plasmid present in the bacterium. P. aeruginosa which was grown at highest concentration of picoline, was cultured at different concentrations of SDS in the medium i.e. 0.1 (lane 2), 0.2 (lane 3), 0.3 (lane 4), 0.4 (lane 5), 0.5 (lane 6), 0.6 (lane 7) and 0.7 mg/ml (lane 8). After overnight incubation culture was used to isolate the plasmid and run on agarose gel electrophoresis. As per the figure 8 it is said that at 0.3 mg/ml concentration of SDS plasmid curing occurred, hence no plasmid observed at this point (lane 4). These plasmid cured bacteria was also unable to grow on picoline containing medium, confirming that picoline remediating activity is plasmid born.

4. Conclusion

As the picoline is considered as potential carcinogen, its removal from the environment has become the prime concern. The present study concludes that naturally developing species of p. *aeruginosa* can consume picoline and effectively eliminate it from its vicinity and this capacity of this organism is encode by its plasmid.

5. References

1. Ghani ANawaz K, Hussain K, Choudary N, Majeed A, Ilyas U,. Eco-friendly role of biodegradation against agricultural pesticides hazards. African Journal of Microbiology Research. **2011**, 5(3): 177-183
2. Diaz E. Microbial Biodegradation: Genomics and Molecular Biology (1st Ed.). Caister Academic Press. **2008**. ISBN 978-1-904455-17-2.
3. Sims GK, Sommers LE. Degradation of pyridine derivatives in soil. Journal of Environmental Quality. **1985**, 14: 580-584.
4. Sims GK, Sommers LE. Biodegradation of pyridine derivatives in soil suspensions. Environmental Toxicology and Chemistry 1986,5: 503-509.
5. John AK, Dua M, Tuteja, D, Saxena R, Saxena DM, Lal R. Genetic manipulations of microorganisms for the degradation of hexa chloro cyclohexane. FEMS Microbiology Review. **2002**, 19: 69-84.
6. Roane TM, Josephson KL, Pepper IL. Dual-bio augmentation strategy to enhance remediation of co-contaminated soil. Applied and Environmental Microbiology. **2001**, 67: 3208-3215.

7. Matsumura F, Benzet FU, Patil KC. Facto affecting microbial metabolism of y-BHC. Journal of Pesticide Science. **1976**, 1: 3-8.
8. Wada H, Senoo K, Takai Y. Rapid degradation of 2,-HCH in upland soil after multiple applications. Soil Science and Plant Nutrition, **1989**, 35: 71-77.
9. Ahmad M Wasi S, Tabrez S. Suitability of immobilized Pseudomonas fluorescens SM is train for remediation of phenols, heavy metals and pesticides from water. Water, Air, and Soil Pollution. **2011**, 220(l): 89-99.
10. Cho YS, Kahng HY, Kirn CK, Kukor JJ, Oh KH. Physiological and cellular responses of the 2,4-Ddegrading bacterium, Burkholderia cepaciaYK-2, to the phenoxyherbicides 2,4-D and 2,4,5-T.Current Microbiology. **2002**, 45(6): 415.
11. Doddamani HP, Ninnekar HZ. Biodegradation of carbaryl by a Micrococcus species. Current Microbiology. **2001**, 43: 69-73.
12. Barragan-Huerta BE, Costa-Perez C, Peralta-Cruz J, Barrera-Cortes J, Esparza-Garcia F, Rodriguez-Vazquez R. Biodegradation of organochlorine pesticides by bacteria grown in microniches of the porous structure of green bean coffee. *Int. Bio deterioration and Biodegradation*. **2007**, 59: 239-244.
13. Wasi S, Jeelani G, Ahmad M. Biochemical characterization of a multiple heavy metal, pesticides and phenol Resistant Pseudomonas fluorescens strain. Chemosphere. **2008**, 71: 1348-1355.