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Growth in The Presence of Organic Solvents in *Pseudomonas Aeruginosa* and Its *Vitreoscilla* Hemoglobin (Vhb) Recombinant Strain (Pajc)

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Abstract

This study is concerned with the potential use of *Pseudomomas aeruginosa* and its recombinant strain carrying *Vitreoscilla* hemoglobin gene (*vhb*) for degradation of important harmful aromatic compounds as toluene, xylene, benzene and 1-Cl -2,4-Dinitrobenzene (CDNB). *P. aeruginasa* and recombinant strain were grown in 10 ml LB overnight at 37°C in a shaking water-bath at 200 rpm. High aeration/high agitation cultures were incubated in a gyrator water-bath at 200 rpm in flasks containing 20 ml medium. *P. aeruginosa* and its various strains are known for their ease of use characteristics allowing gene transfer, expression and capabilities of degrading the heterocyclic aromatic compounds which are mostly harmful to all organisms. In this study, degradation of these single ring aromatic compounds by the recombinant strain that bears the *vhb* and its parental wild-type strain *P. aeruginosa* was carried out.

Keywords: Pseudomonas Aeruginosa, Vitreoscilla Hemoglobin, Organic Solvents.

Pseudomonas Aeruginosa ve *Vitreoscilla* Hemoglobini (Vhb) Taşıyan Rekombinant Suşunun (Pajc) Organik Solventlerin Varlığında Büyümesi

Özet

Pseudomomas aeruginosa ile onun *Vitreoscilla* hemoglobin geni (*vhb*) klonlanmış rekombinant suşunun önemli endüstriyel aromatik kirleticilerden olan toluen, 1-Cl -2,4- Dinitrobenzen (CDNB), ksilen ve benzen yıkımı çalışılmıştır. *P. aeruginosa* ve rekombinat suşu, 200 rpm 37° C'de 10 ml LB ortamında gece boyu üretildi. Yüksek havalandırma koşulları için 200 rpm ve 20 ml erlende büyütüldüler. *P. aeruginosa* ve çeşitli suşları gen transferi, ekspresyonu gibi çalışmalarda oldukça yaygın olarak kullanılan ve canlılar için çoğu zaman zararlı ve canlılar tarafından yapılmayan birçok heterohalkasal bileşikleri doğal olarak yıkma kabiliyeti ve yüksek solvent direnci ile bilinmektedir. Bu çalışmada *Vitreoscilla* sp.'den elde edilen *vhb* kopyalanmış rekombinant suş ve onun konakçısı yabanıl tip *P. aeruginosa*'nın solvent yıkma kapasiteleri incelenmiştir.

Anahtar Kelimeler: Pseudomonas Aeruginosa, Vitreoscilla Hemoglobini, Organic Solventler.

1. Introduction

Pollution arising as a result of modern life's been focused on solutions today, and at least one of the issues that can be. One of these kinds of impurities and the like contained in the oil, known as BTEX briefly dramatically in its refined products and its alkylated derivatives with toluene, ethyl benzene and xylene is caused. These substances are the most widely known examples of typical aromatic ring compound. Essentially all the aromatic hydrocarbon is a benzene derivative. These compounds as they show similarities although alkenes annular structures also are more stable and do not undergo the reactions of alkenes suffered.

Therefore, they are seen as more dangerous environmental pollutants. With these substances are not made by living creatures just carry toxic to all living things. These substances are applied to the contaminated environment with physical and chemical decontamination methods are far from being a complete and effective solution. Micro molar concentrations of the residue from treatment with these types of technology are even show toxic to animals. Potential availability

of biochemical methods for such transactions in recent years due to have been investigated, and in particular substance has been determined to find alternative uses of *Pseudomonas* bacteria species equipped with the necessary gene systems to break down into less harmful compounds.

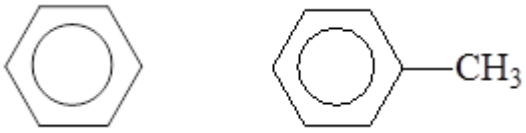
The hemoglobin (VHb) of the bacterium *Vitreoscilla* is the first discovered and probably best characterized of the microbial hemoglobin's [1]. The *in vivo* role of VHb in its native host is suggested oxygen trapping/transfer, allowing this bacterium to survive and grow in micro aerobic environments [2]. Priority pollutants such as benzene, nitrobenzene, toluene and xylene (BTX) are common hydrocarbon constituents produced by petrochemical companies in vast amounts each year. BTX are also common ground water contaminants often found in oxygen-limited aquifers are classified as priority pollutants by the U.S. Environmental Protection Agency (EPA). *Pseudomonas aeruginosa* strains are typically active denitrifies and produce biosurfactants which are very beneficial for bioremediation, as they solubilize and mobilize hydrocarbons.

Having these versatile and unique metabolic characteristics, *P. aeruginosa* is one of the most suitable bacteria in initial studies of sequential hydrocarbon metabolism [3]. Given that *Pseudomonas* sp. are able to degrade a wide variety of organic chemicals, engineering them to produce VHb for better oxygen uptake for growth and metabolism may be of particular usefulness in the bioremediation of toxic wastes in situ where oxygen is limiting [4]. Attempt has been made to overcome the oxygen limitation problem by genetically engineering microorganisms to enhance their survival, growth and ability to degrade aromatic compounds under oxygen limiting conditions [5, 6].

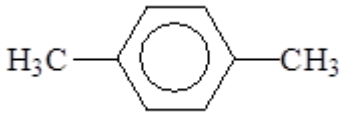
2. Materials and Methods

2.1. Solvents

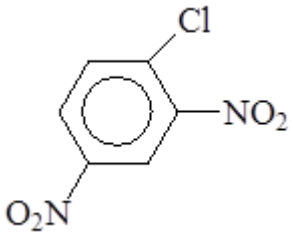
1 –Cl -2,4- Dinitrobenzene (CDNB) (Aldrich Chemicals Co.). Benzene (Riedel-de Haën), Xylene and Toluene (Merck Chemicals Co.) and all other chemicals were of analytical grade.



Benzene Toluene



Xylene



1 -Cl -2,4- Dinitrobenzene

Figure 1. Structure of solvents

2.2. Bacterial strains and growth media

Both *P. aeruginosa* (NRRL B-77) and its transposon-mediated *vhb* transferred recombinant strain named PaJC [6]. The Cells were maintained on LB agar plates at 4 ^{0}C with transfers at monthly intervals. The liquid media used throughout the study were Luria-Bertani (LB) broth. The final pH's of both media were adjusted to 7.0. The concentrations of these compounds, based on our preliminary experiments, in LB were 9 mM, 0.05 mM, 15 mM and 3 mM for benzene, CDNB, toluene, and xylene, respectively.

2.3. Growth conditions

Growth of non-transformed and recombinant cells was compared in LB media. *P. aeruginasa* and recombinant strain were grown in 10 ml LB overnight at 37°C in a shaking water-bath at 200 rpm. A 1/100 inoculum of overnight cultures grown in a LB medium was made in 20 ml of the same medium

in 125 ml Erlenmayer flasks and inocula in flasks were grown for different periods of time at 37°C. High aeration/high agitation cultures were incubated in a gyrator water-bath at 200 rpm in flasks containing 20 ml medium [7]. Growth was monitored by both OD_{600} (cell mass) measurements.

2.4. Statistical analysis

The data represent the arithmetic averages of at least three replicates. The analyses were carried out using *t*- test (95% confidence limit). For clarity, no error bars are given, but they are mostly less than 10 % of the respective data point.

3. Results and Discussion

Benzene, toluene, CDNB, xylene (BTX); an important family of organic pollutants due to their toxicity and constitute a significant risk to organisms and the environment. BTX compounds are also the best solves can of gasoline compounds and often underground storage tanks, pipelines, sea spilled oil by accident, is consider the most common pollutants to which they penetrate the irregular waste storage and liquid due to leakage in the filter that land fill groundwater. These aromatic compounds are of high viscosity. The growth dynamics of *P. aeruginosa* and its *vhb* recombinant, PaJC, in the LB containing any one of BTX compounds are summarized in Figs. 1-4. *P. aeruginosa* and its *vhb* bearing strain (PaJC) were cultivated in shake flasks under 200 rpm agitation rates and 37 °C. The biomass level was determined cultures harvested at 10 h.

In the presence of benzene, the highest level of biomass ratio was *P. aeruginosa* 3,44 (Fig. 1), while this highest value was 3,457 PaJC (Fig. 2) in presence of CDNB, respectively. In the presence of toluene, the lowest level of biomass ratio was both *P. aeruginosa* 2,183 and 2,685 PaJC (Fig. 4), respectively.

In LB medium used to control wild type it was reached higher biomass values *(P. aeruginosa* 4,485 and 3,88 PaJC (Fig. 5), respectively).

Benzene and xylene is the least difference between the two bacteria (Fig. 1 and 3).

Biomass values, about the total cell density information in order to have been considered. CDNB, toluene, xylene environment of the PaJC in accordance with wild type was shown to achieve higher biomass values. CDNB, toluene, xylene environment of the PaJC in accordance with wild type was shown to achieve higher biomass values. Significant differences were not observed in all other media. *Pseudomonas aeruginosa* and *Pseudomonas putida* with a similar study, there was no significant difference between the wild type and recombinant bacterial biomass values in the first 48 hours [8]. Statistical significance of the differences was in the times to be shown on the chart. Most statistically significant difference is observed in the presence of xylene (Fig 3). However, recombinant bacteria reached more biomass values in the presence of aromatics compared to wild bacteria. In this regard, the cleaning of the media may be contaminated with aromatics recombinant bacteria efficiently.

Microorganisms; aerobic, microaerobic, under hypoxic or anaerobic conditions BTX can destroy. BTX's biological degradation has been studied widely and is presently being studied. It is usually faster than anaerobic degradation of the aerobic degradation was observed [9].

Effective as BTX's known to break down all the components are not pure strains of any bacteria. Especially the xylenes, are known to be resistant to bacterial destruction. *Pseudomonas stutzeri* is one of the few organisms that can break down xylene. These compounds are destroyed by the slow destruction that can cause toxicity and microorganisms; they can be successful in the destruction of only low concentrations [10, 11].

The biodegradation of alkyl benzene such as toluene under aerobic conditions it is easier. In bacterial BTX degradation media has been reported to increase the inhibitory effect of coexistence of these substances. Again, the biological degradation of organic contaminants could be observed only during secondary metabolism was induced by nitrogen or carbon starvation [9, 12-14]. In the studies, some of *Pseudomonas*, under hypoxic conditions using nitrate as the terminal electron acceptor has been shown to break down the BTX. However,

compared to benzene, toluene has been shown in these studies faster destroyed [15,16].
However, destruction of the molecular oxygen is needed for the hydroxylation of aromatic rings [17].

Pseudomonas species have been performing the biological degradation of toxic chemicals show great diversity [8, 18-19]. Some of *Pseudomonas* species in the aerobic and facultative chemolithotrophs, chemoorganotrof is heterocyclic and aromatic substances are capable of destroying or using the cellular energy and materials [20].

Pseudomonas the SNF made in some studies to increase the bioremediation has been shown to have significant potential [21, 22].

One of the most important reasons is that they lead to higher uptake of oxygen species transferred VHb compared to wild-type [18].

PaJC was advantageous biomass production in the presence of CDNB. Because often limiting oxygen plays a role in this type of bioprocesses. We have done so far in the literature for similar studies could not find work.

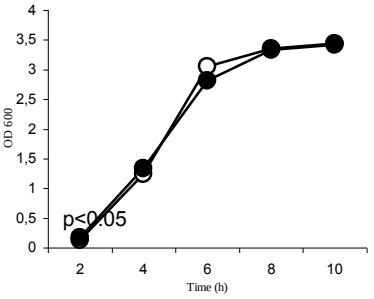


Figure 1. Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in LB medium + **Benzene**

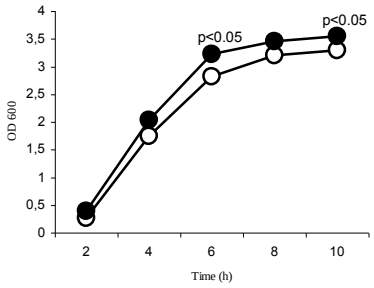


Figure 2. Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in LB medium + **CDNB**

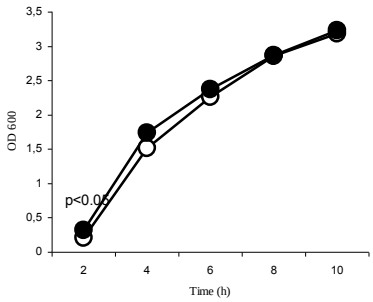


Figure 3. Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in LB medium + **Xylene**

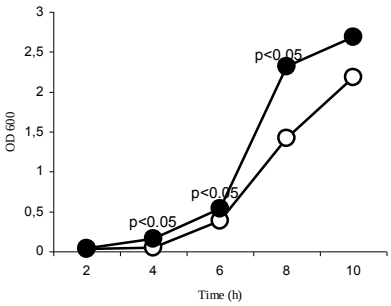


Figure 4. Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in LB medium + **Toluene**

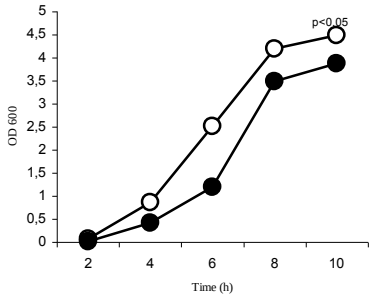


Figure 5. Viable cell mass of *P. aeruginosa* (open symbols) and its recombinant PaJC (filled symbols) strain grown in **LB** medium

4. Acknowledgements

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5. References

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