Aerobic Biodegradation of BTX by Halophilic Planococcus sp. Strain TS1 Isolated from Egypt

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ABSTRACT

Aims: The purpose of the present study is to isolate pure halophilic bacteria potentially able to degrade BTX compounds as a sole carbon source under aerobic condition. Such these studies are necessary for bioremediation application of hydrocarbons in our ecosystem.

Study Design: Isolation of halophilic bacterial strains from hypersaline soil of Wadi An- Natrun, in Egypt. Selected isolates able to utilize BTX compounds as the only carbon and energy source. Efficiency of biodegradation ability will be enhanced via optimizing microbial growth condition. Most potent isolate will be identified through phenotypic and phylogenetic characterizations.

Place and Duration of Study: The study was performed in Physiological Lab in Botany and Microbiology Department in Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt from May 2013 until October 2015.

Methodology: The halophilic bacterial strains were selected based on its ability to grow in the presence of high salt concentration and undiluted Toluene (~222 µmol) which served as the only source of carbon. Subsequently select the most potent isolate and identify it. Thereafter, study...
some factors affecting on the biodegradation of toluene and estimated the biodegradation ratio of BTX by Purge-Trap GC-MS.

**Result:** Fifty halophilic bacteria isolates are capable of utilized toluene as the only source of carbon and energy isolated from alkaline soils in Al-Hamra Lake, Wadi An Natrun, Egypt. One isolate was selected as the most potent strain. Based on the 16S rDNA gene sequence and phenotypic characterizations the strain TS1 was most closely related to the *Planococcus maritimus* with similarity 95% which belong to family Planococcaceae. Strain TS1 could grow at temperatures between 20 up to 40°C, pH 5 to 8 and salt concentrations from 5 to 20%. Its optimal conditions for biodegradation of Toluene were 30°C, pH 8 and 10% salt concentration. Purge-Trap GC-MS analysis showed that, strain TS1 has the ability to degrade 25.33% of toluene and 46.67% of xylene in addition 47.55% and 46.84% of benzene and toluene mixture respectively, during first 24 h of incubation. This study suggests that strain *Planococcus* TS1 may play an important role for biodegradation of BTX in different marine contaminated sites.

**Keywords:** Halophiles; alkalophiles; monoaromatic; BTX biodegradation; Planococcus sp.

**ABBREVIATIONS**

BTEX: Benzene, Toluene, Ethyl benzene and Xylenes; MSM: Mineral Salt Media; EPA: Environmental Protection Agency.

**1. INTRODUCTION**

The monoaromatic hydrocarbons, abbreviated BTEX, which refers to benzene, toluene, ethyl benzene and xylenes, are the most common groundwater, soil and air pollutants. They are also used as industrial solvents for the synthesis of several organic compounds (e.g. plastics, synthetic fibers, and pesticides) and are the major aromatic components in many petroleum products [1,2]. BTEX compounds are high solubility in water relative to other petroleum hydrocarbons; these compounds account for as much as 90% of the gasoline components found in the water soluble fraction, and groundwater can transport them from tens to hundreds of meters down gradient of the contamination source [3,4].

BTEX contamination of soil and groundwater is usually related to petroleum leakages and fuel oil from underground storage tanks, manufacturing of solvent-based paints, lacquers and varnishes and the activities of manufactured gas plants. Significant quantities of these contaminants inevitably enter the environment during the production process [5,6]. BTEX compounds are toxic to humans and are confirmed carcinogens; these toxic substances easily move in air, they have direct and indirect impacts on human health. Short term (acute) hazards of BTEX include potential acute toxicity to aquatic life in the water column (especially in relatively confined areas) as well as potential inhalation hazards. Long term (chronic) potential hazards of these compounds include changes in the liver and harmful effects on the kidneys, heart, lungs, and nervous system [7].

Human exposure to these compounds as a mixture can lead to neurological, respiratory, genetic and excretory system damage and other health problems ranging from irritation of the eyes, mucous membranes and skin, to weakened nervous systems, reduced bone marrow function and cancers. Usage of BTEX has persisted despite all these adverse effect because of the extent of applications [7]. The very harmful effects of these compounds on the environment including soil and groundwater in addition to toxicity caused on living organisms, makes governments in many parts of the world have been implementing very stringent environmental standards. Thus, the United States EPA classifies them as environmental priority pollutants, making their removal from polluted environments critical [8,9].

There is an urgent need for the development of efficient methodologies that are able to minimize or eliminate the harmful effect of these compounds. Conventional treatment techniques such as absorption, adsorption, combustion and condensation suffer from several drawbacks, including high capital, operating and maintenance costs, high energy input, difficulty in handling low concentration pollutants, and production of toxic byproducts [2].
Recently, biological treatment processes that use the natural capability of microorganisms to degrade pollutants to less harmful products and utilize the carbon contained in these toxic compounds are believed to be an attractive alternative. The numerous advantages of biological methods include direct degradation, thus preventing the increase in contamination of the environment; reduction of the pollutants into less harmful reaction products (biomass, CO₂, H₂O and salt). The energy source for contaminant decomposition is provided by the contaminant themselves and investment and operating costs are low compared with other technologies. These can also be very effective for treating contaminants with high flow rates and low pollutant concentrations [2].

It has been reported that roughly 25% of all petroleum contaminated land is being bioremediated using natural attenuation processes this confirmed the importance of microorganisms in remediation strategies [10]. Therefore, bioremediation technology utilizes microorganisms to degrade toxic pollutants to harmless products such as CO₂, H₂O, and other inorganic compounds the processes are environmentally safe and cost efficient [11].

However, application of non halophilic microbial technologies for treating contaminated high salinity or fluctuating salinity environment is limited due to the detrimental effects of salt on microbial life including disruption cell membrane, denaturation of enzymes, low solubility of oxygen, low solubility of hydrocarbons, and desiccation [12]. Therefore, bioremediation of saline environments without costly dilution of salt-laden soil and water could be require halophilic or halotolerant organisms that tolerate high salt concentrations.

Metabolic pathways for the degradation of BTEX are provided by two enzymatic systems: dioxygenases and monooxygenases. The monooxygenase, also referred to as "tol" pathway, attacks methyl or ethyl substituents of the aromatic ring which are subsequently transformed by several oxidations to corresponding substituted pyrocatechols or phenyl glyoxal, respectively. The dioxygenase, also referred to as the "tod" pathway, attacks aromatic ring with formation of 2-hydroxy substituted known as catechol intermediate compounds [13,14,15].

Subsequently, these catechol intermediates are mineralized by catechol 1, 2-dioxygenase or catechol 2, 3-dioxygenase enzymes. The ring is opened and then degraded, finally, producing low molecular weight compounds such as pyruvate and acetaldehyde, which can be further oxidized via the Krebs cycle [16,17,15,18,19,20]. This process requires dissolved oxygen which utilized for both ring activation and cleavage of the aromatic nucleus and as the electron acceptor for its complete degradation by bacteria [21,19].

Halophiles are classified into three groups according to their optimal salt concentration for growth: slightly halophilic (1–3% w/v), moderately halophilic (3-15% w/v), and extremely halophilic (15–32% w/v) [22,23]. There are a few published reports about BTEX degradation under hypersaline conditions. The first time that BTEX can be degraded by microorganisms present in hypersaline environments was reported by Nicholson and Fathepure. They are developed BTEX degrading enrichment cultures used in isolation of *Arthomonas* sp. strain from soil sample and sediment sample from an oilfield in Oklahoma at high salinity. This strains rapidly degraded benzene and toluene as the sole sources of carbon in the presence of 3–23% NaCl [24,25].

Li and his team have been isolated a Planococcus sp. strain ZD22 using a contaminated soil collected from a petroleum refinery effluent in China. The organism is a moderate haloalkaliphilic able to degrade BTEX until 20% salt [26].

Recently *Alcanivorax* sp.HA03 isolated from soda lakes in Wadi An Natrun capable of degrading benzene, toluene, and chlorobenzene as the sole sources of carbon at salinity ranging from 3 to 15% NaCl while *Marinobacter* bacteria that isolated from hyper saline areas along the Arabian Gulf coasts able to degrade benzene as the sole carbon source in the presence of 6% NaCl [27,28]. There are only very few information available concerning BTEX biodegradation by pure cultures of halophilic bacterium because many existing reports on aerobic biodegradation of BTEX by halophilic and halotolerant bacteria used microbial consortium rather than pure culture. Therefor isolation of bacteria with degradation potential in the presence of various salt concentrations is important for developing biological catalysts serve in removing or minimizing petroleum hydrocarbons pollutants from hypersaline environments and for monitoring and studying the presence of such bacteria in hypersaline environments. BTEX are
toxic compounds produced in huge amounts from numerous sources and classified as priority pollutants by the Unitate State Environmental Protection Agency. Concentrations of these pollutants were increased in seas especially in coastal seawaters, lakes, ground water and hypersaline environments.

The purpose of the present study is to isolate halophilic bacteria that able to degrade BTX compounds as a sole carbon source from Wadi An Natrun, Egypt and identify the most potent isolate through phenotype, phylogeny and study the culture condition characterizations of this isolate, as well determine the degradation ratio for each substrate by the most potent isolate.

2. MATERIALS AND METHODS

2.1 Soil Samples Collection

Forty soil samples were collected from Al- Hamra Lake, Wadi An Natrun, Egypt. Two hundred grams of each sample were taken from 0 to 5 cm depth and placed in sterile plastic bags and transported immediately in cold storage containers to laboratory for isolation of BTX degrading bacteria or kept in refrigerator at temperature 4ºC until further studies.

2.2 Chemicals

Toluene and Xylenes were purchased from SIGMA- ALDRICH COMPANY with purity ≥99.7% and CAS. Number 108-88-3 for toluene and purity ≥99% and CAS. Number 1330-20-7 for mixture of o, m-xylenes while Benzene was purchased from Biotech For Laboratory Chemical Company with purity ≥99%.

2.3 Enrichment Culture and Isolation of BTX Degrading Halophilic Bacteria

The enrichment was initiated by adding 1 g of soil samples (wet weight) to 100 ml of mineral salt medium (MSM) which contained (in grams / liter): NaCl, 145; MgCl₂, 0.5; KH₂PO₄, 0.45; K₂HPO₄, 0.9; NH₄Cl, 0.3; KCl, 0.3. The air in the headspace of serum bottles served as the source of oxygen. One hundred microliter pipettes was used to introduce 20 µl of undiluted toluene (~222 µmol) to each bottle which served as the only source of carbon. The inoculated bottles were closed with rubber septa and aluminum caps and incubated under shaking conditions at 100 rpm in the dark at 30°C. After one month of cultivation 10% of the culture was transferred to fresh medium contained 20 µl of undiluted toluene as the only source of carbon and cultured for another one month. BTX degrading halphilic bacterium was isolated on mineral salts medium containing 1.5% agar and 20 µl of toluene per plate injected between the dish bottom and the agar layer. Colonies grown on the plates was streaked pure by serial dilutions. Finally colonies exhibiting well growth on MSM agar with BTX compounds were selected as most potent isolate and aseptically transferred to liquid culture 50 ml of sterile MSM supplemented 20 µl of toluene for further characterization [24].

2.4 Phenotypic and Phylogenetic Characterization of Strain TS1

2.4.1 Phenotypic characterization of strain TS1

Morphological studies were performed including Gram staining and motility test through standard procedures according to Barrow and Feltham [29]. Cell shape and diameter were investigate and determined after colonies cultivated on rich medium under two conditions, one of them in presence of 10% NaCl only and the second one in presence of 10% NaCl in addition to BTX by scanning electron microscopy, in which samples were metalized with a thin gold film using sputtering device (JFC-1100 E JOEL, USA) for 12 min. Scanning electron microscopy was performed with JSM 5300 JOEL, USA Scanning Electron Microscope at 20 kV in the center laboratory, City of Scientific Research and Technological Applications. Biochemical characterization such as catalase, oxidase, H₂S and indole production, methyl red test, Voges Proskauer reaction, citrate utilization, nitrate reduction and other various biochemical tests also performed. Susceptibility and resistance toward different antibiotics by strain TS1 also studied by disc diffusion method using commercial paper discs impregnated with antibiotics from Bioanalyse Company. The previously mentioned tests were performed according to standard procedures described by Barrow and Feltham [29].

2.4.2 Phylogenetic analysis of bacterial strain TS1

Phylogenetic identification of the BTX degrading halophilic bacteria isolate was done by means of
sequence analysis of the 16S rRNA gene. Partial 16S rDNA sequence of bacterial isolate was carried out in Sigma Research Company, Cairo, Egypt. DNA was extracted using protocol of Gene Jet genomic DNA purification Kit (Fermentas) and amplified using Maxima Hot Start PCR Master Mix (Fermentas). PCR product was purified using Gene JET PCR Purification Kit (fermentas). The forward and reverse primers used for PCR amplification were 27f (5′-AGAGTTTGATCCTGGCTCAG -3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′) (16S rDNA universal primer). Sequencing of the PCR product was carried out in GATC (Guanin Adenin Thymin Cytosin) German Company using ABI 3730xl DNA sequencer.

By using 16S rRNA gene sequences, the strain was identified by BLAST search (blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of closely related type strains were retrieved for constructing the phylogenetic trees to confirm similarities of most potent strains with other related groups.

2.5 Effect of Culture Conditions on the Growth of Bacterial Strain TS1

The most potent BTX utilizing halophilic bacterial isolate was selected for investigating the effect of different pH, temperature, sodium chloride concentration. The bacterial culture (Fresh culture, from 12 to 16 h old) were harvested by centrifugation at 5000 rpm for 20 min, washed twice in sterile MSM and resuspended in the previous medium. This concentrated cell suspension was used as inoculum for subsequent experiments. To determine the suitable pH of biodegradation, 50 ml of sterile MSM supplemented 22 µl (~245 µmol) of pure toluene was prepared at pH 5, 6, 7, 8 and 9 using 0.1N HCl and 0.1N NaOH. The inoculated bottles were incubated under shaking conditions at 100 rpm at 30ºC for 72 h. The bacterial growth was determined spectrophotometrically at 600nm. An increasing in the OD_{600nm} in substrate containing cultures compared with control lacking substrate was considered as positive growth. The effect of different temperatures (20, 30, 35, 40 and 50ºC) on the growth of the most potent toluene degrading bacterial cultures was investigated as previously mentioned above. The effect of salt on biodegradation of toluene was determined at various concentrations of NaCl (2.5, 5, 10, 15, 20% (w/v). All experiments were performed in triplicate and results were recorded as described previously [26,30].

2.6 Analytical Method

All biodegradation ratios were conducted using purge and trap analyzed by gas chromatograph equipped with 5975c mass spectrometer with triple axis detector (Purge-Trap GC-ELCDMS) at water analysis lab of Ministry of defense, Egypt. Liquid Autosampler was programmed to automatically dispense 5 ml sample aliquots into purging device. The sample was purged with a stream of Helium (carrier gas) at 30–45 ml /min for 10–15 min at ambient temperature. After sample loading, the trapped sample components were desorbed by heating the cartridges at 225ºC and passing helium gas at 3 ml / min during 3 min and the injector was set in the splitless mode, and helium flow-rate was decreased from 3 to 1 ml / min in 1 min. fused-silica capillary column with a 3-mm film thickness was used and helium was used as carrier gas at 1 ml /min. The oven was set at 35ºC (5 min) and raised to 160ºC at 5ºC/min, letting it stay for 1 min and to 210ºC at 5ºC/min. The final temperature was maintained for 5 min and the total run time was 46 min. Retention times and peak areas were determined by using MS Chemstation software. Biodegradation ratio of BTX compounds by strain TS1 was determined by comparing the inoculated samples results to control.

3. RESULTS AND DISCUSSION

3.1 Isolation and Characterization of BTX-Degrading Strain TS1

The isolation procedures resulted in fifty pure bacteria cultures capable of growing on MSM medium supplemented with toluene as the only source of carbon and energy from alkaline and saline soils in Al- Hamra Lake, Wadi An Natrun, Egypt. The strains were isolated after serial enrichment with toluene as a sole carbon and energy sources. Initial subcultures took longer to degrade toluene and did not have high numbers of the toluene degrading colonies, whereas subsequent subcultures degraded toluene more quickly and had high numbers of toluene degrading colonies when plated on MSM containing toluene. Five bacterial isolates were selected as most potent toluene degrading strains on the basis of morphological characteristics on MSM agar medium, and their capabilities to grow on toluene as a sole carbon and energy sources. Finally, one strain showing highly ability of growing on and degrading toluene was designated TS1 and selected for further investigation.
3.2 Identification of BTX Degrading Strain TS1

3.2.1 Morphological characteristic of strain TS1

Strain TS1 formed Gram-positive reaction with cocci shape cells, when grown in enrich medium containing 10% salt only and produced a yellow-orange, water-insoluble pigment. SEM investigation illustrated changes in bacterial cell size, shape as well as cell wall surface in case of 10% salt and BTX (Fig. 1B) rather than 10% salt only (Fig. 1A).

3.2.2 Biochemical characteristic of strain TS1

Biochemical properties of strain TS1 were as follows (Table 1): catalase, oxidase, Voges Proskauer, amylase and gelatinase production, and nitrate reduction positive; urase, spore-forming, indole and H2S production, methyl red test, lipase and pectinase negative. The strain TS1 has the ability to produce acid under aerobic condition from glucose, sucrose, manose, rhamnose, raffinose, manitol, cellobiose and starch. Controlling the growth of isolate TS1 was found against of all commercial antibiotics paper discs used including, Ampicillin (10 µg), Penicillin (10 µg), Cefoperazone / sulbactam (105 µg) and Ciprofloxacin (5 µg), Rifamycin (30 µg), Neomycin (30 µg), Tetracycline (30 µg) and Chloramphenicol (30 µg).

3.2.3 Phylogenetic analysis of bacterial strain TS1

Phylogenetic analysis based on the 16S rDNA gene sequence showed that strain TS1 was most closely related to the Planococcus, Chryseomicrobium, and Planomicrobium species and all belong to family Planococcaceae, with similarity 96 %, 95% and95 % respectively. The partial 16S rDNA gene sequence (1162 bp) of strain TS1 was compared with closely related sequences of reference organisms from NCBI network service (blast.ncbi.nlm.nih.gov/Blast.cgi). PCR product of 16S rDNA gene for the isolate TS1 is shown in (Fig. 2).

The phylogenetic, morphological and biochemical characteristics of isolate TS1 suggested that; this isolate have high similarity with reference strain Planococcus sp. Differential phenotypic characteristics of strain TS1 and closely related Planococcus sp. As a result the present study showed that the isolate TS1 was highly coupled with Planococcus sp which isolated by Yoon et al. [31] as a Gram positive non spore forming cocci, motile, aerobic, their colonies distinguished by yellowish orange pigmentation, it was able to grow up to 40°C, pH range 6-9 and salt concentration up to 20%. Also TS1 like Planococcus sp able to secrete gelatinase enzyme in addition to production of acids from glucose, mannitol and raffinose [31].
### Table 1. Differential phenotypic characteristics of strain TS1 and closely related Planococcus sp. [31]

| Character                | Isolate TS1 | Planococcus maritimus |
|--------------------------|-------------|-----------------------|
| Cell shape               | Cocci       | Cocci                 |
| Colony pigmentation      | Yellow-Orange| Yellow-Orange         |
| Gram reaction            | +           | +                     |
| KOH (3%)                 | −           | −                     |
| Motility                 | Motile      | Motile                |
| Spore formation          | −           | −                     |
| Catalase                 | +           | +                     |
| Oxidase                  | D           | −                     |
| Relation to oxygen       | Aerobic     | Aerobic               |
| Salt range (%, w/v)      | Up To 20    | Up To 17              |
| Temp. range (˚C)         | Up to 40 (optimum 30) | Up to 41 (optimum 30) |
| pH range                 | 6−9         | 6–8                   |
| Indol production         | −           | ND                    |
| Methyl red               | −           | ND                    |
| Voges-Proskauer          | +           | ND                    |
| Citrate utilization      | −           | ND                    |
| Nitrate reduction        | +           | −                     |
| Urease                   | −           | ND                    |
| H₂S formation            | −           | ND                    |
| **Aerobic acid production** |          |                       |
| Glucose                  | +           | +                     |
| Galactose                | −           | −                     |
| Rhamnose                 | +           | −                     |
| Xylose                   | −           | −                     |
| Arabinose                | −           | −                     |
| Mannitol                 | +           | +                     |
| Lactose                  | −           | −                     |
| Cellobiose               | +           | −                     |
| Maltose                  | −           | −                     |
| Sucrose                  | D           | −                     |
| Sorbitol                 | −           | −                     |
| Raffinose                | +           | −                     |
| Mannose                  | +           | −                     |
| O/F test                 | D           | ND                    |
| **Extracellular enzymes** |          |                       |
| Amylase                  | +           | −                     |
| Lipase                   | −           | ND                    |
| Cellulase                | −           | ND                    |
| Gelatinase               | +           | +                     |
| Pectinase                | −           | ND                    |

(+) Positive, (−) Negative, (O/F): Oxidation fermentation test, (D): Doubtful, (ND): Not detected

Identification of strain TS1 as a Planococcus isolate is appropriate and consistent with the physiological properties and habits of such organisms, since other Planococcus strains have been isolated from marine environments and Antarctic sea ice brine according results reported by [32,33,26]. Dendrogram tree was illustrated in (Fig. 3) showing the phylogenetic relationship of TS1 with related taxa.

**3.3 Effect of Culture Conditions on Growth of Planococcus TS1 Strain and Toluene Degradation**

The influence of different culture conditions were necessary to carry out on the biodegradation rate of toluene by most potent bacterial strain Planococcus TS1. Three parameters were studied effect of different initial pH values,
temperature ranges and sodium chloride concentrations. The biodegradation of toluene was directly correlated with bacterial growth hence, an increase in bacterial cell biomass of strain Planococcus TS1 accompanying with a decrease in the tested substrate concentrations. Therefor all previously mentioned parameters were determined by bacterial growth rate (OD at 600 nm).

Effect of initial pH values: This experiment was performed to examine the optimum pH for the most potent bacterial isolate TS1 at which the highest degree of toluene biodegradation can be obtained. The results observed in (Fig. 4) showed that strain TS1 degraded 22 µl (~245 µmol) of toluene at pH between 6 and 9 for 24 h, with optimum pH value 8 at which the bacterial strain Planococcus TS1 gave high growth rate indicating that this strain is an alkaliphilic bacterium and these results were recorded by Li et al. [26], they isolated alkaliphilic Planococcus sp. ZD22 from a petroleum refinery effluent in China and with results reported by Romano et al. [34] whose isolated Halomonas campaniesis sp. nov., a halokaliphilic bacterium able to degrade aromatic hydrocarbon under alkaline condition.

Our present results are consistent with most recorded observation by authors; for example Stenotrophomonas maltophilia T3-c, isolated from a biofilter by Lee et al. [35] able to grow in a mineral salt medium containing toluene, benzene, or ethyl benzene as the sole source of carbon with optimum temperature 30°C in addition to 25 strains corresponded to Planococcus halophilus were isolated at mesophilic temperature by Ventosa et al. [36]. Overall, most of BTEX degrading bacteria are mesophilic except strain Planococcus sp. ZD22 recorded by Li et al. [26] showed the ability to degrade benzene faster at 15 to 25°C than at 30°C and mineralized benzene at a relatively low and acceptable rate at 8°C and this is contrast with our results.

The capabilities of Planococcus sp. TS1 to degrade toluene as a sole carbon source with different salinities ranging from 5 to 20% were performed. Results illustrated in (Fig. 6) showed that Planococcus sp. TS1 degraded (~245 µmol) of toluene in MSM containing broad NaCl concentrations ranging from 5 to 20% for 24 h, with an optimal NaCl concentration of 10%. Our observation is appropriate and consistent with those noted in moderately haloalkaliphilic Planococcus sp. strain ZD22 fully degraded 2 mM benzene in media containing NaCl concentrations ranging from 5 to 20% for 3 days, with an optimal NaCl concentration of 10% [26].

Also our finding is in agreement with the observation reported by Nicholson and Fathepure as well Azetsu et al. [37,38] they isolated two strains of gamma-proteobacteria identified as Arhodomonas sp. strain Seminole and Arhodomonas sp. strain Rozel from enrichments developed using a soil sample from an oilfield in Oklahoma and a sediment sample from Rozel Point, respectively. Both strains rapidly degraded benzene and toluene as the sole sources of carbon in the presence of broad concentration of NaCl from 3 to 23%. The ability of bacterial strain to maintain its growth and degradation efficiency of aromatic compounds under varying salinities is an excellent feature that improvement the bioremediation application in environments containing a wide salinity ranges.

3.4 Analytical Method

The degradation ratio of BTX compounds by Planococcus sp. TS1 was determined at previously mentioned optimum conditions by

![Fig. 2. PCR product of 16S rDNA gene for the isolate TS1 at lane (1) and (L); DNA ladder (marker)](image)
using (Purge-Trap GC-ELCDMS). The biodegradation of BTX were done by using ~450 µmol of pure toluene or xylene and mixture of toluene with benzene together. Our results showed that the Planococcus sp. TS1 strain has different degradation ratio toward BTX compounds, in which 25.33% of toluene was utilized by this strain during 24 h of incubation and xylene consumed by 46.67% after 24 h of incubation passing. In binary mixture of toluene and benzene, the presence of benzene enhanced the degradation rate of toluene in which the benzene and toluene were degraded by 47.55% and 46.84% respectively within first 24 h. Biodegradation ratio of BTX by Planococcus sp. TS1 shown in (Table 2)

![Phylogenetic tree](image)

**Fig. 3.** Phylogenetic tree based on 16S rRNA sequences, constructed by the Neighbor-joining method, showing the position of strain TS1 (Query) and representatives of some related taxa

![Growth graph](image)

**Fig. 4.** Growth of Planococcus sp. TS1 at different pH values on (~245 µmol) of toluene as a sole carbon source
therefore *Planococcus* sp. TS1 appeared highly efficiency to utilized all amount of toluene within 3 to 4 days if compared with *Alcanivorax* sp. HA03 isolated from soda lakes in Wadi El Natrun which needed almost 4 weeks for degrading toluene and observation noted for *Marinobacter hydrocarbonoclasticus* that degraded 10% of benzene, 20% of toluene in 7 days as the sole sources of carbon [39,27].

**Fig. 5.** Growth of *Planococcus* sp. TS1 at different temperature ranges on (~245 µmol) of toluene as a sole carbon source

**Fig. 6.** Growth of *Planococcus* sp. TS1 at different sodium concentrations chloride on (~245 µmol) of toluene as a sole carbon source
The results that obtained by Planococcus sp. TS1 was similar to Marinobacter vinifirmus that able to degrade all the added benzene and toluene in 3 days [37].

Table 2. Biodegradation ratio of BTX by Planococcus sp. TS1

| BTX compounds     | Biodegradation ratio / day |
|-------------------|---------------------------|
| Toluene (~450 µmol) | 25.33%                    |
| Xylene            | 46.67%                    |
| Mixture of BT     | 47.55%                    |
| Toluene           | 46.84%                    |
| Benzene           |                           |

4. CONCLUSION

The appearance of BTX compounds in our environments with highly concentrations is usually associated with the discharge of petroleum products and synthetic chemicals in the form of herbicides, pesticides and industrial effluents and recognized as common organic contaminants so that, these compounds classified as main pollutants by the US Environmental Protection Agency. Microorganisms including eubacteria, archaea, fungi and microalgae utilized monoaromatic pollutants as carbon source and hence these microorganisms play a critical role in monoaromatic removal through in situ bioremediation processes. Therefore, there is still the need to isolate new BTX degrading halophilic bacteria that can aerobically grow at elevated concentration of salt.

Our results have demonstrated the ability of genus Planococcus represented by Planococcus sp. TS1 isolated from soda lakes in Wadi E1Natrun to degrade low molecular weight hydrocarbons (BTX) with high biodegradation activity under multiple extreme conditions (high salinity and alkaline pH), make it a good candidate for use in bioremediation potential of hydrocarbons at Wadi An Natrun and other salty hydrocarbon contaminated sites. More study should be recommended including the ability of Planococcus sp. TS1 to degrade other various aromatic compounds as well as elucidation the catabolic pathway and enzymatic gene regulation mechanisms of these compounds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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