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Research Article

COMPARISON OF FREQUENCY ANILINE DEGRADING BACTERIA IN THE SOIL AROUND SHIRAZ REFINERY IN SUMMER AND AUTUMN

***Farshid Kafilzadeh and Azadeh Khezri**
Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran
**Author for Correspondence*

ABSTRACT

Aniline is one of the serious hazardous materials for life due to toxicity and carcinogenic effects. It is widely distributed in the soil and surrounding environment. Microbial degradation is one of the main mechanisms to remove it from environment. The aim of this research is to detect the indigenous aniline resistance bacteria from soil around Shiraz Refinery (Iran) and compare these for the term of two seasons in order to select the most powerful aniline resistant bacteria for bioremediation and clean a polluted soil. Sampling from the soil around Shiraz Refinery was done during summer and autumn and cultured in a medium containing 0.1 g/l aniline. Isolated bacteria were identified using the standard methods, these are known to be: *Delftia acidovorans, Enterobacter ludwigii, Raoultella planticola, Alcaligenes faecalis, Serratia marcescens, Microbacterium barkeri, Pseudomonas acidovorans, Erwinia* sp., *Rhodococcus* sp. Findings showed all of these bacteria were able to degrade aniline from the soil around Shiraz refinery and *Delftia acidovorans* and *Rhodococcus* sp. which was found during both time periods, had the highest ability to eliminate aniline.

Keywords*: Aniline, Bioremediation, Delftiaacidovorans, Shiraz Refinery*

INTRODUCTION

Aniline is a vastly distributed pollutant in the environment that results from the manufacturing of dye and agricultural material such as herbicides (Konopka *et al.,* 1989; Kearney and Kaufmann, 1975). Thus, aniline is one of the increasing threats for both environment and human health and becoming a major concern among waste materials (Anson *el al.,* 1984).

Microbial degradation is major mechanism to destroy aniline from the environment (Obinna *et al.,* 2008). Bacterial species such as Pseudomonas (Hinteregger *et al.,*1992), Acinetobacter (Kim *et al.,* 1997), Rhodococcus (Aoki *et al.,* 1983), Frateuria (Murakumi *et al.,* 1999), Moraxella (Zeyer *et al.,*1982), Candida tropicalis (Wang *et al.,* 2011) and Nocardia (Bachofer *et al.,* 1975) have been shown to be able to degrade aniline and its derivatives. Up to now, researchers found that aniline can be removed by aerobic biological treatment (Takeo *et al.,* 1998; Zhang *et al.,* 2008; Chengbin *et al.,* 2009).

It was demonstrated that the soil microorganisms have their metabolic pathways for effective utilization of synthetic compounds, during their mutation or adaptation (Obinna *et al.,* 2008). Previous studies on the aerobic microbial degradation of aniline have indicated that it can be metabolized to catechol as the first intermediate, liberating ammonia, and subsequently undergoing metabolic transformations through ortho- or meta-ring cleavage pathways (Kahng *et al.,* 2000).

Chengbin *et al.,* in 2009 reported a new species of *Delftia* XYJ6 which was involved in the degradation of aniline. A new strain from rhizospheric soil samples was isolated from an agricultural site near the industrial area of Faisalabad, with the ability of degrading aniline with its maximum activity. This strain was known as *Staphylococcus aureus* ST1 (Ahmed *et al.,* 2010). In 2000 a novel microorganism characterized by Kahang *et al.,* Strains HY 99 found to be capable of degradation of aniline in aerobic and anaerobic situation.

In the current research, indigenous aniline resistant bacteria and the most powerful of them, were isolated and identified during summer and autumn, from the soil around Shiraz Refinery (Iran) for bioremediation, instead of using hazardous chemical materials.

MATERIALS AND METHODS

Sampling: Soil sampling around Shiraz Refinery was done during summer and autumn, from three stations A, B, C and from each station 3 times (Table 1). Soil sampling depth was 3 to 5 cm and done by sterile dishes and were transported to the laboratory within 3 hours in ice containers.

Table 1: Geographical location of stations

Station	Geographical location
A	(29˚ 43′ 54.95″ N) , (52˚ 39′ 12.65″ E)
B	(29˚ 43′ 59.19″ N) , (52˚ 39′ 37.5″ E)
C	(29˚ 44′ 00.47″ N) , (52˚ 39′ 34.44″ E)

Counting bacteria: The bacteria were counted by viable plate count method. Dilutions of 10^{-1} to 10^{-9} were prepared by sodium chloride solution from the soil samples. After that, diluted samples were cultured on nutrient agar containing 0.1 g/L of aniline and nutrient agar without aniline. The cultured plates were put in to the 30 °C incubation. Then the colonies were counted after 48 hours.

Bacterial enrichment: Salt based medium was used for enrichment. For this step, 90ml of the basic medium and 10g of the contaminated soil were poured into flasks; then, 0.1 ml of aniline was added to the samples by syringe filter. Enrichment medium was placed in a shaking incubator at 30˚C. After that, enrichment of bacteria was done during the process of enriched medium transference to the fresh medium and adding again the inoculums aniline after seeing the turbidity (Dehghani *et al.*, 2007).

Isolation and identification of aniline-degrading bacteria: Salt based medium with 0.1 g/L aniline was used for the isolation. The bacteria were transferred from enrichment medium to the solid medium and were cultured by sterile swab. Then incubated for 3-5 days at 30˚ C and resistant colonies were purified on blood agar medium. Isolated colonies were identified by various biochemical tests such as oxidase, catalase, Gram stain, TSI, urease, citrate, PD, OD, LD, MRVP, SIM (Schaad *et al.*, 2011).

Statistical analysis: The results were analyzed using SPSS software and analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Counting bacteria

There are differences between stations in terms of the average of bacterial counts. According to the figure1 the maximum number of bacteria is located in station B which is 4.829 ± 0.014 (cfu/ml), and the minimum number of bacteria is located in station A which is 4.669 ± 0.013 (cfu/ml). According to the figure 2 average of bacterial count in summer in presence of aniline shows4.858 ± 0.017(cfu/ml) and without aniline in the same season is 5.226 ± 0.014(cfu/ml). This amount in presence of aniline in autumn is4.203 ±0.017 (cfu/ml) and without aniline shows 4.461± 0.016(cfu/ml).All stations showed a significant difference of five percent by Duncan test. *Delftiaacidovorans* with 25.8% frequency and *Pseudomonasacidovorans* with 19.9% frequency shows the highest frequency compared to other resistant bacteria according to figure3in summer. In autumn the highest frequency according to figure4 belongs to both *Delftiaacidovorans* and *Pseudomonasacidovorans* with 24 % frequency.

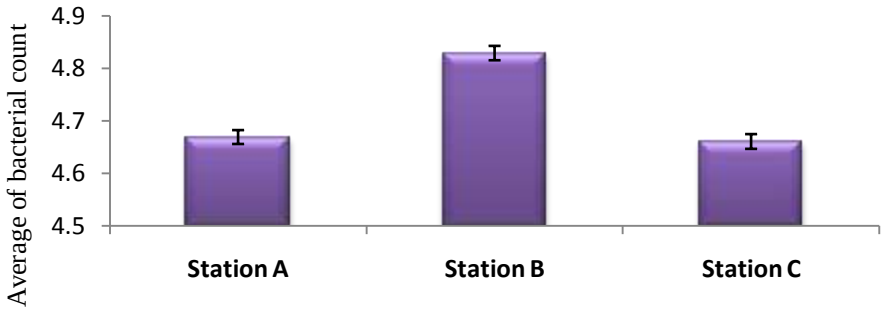

Figure 1: Average of bacterial count in different stations

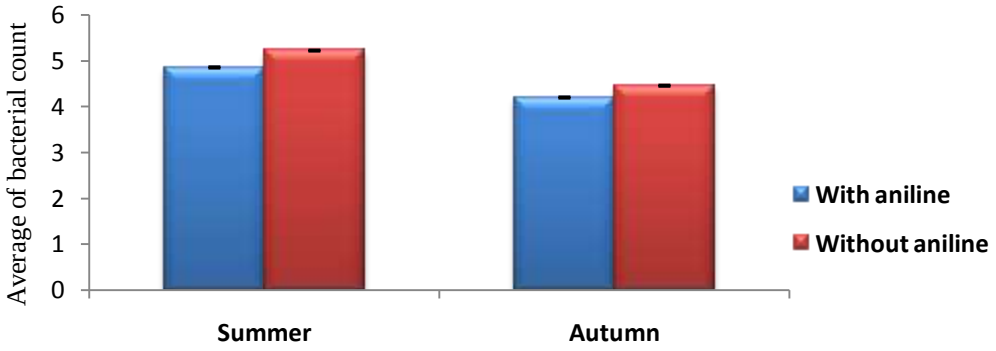

Figure 2: Average of bacterial count in summer and autumn seasons

Isolation and characterization of aniline degrading bacteria:

Nine strains were isolated in summer and six strains in autumn. All of them had well growth, in the presence of aniline. After 3 to 5 days, their colonies appeared in the aniline agar medium, due to the good ability to use this material as the sole carbon and energy source. The isolated strains in summer were identified as *Delftiaacidovorans, Enterobacterludwigii, Raoultellaplanticola, Alcaligenesfaecalis, Serratiamarcescens, Microbacteriumbarkeri, and Erwinia* sp., *Rhodococcus* sp. and *Pseudomonasacidovorans* (figure 3). The isolated strains in autumn were identified as *Delftiaacidovorans, Enterobacterludwigii, Raoltellaplanticola, Alcaligenesfaecalis, and Rhodococcus* sp., *Pseudomonasacidovorans* (figure 4). From the present study, it is concluded that *Delftiaacidovorans, Enterobacterludwigii, Raoultellaplanticola, Alcaligenesfaecalis, Serratiamarcescens, Microbacteriumbarkeri, Pseudomonasacidovorans, Erwinia* sp., *Rhodococcus* sp. have ability for degradation of aniline from soil around Shiraz refinery.

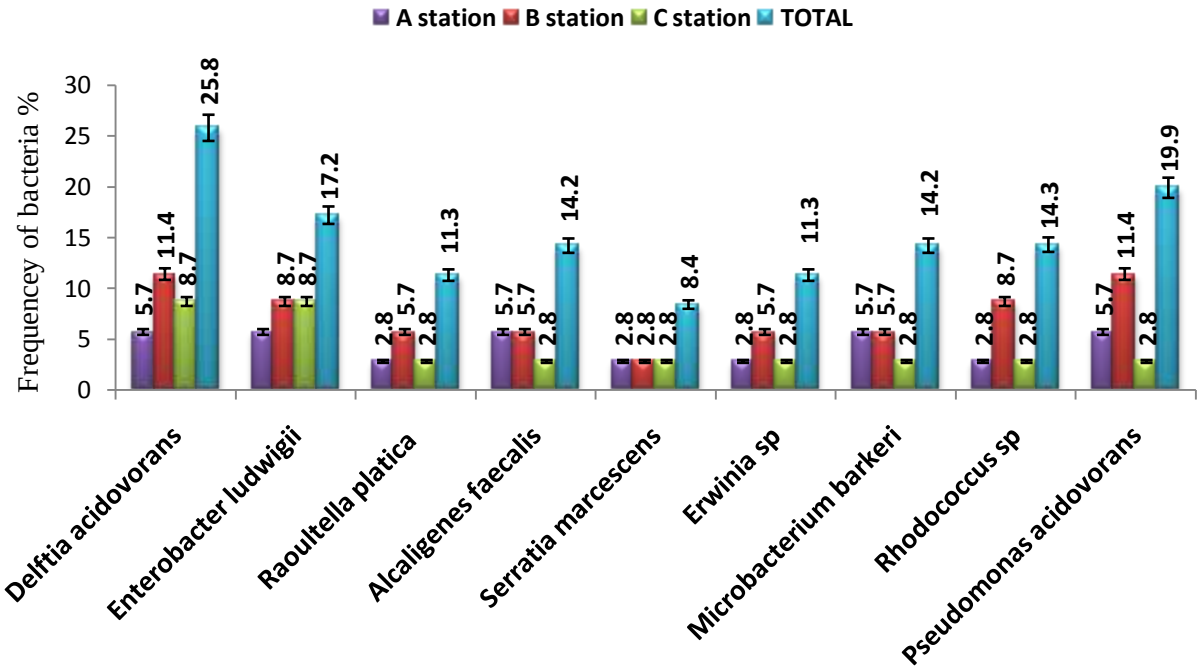

Figure 3: Frequency of various bacteria in summer

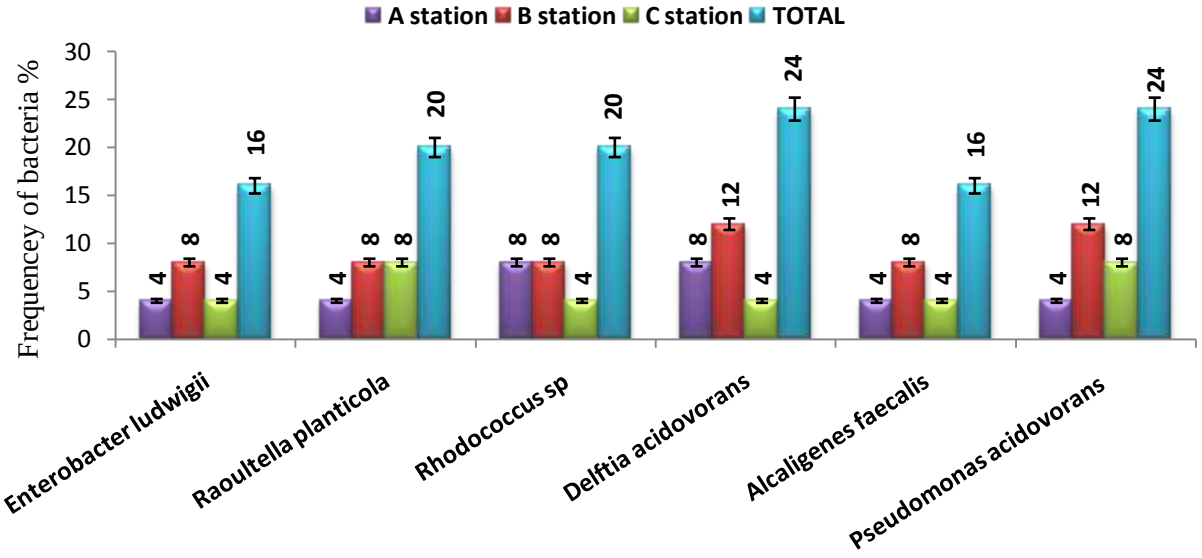

Figure 4: Frequency of various bacteria in autumn

Discussion

Aromatic amines such as anilines and its derivatives are largely used in chemical industrial manufacturing and known as a significant group of serious environmental pollutants (Sihtmaee *et al.*, 2010). From the beginning of the previous decade, the bacterial decomposition of aromatic compounds has been shown to be plasmid encoded (Anson *et al.*, 1984), furthermore many researchers have investigated to eliminate these compounds from the environment (Talaii, 2010).

Climate changing has a significant effect on microbial abundance (Penuelas *et al.*, 2011). In the present study, summer and autumn periods were selected for soil sampling; summer due to the warm weather conditions would provide high diversity of bacteria, whilst with autumn and the rainy season, low temperatures offer a limited growth period for bacteria. In 2010, Castro *et al.*, stated that increasing temperatures can cause faster growth rates. However the exact details of studies are rare in soil microorganisms. Following this, average of bacterial count during summer in presence of aniline and without aniline is higher than average of bacterial count in autumn in presence of aniline and without aniline. Studying the average of bacterial count in presence of aniline and without aniline it can be concluded that non-resistant bacteria to aniline increase the log of bacterial count in the absence of aniline. According to the current study *Delftiaacidovorans* and *Pseudomonasacidovorans* were found in both seasons with the highest frequency compared to other bacteria. Thus, this shows that these two bacteria have a wide range for aniline degradation in the soil. Loidel *et al.*, in 1990 found four *Pseudomonas acidovorans* strains that could use aniline and its derivatives as carbon, nitrogen and energy sources.Six bacterial strains, able to use aniline, were isolated in 1992, belonging to *Pseudomonasputida*, *Pseudomonasacidovorans*, *Achromobactergr.D.V*, *Achromobacterxylosoxidans*, *Moraxella* sp. K21 and *Moraxella* sp. K22 (Kahng *et al.*, 1992). Among the listed bacteria *Pseudomonas acidovorans* was found in the present research. *Candida tropicalis* is isolated from an activated sludge in 2010. Optimal degrees for biodegradation of aniline by this reported strain was 28 to 35°C at pH 7.0 (Wang *et al.*, 2011). In the present study no *Candida tropicalis* was identified. *Burkholderia* sp. is new aniline utilizing bacteria which was reported by Kahng *et al.*, in 2000 from Orchad soil. They revealed that this strain has a good ability to degrade the aniline. In the current study no *Burkholderia* sp. was found. Li *et al.*, (2009) isolated *Erwiniaamylovora*as aniline degrading bacterium, being a slightly halophilic bacterium that can use aniline as carbon source and electron donor. *Erwinia* sp., was isolated in the current research as well.

Rhodocuccos species were illustrated their ability to degrade the aniline in 2008 (Obinna*et al.*, 2008), being isolated and characterized at Shiraz refinery as well. *Ahmad et al.*, identified *Staphylococcus aureus* as aniline degrading bacterium in 2009. This strain showed ability to utilize aniline up to 2000 ppm on mineral salt media. No *Staphylococcus aureus* was found in the present study. Newly isolated aniline-tolerant bacterial strain was found by Liu *et al.*, *Delftia* sp.AN3 was isolated from activated sludge in 2002.This strain was capable to use aniline and acetanilide as sole nitrogen and energy and carbon source. Kahng *et al.*, (2000) characterized *Delftiaacidovorance* as aerobic and anaerobic aniline degradation bacteria. *Delftiaacidovorance* was one of the isolated aniline degradation bacteria, being isolated under aerobic situation. The identified results prove its aniline utilizing capability as the sole carbon and energy source, with the highest frequency, comparatively to the other isolated bacteria. In addition, it is noticeable that indigenous bacteria are more efficient to eliminate aniline from soil around Shiraz Refinery.

Conclusion

In this research *Pseudomonasacidovorans* and *Delftiaacidovorans* were isolated and identified in several sampling and during both seasons with the highest frequency. This fact indicates high aniline degradation ability for these two bacteria; thus being good choices to eliminate aniline from soil around Shiraz refinery.

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