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Isolation, identification and characterization of BTX degrading *Stenotrophomonas* sp. TS48 strain obtained from Egyptian saline soil

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Abstract

BTX compounds are monoaromatic hydrocarbon pollutants that are toxic to the human health and environment. It is one of the most stable compounds in soils, ground and surface waters, therefore its biodegrading is worthy to be undertaken. In the present investigation, thirty nine halophilic bacterial isolates capable to utilize toluene as the only carbon source. They were isolated from alkaline soils in Al- Hamra Lake, Wadi ElNatrun, Egypt. Based on the highest degradation of toluene, isolate TS48 was selected as the most potent isolate. The isolate is belonging to family *Xanthomonadaceae* of the subclass *Proteobacteria* and was identified as *Stenotrophomonas* sp. TS48 according to the 16S rRNA gene sequence and phenotypic characterizations. Strain TS48 was very closely related to *Stenotrophomonas acidaminiphila* AMX 19, *Stenotrophomonas nitritireducens* L2 and *Stenotrophomonas terrae* R-32768 with similarity levels at 97, 96 and 96% respectively. Strain TS48showed better growth at a wide range of temperatures between 20 up to 40 ºC, pH values 6 to 9 and salt concentrations from 2.5 to 10%. Its optimal conditions for toluene biodegradation were exhibited at temperature 35ºC, pH 7.0 and 5.0 % salt concentration. Purge-Trap GC-MS results showed that strain TS48 has the ability to degrade 24.5%, 31.2% and 40.0% of toluene, xylene and benzene, respectively within 24 h of incubation. This study suggested that strain *Stenotrophomonas* TS48considered as effective marker for biodegradation of BTX in contaminated saline sites.

Keywords: Halophiles, *Stenotrophomonas*, Monoaromatic, BTX biodegradation.

Introduction

BTEX compounds (benzene, toluene, ethylbenzene, and xylene isomers) are volatile monoaromatic hydrocarbons which are commonly found in crude petroleum and petroleum products and make up about 18% (*w/w*) in standard gasoline **(Budavari, 1996)**.Leakages in fuel storage tanks, pipelines, and landfills causes release of BTEX compounds which resulted in soil contamination. BTEX are soluble compounds transported downstream of the contaminant sources and contaminate the groundwater.

BTEX compounds are classified as priority pollutants by the U.S. Environmental Protection Agency **(Eriksson *et al*., 1998).**Exposure of human to these compounds 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 **(Irwin, 1997)**.

Thermal, chemical, and mechanical strategies are presently used to remove hydrocarbons from contaminated sites. However such technologies are expensive, result in incomplete decomposition in addition to the production of by-products which have negative impacts on both environment and public health **(Taiwo, 2011).**Subsequently, bioremediation using biological transformation is the most emerging

strategy for treatment of hydrocarbon contaminated sites. It is advantageous in cost-effective and lead to degradation or complete mineralization of organic contaminants into carbon dioxide, water and inorganic compounds **(Medina-Bellver *et al.*, 2005**; **Beller *et al*., 1996; Coozzarelli *et al*., 1990).**

Microbial biodegradation depends on several factors, such as pH, temperature, salinity and pressure. Degradation of BTEX in extreme conditions such contaminated water or high salt soil is of great interest because most of BTEX pollutants occur near to sea or saline soils. Consequently, the development of optimization of biodegradation processes for hydrocarbons at extreme environments in saline environments require halophilic or halotolerant microorganisms that tolerate or adapt with high salt concentrations **(Baraniecki *et al.*, 2002; Margesin and Schinner, 2001)**. Halophilic bacteria may be presence in one of 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 %,*w/v*) **(Oren, 2002; Ventosa and Nieto, 1995).**

Biodegradation of BTEX compounds under saline condition have been reported by several authors (**Nicholson and Fathepure; 2004, 2005; Li *et al.*, 2006)**.The most promising strains in halophilic bacteria, members of the genera *Halomonas*, *Marinobacter*, and *Alcanivorax* have been widely reported to degrade aliphatic and aromatic compounds over a broad range of salinities. Also, *Marinobacter* and *Alcanivorax* have been showed the capabilities to degrade mainly aliphatic compounds, BTEX, and PAHs (poly aromatic hydrocarbons), but not much is known about their ability to degrade phenols and benzoates under moderate to high salt conditions **(Patzelt, 2005; Fathepure, 2014; Sorokin *et al.*, 2011).** Recently, searching for a novel bacterial strain with high degradation capability to either aliphatic or aromatic pollutants and developing an effective consortium to be available under wide environmental conditions are of great interest.

The aim of this study was to isolate a new potent halophilic bacterial strains capable to degrade BTX compounds from hypersaline sites (Wadi ElNatrun, Egypt) and to identify the most potent isolate using morphological, biochemical and phylogenetic characterizations and to study the effect of environmental factors on the growth of this isolate. Finally, the degradation ratios for each substrate by the most potent isolate were investigated under optimum conditions.

Materials and Methods

Samples collection and chemicals

Forty soil samples were obtained from Al- Hamra Lake, Wadi ElNatrun (alkaline inland saline lakes, had pH values of 8.5-11 and salinity ranging from 283 to 540 g/L **(Amany, 1999**) located about 90 km northwest of Cairo, Egypt. Samples were collected from surface to 5 cm depth and placed in sterile plastic bags, then carried immediately to the laboratory on ice for isolation of BTX degrading bacteria or kept at 4 ºC until they were inoculate into specific media.

Toluene and xylenes were purchased from SIGMA-ALDRICH (Germany) with purity ≥99.7% and ≥99% for toluene and mixture of ortho, meta-xylenes respectively. Benzene was purchased from Biotech For Laboratory Chemical Company with purity ≥99 %.

Isolation medium

Mineral salt medium (MSM) which modified by Nicholson and Fathepure (2004) was used for isolation procedures. This medium contains the following ingredients (grams/liter): NaCl, 100; $MgCl_2$, 0.5; KH_2PO_4, 0.45; K_2HPO_4, 0.9; NH_4Cl, 0.3; KCl, 0.3 and20 µl toluene (first isolation) was added to MSM as the only source of carbon and energy **(Nicholson and Fathepure, 2004)**.

Isolation of BTX utilizing halophilic bacteria

The enrichment steps have been accomplished by adding 1 g of each soil sample(wet weight)was inoculated into 100 ml of mineral salt medium (MSM) supplemented with 20 µl of undiluted toluene (~222 µmol) as the only source of carbon. The inoculated bottles were closed well and incubated under shaking conditions at 100 rpm in the dark at 30°C. After 10 days of cultivation, 10 % of the culture was transferred to bottles contain same for another10 days. BTX degrading halphilic bacterium was isolated on mineral salts medium containing 1.5 % agar and 20 µl of toluene per plate. Colonies grown on the plates were picked up and further purified by repetitive streaking on previous mentioned agar medium. After purification technique, colonies exhibiting good growth were selected as most potent isolate and transfer under aseptic conditions to 100 ml of sterile

MSM supplemented with 20 µl of toluene for further investigation **(Nicholson and Fathepure, 2004)**.

Morphological and biochemical properties of strain TS48

Morphological studies were achieved by using standard procedures described by **Barrow and Feltham (1993)** including Gram staining and motility. Cell shape and diameter size were determined for strain TS48 in presence of 10% NaCl only and the in presence of 10% NaCl puls BTX using 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 was performed with JSM 5300 JOEL, USA Scanning Electron Microscope at 20 kV in the center laboratory, City of Scientific Research and Technological Applications, Alexandria, Egypt.

Biochemical characterizations including catalase, amylase, lipase, oxidase, H_2S and indole production, methyl red test, Voges Proskauer reaction, citrate utilization, nitrate reduction and other various biochemical tests also performed according to standard procedures described by Barrow and Feltham and Bergeys Manual of Systematic Bacteriology (**Barrow and Feltham, 1993; Holt *et al.*, 1994**).

Susceptibility of strain TS48 toward eight antibiotics was tested by disc diffusion technique using commercial paper discs impregnated with antibiotics purchased from Bioanalyse^R Company. Inhibition diameters were recorded after 24 h of incubation of strain TS48 on nutrient agar medium at 37°C under aerobic conditions. The strain was classified according to its response as sensitive or resist.

16S rRNA sequencing and phylogeny analysis of bacterial isolate TS48

Partial 16S rRNA sequence of bacterial isolate was performed 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 universal primers used for PCR amplification were 27^f (5′-AGAGTTTGATCCTGGCTCAG -3′) and 1492^r (5′-GGTTACCTTGTTACGACTT-3′).Sequencing of the PCR product was carried out in GATC German Company using ABI 3730xl DNA sequencer. The determined 16S rRNA gene nucleotide sequence was identified by BLAST (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.

Parameters controlling the biodegradation of BTX compounds by strain TS48

The effect of pH, temperature and different sodium chloride concentrations on the growth of isolate TS48with toluene was investigated. Bacterial cells grown in MSM medium incubated for (12 -18 h) harvested by centrifugation at 5000 rpm for 20 min, washed twice by sterile MSM and suspended again in the same medium to be used as inoculum for subsequent experiments.

The effect of initial pH on the growth of TS48in MSM containing toluene at~245 µmol was investigated 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 spectrophotometricaly at 600 nm as a primary indication for toluene biodegradation (**Bayoumi and Abul-Hamd, 2010; Li *et al.*, 2006**).

The effect of different temperatures (20,30,35,40 and 50 ºC) on the growth of the most potent toluene utilizing strain TS48 was investigated using MSM medium supplemented with ~245 µmol toluene at optimal pH value. The growth of most potent bacterial isolate was determined as previously mentioned above.

The effect of different sodium chloride concentrations viz.,2.5, 5, 10, 15 and 20% (w/v) on the growth of strain TS48 was investigated. Salt was added to MSM medium supplemented with ~245 µmol of toluene as the only carbon source. The inoculated bottles were incubated under all obtained optimal conditions and growth was determined as previously mentioned. All previous parameters were performed in triplicate.

Biodegradation analysis

Optimized MSM medium supplemented with ~450 µmol of toluene or xylene isomers or benzene (sublethal concentration) as only carbon sources were inoculated by strain TS48 and incubated under optimal biodegradation condition. Samples withdrawn from the bottles were analyzed for BTX 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. Retention times and peak areas were determined by using MS Chemstation software. Biodegradation ratio was determined by comparing the results recorded by inoculated samples to uninoculated control samples.

Results and Discussion

Isolation of BTX degrading halophilic bacteria

Thirty nine pure isolates were obtained from saline soil samples in Al- Hamra Lake, Wadi ElNatrun, Egypt using serial of enrichment cultures and purification steps as described in "Materials and Methods". These isolates were able to grow on MSM and utilize toluene as a sole source of carbon and energy. Primary subcultures showed longer growth time and low numbers of colonies were appeared, while next subcultures showed quick degraded and high numbers of toluene degrading colonies on MSM agar plates. Among these isolates, TS48 was selected as the most potent toluene degrading strain which exhibited well growth on MSM-toluene agar plates.

Identification of BTX degrading strain TS48

Morphological characteristics of isolate TS48

Cells of isolate TS48 were straight to curved rods shape, that stained Gram-negative and its colonies appeared with pale yellow color on nutrient agar medium. Spore formation was not observed. The cells occurred singly or in pairs. Isolate TS48 cells showed well growth as well as their cells appeared normal rod shaped without any distortion when grow in enrich medium supplemented with 10% sodium chloride or 10% sodium chloride plus each of one BTX compounds. Cells of isolate TS48 were 0.81-0.88 µm in diameter and 2.31-2.61 µm in length under effect of 10% sodium chloride as shown in scanning electron microscopy photograph **(Fig. 1A).** On the other hand, the cells were 0.64- 0.86 µm in diameter and 1.34 - 3.01 µm in length under effect of 10% sodium chloride plus BTX compounds as illustrated in scanning electron microscopy photograph **(Fig. 1B).** Therefore the isolate TS48 not affected by presence of high salt concentration and/or BTX compounds and these results might indicate that strain TS48 was adapted to growth under stress conditions.

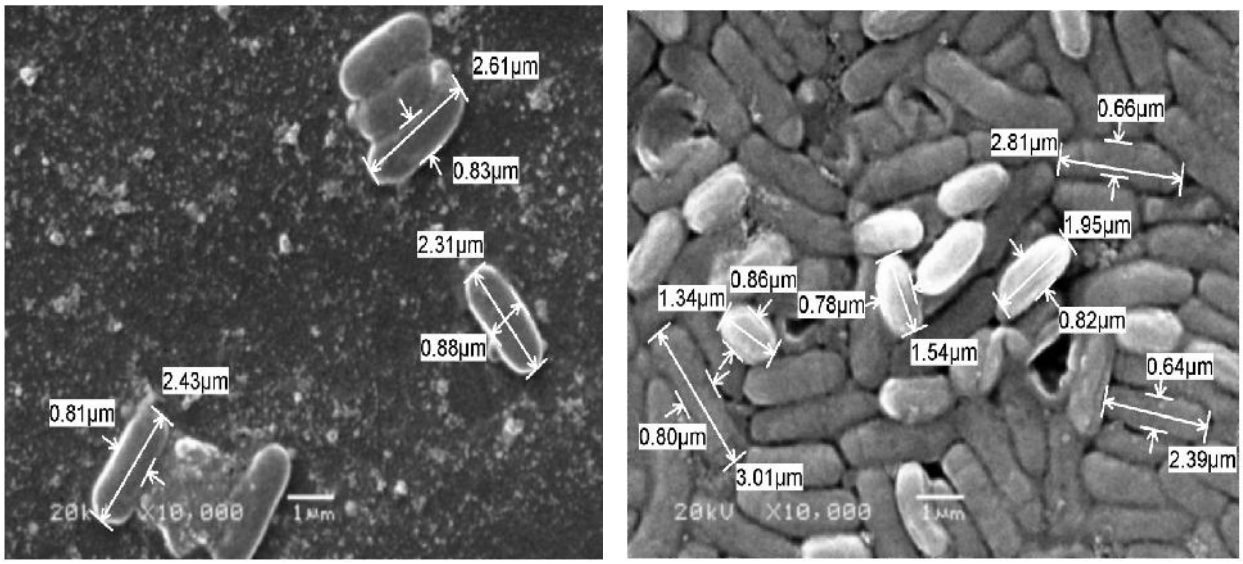

Fig. 1: Scanning electron microscopy showing the growth of strain TS48 (A) in presence of 10% NaCl, (B) in presence of 10% NaCl, plus BTX compounds

Morphological and Biochemical characteristics of isolate TS48

Isolate TS48 is strictly aerobic, Gram-negative, straight to curved rod-shaped bacteria. It showed optimum growth temperature 30 to 40°C. Isolate TS48 showed positive results for catalase, oxidase, Voges Proskauer, weak lipase production, and nitrate reduction. On the other hand, it showed negative results for amylase, gelatinase, pectinase, cellulase,

urease indole and H_2S production. It is methyl red negative and non-endospore forming strain. The isolate can utilize various sugars with acid formation from glucose, sucrose, mannose, rhamnose, and raffinose, whereas starch, mannitol, cellobiose, sorbitol, xylose, lactose and maltose cannot be utilized by this strain. These results indicate that the isolate is related to genus *Stenotrophomonas* sp. The morphological and biochemical characteristics of most potent isolate TS48 in relation to other *Stenotrophomonas* sp. were summarized in **Table 1**.

Table 1: Differential morphological and biochemical characteristics of strain TS48 and closely related species **(Wolfgang *et al*., 2000; Assih *et al*., 2002; Brenner *et al*., 2005)**.

Character	Isolate TS48	*Stenotrophomonas acidaminiphila*	*Stenotrophomonas nitritireducens*
Cell shape	Straight to curved rod	Straight to curved rod	Straight rod
Colony pigmentation	Pale yellow	Yellow	Pale yellow
Gram reaction	−	−	−
KOH (3%)	+	+	+
Motility	D	Motile	Motile
Spore formation	−	−	−
Catalase	+	+	+
Oxidase	+	+	+
Relation to oxygen	Aerobic	Aerobic	Aerobic
Salt range (%, *w/v*)	up to 10 (optimum 5)	ND	ND
Temp. range (˚C)	up to 40 (optimum 35)	up to 41(optimum 30-35)	35
pH range	6−9 (optimum 6-7)	5−9 (optimum 6-7)	
Indol production	−	ND	−
Methyl red	−	ND	ND
Voges-Proskuaer	+	ND	ND
Citrate utilization	+	−	+
Nitrate reduction	+	+	−
Urease	−	ND	+
H_2S formation	−	ND	ND
	Acid formation from sugars		
Glucose	+	+	+
Galactose	−	−	−
Rhamnose	+	−	−
Xylose	−	−	−
Arabinose	+	−	−
Mannitol	−	−	−
Lactose	−	−	−
Cellobiose	−	−	−
Maltose	−	−	+
Sucrose	+	−	+
Sorbitol	−	−	−
Raffinose	+	+	−
Mannose	+	+	+
O/F test	D	ND	ND
	Extracellular enzymes		
Amylase	−	−	−
Lipase	±	+	ND
Cellulase	−	ND	ND
Gelatinase	−	−	−
Pectinase	−	ND	ND

(+): Positive, (−): Negative, (O/F): Oxidation Fermentation test, (D); Doubtful,(ND); Not Detected, (±): Weak Production.

Susceptibility of the sensitivity of isolate TS48 to antibiotics was observed with all the aminoglycosides, quinolones and β-lactams. The tested antibiotics cover the majority mode for mechanisms including inhibition of synthesis of peptidoglycan, proteins and nucleic acids. This strain appeared high response toward antibiotic under investigation. The response of strain TS48 to previous mention antibiotics is in agreement with sensitivity reported by Assih *et al*. (2002)who reported that strain *Stenotrophomonas acidaminiphila* sp. nov., had the susceptibility toward all the aminoglycosides, fluoroquinolones, polypeptides and sulfamides tested **(Assih *et al*., 2002)**. Sensitivity of strain TS48 against different antibiotics represented in form of inhibition zone as showing in **Table 2**. These result confirmed that the isolate is related to *Stenotrophomonas* sp.

Table 2: Susceptibility of strain TS48 towards different antibiotics.

No.	Antibiotic	Symbol	Load / disc	Zone of inhibition (mm)
1	Ciprofloxacin	CIP	5 µg	28 (S)
2	Cefoperazone/sulbactam	CES	105 µg	22 (S)
3	Rifamycin	RF	30 µg	40 (S)
4	Neomycin	N	30 µg	20 (S)
5	Tetracycline	TE	30 µg	25 (S)
6	Chloramphenicol	C	30 µg	20 (S)
7	Ampicillin	AM	10 µg	34 (S)
8	Penicillin	P	10 µg	46 (S)

mm; millimeter S; Sensitive

Phylogenetic analysis of strain TS48

PCR product of 16S rRNA gene for the isolate TS48 is shown in **Fig. 2.** Phylogenetic analyses of 16S rRNA partial gene sequence (1109 bp) was analyzed and compared with closely related sequences of reference organisms from NCBI network service (blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic analysis revealed that the closest relatives to isolate TS48 were *Stenotrophomonas acidaminiphila* strain AMX 19, *Stenotrophomonas nitritireducens* L2 and *Stenotrophomona sterrae* R-32768 with similarity levels 97, 96 and 96% respectively. In addition it showed 95% similarity with other *Stenotrophomonas* species.

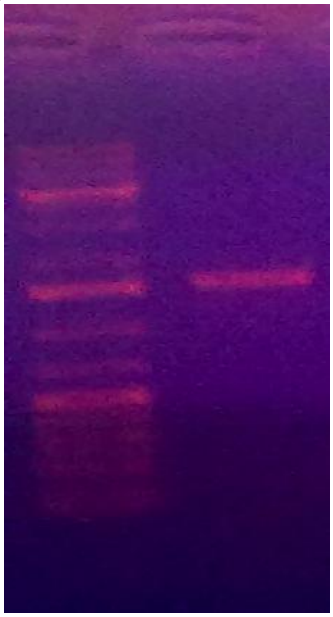

Fig. 2: PCR product of 16S rRNA gene for the isolate TS48 at lane (1) and (M); DNA marker (Ladder).

These results indicate that TS48 was affiliated to genus *Stenotrophomonas* which belonged to family *Xanthomonadaceae* of the subclass of the *Proteobacteria.* The phylogenetic tree based on the 16S rRNA gene of strain TS48 depicts its relationship within the genus *Stenotrophomonas* and other some related taxa is shown in **Fig. 3.**

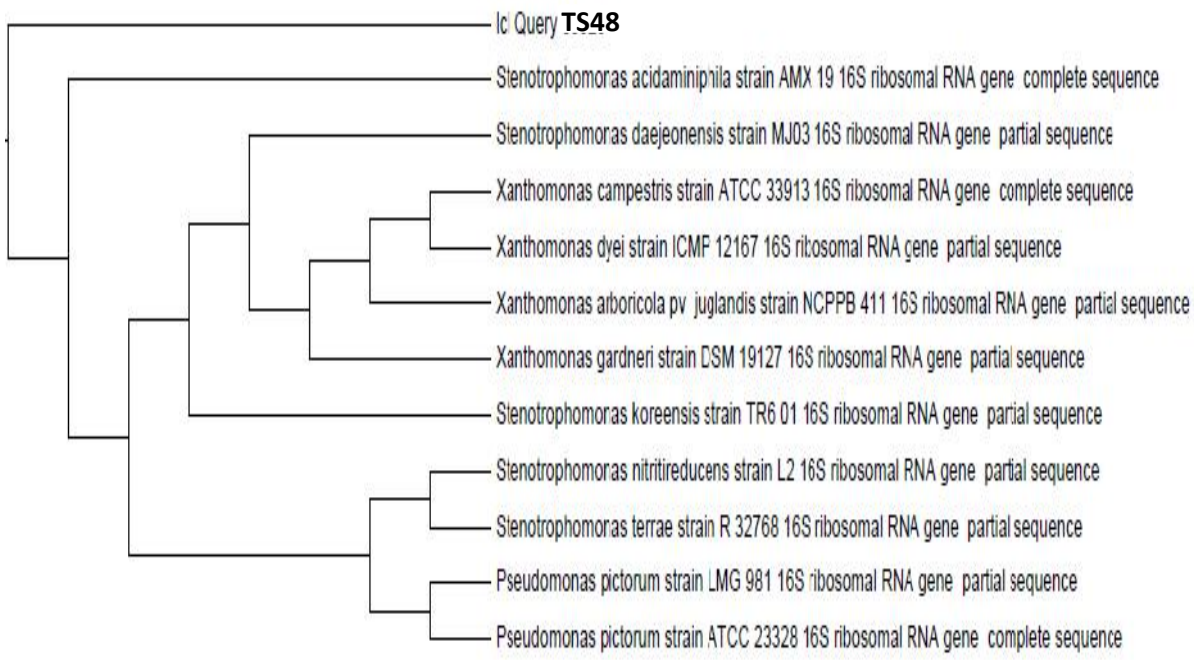

Fig. 3: The phylogenetic tree based on the 16S rRNA gene of strain TS48 (Query) depicts its relationship within the genus *Stenotrophomonas* and other some related taxa (blast.ncbi.nlm.nih.gov/Blast.cgi).

Based on these observations as well as the phylogenetic analysis based on the 16S rRNA gene, the isolate TS48 was putatively identified as *Stenotrophomonas* TS48, therefore, this result was consistent with results reported by several authors **(Palleroni and Bradbury, 1993; Vauterin *et al*., 1995; Assih *et al*., 2002; Yang *et al*., 2006; Liu *et al*., 2007).**

Parameters controlling the biodegradation of BTX compounds by strain TS48

The effect of different environmental factors on the growth rate of strain *Stenotrophomonas*TS48 during degradation of toluene was investigated. Hence the consumption (biodegradation) of toluene as the sole carbon source was directly correlated with bacterial growth in mineral salt medium therefore an increase in bacterial cell biomass of strain *Stenotrophomonas* TS48is corresponding to a decrease in the tested substrate concentrations. The overall factors were determined by analyzing bacterial growth (OD at 600nm).

To determine the optimum pH for the highest rate of toluene biodegradation by *Stenotrophomonas* TS48, an experiment was done at different pH values (pH 5-9) as represented in **Fig. 4**. The results showed that *Stenotrophomonas* TS48did not show any growth when pH was adjusted to 5.0 as there is no increase in OD from 0-72h, while it utilized 22 µl (~245 µmol) of toluene at pH between 6 and 9 with the highest growth obtained after 24 h. The highest growth was obtained pH 7.0 after 24h that exhibited the optimal value among all tested values.

The previous results are almost in agreement with Lee *et al.* (2002) who isolated *Stenotrophomonas maltophilia* T3-c, from a biofilter able to grow in a mineral salt medium containing toluene and benzene as the sole source of carbon at a broad pH range from 5 to 8with optimum pH value 7 **(Lee *et al.*, 2002**). Similar findings were also reported by **Assih *et al.*, (2002)** who isolated *Stenotrophomonas acidaminiphila* sp. nov., that had efficiently grow within pH range from 5 to 9 with an optimal growth at pH 6–7.

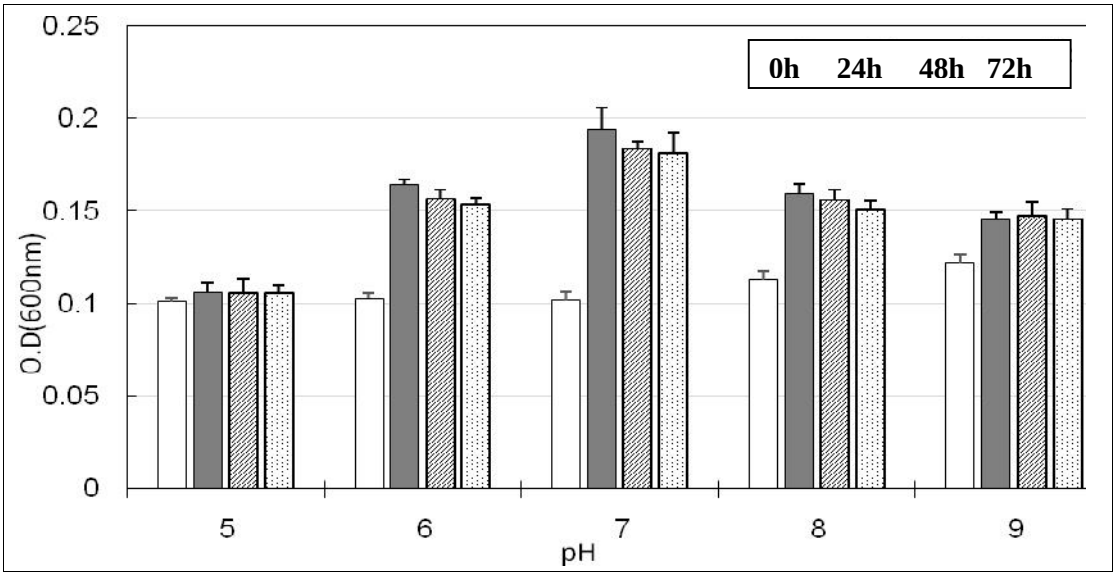

Fig. 4: Effect of different pH values on the growth of *Stenotrophomonas* TS48in MSM supplemented with (~245 µmol) of toluene as a sole carbon source at varying incubation periods (0-72 h).

The influence of different temperatures on growth of *Stenotrophomonas* TS48 was investigated in presence of ~245 µmol of toluene as a sole carbon source and pH 7.0 as shown in **Fig. 5**. The strain showed very weak growth and consequently biodegradation at 20 and above 40 ºC. Higher growth was obtained at 30-40 ºC, with an optimal value at 35 ºC after 24 h incubation period. A decrease in growth was observed after 24 h.

These results are consistent with **Deeb and Alvarez-Cohen (1999)** who reported the optimum temperature for toluene degradation was 35 ºC. **Assih *et al*. (2002)** reported an optimum temperature at 30-35 °C by *Stenotrophomonas acidaminiphila* sp. nov. **Liu *et al*., (2007)** reported that *Stenotrophomonas* LZ-1 strain has an optimal growth temperature of 32 ºC**. Lee *et al*., (2002)** found that *Stenotrophomonas maltophilia* T3-c able to grow in a mineral salt medium containing toluene, benzene, or ethylbenzene between 20 ºC to 42 ºC with an optimal temperature of 30 ºC. Also, Lu *et al.* (1999) reported that toluene removal efficiency by trickle bed biofilter increased with temperatures ranging from 15 to 30 ºC, and decreased from 30 to 50 ºC and the optimum temperature ranged from 25 to 35 ºC **(Lu *et al*., 1999).**

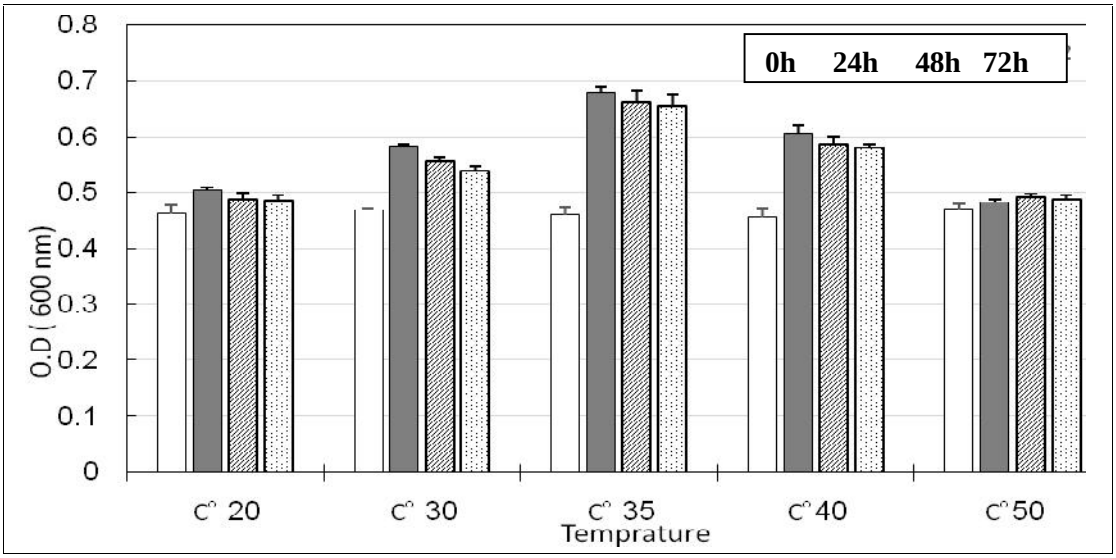

Fig. 5: Effect of different temperature on the growth of *Stenotrophomonas* TS48 in MSM supplemented with (~245 µmol) of toluene as a sole carbon source at varying incubation periods (0-72 h).

The abilities of *Stenotrophomonas* TS48to utilize toluene as a sole carbon source at different salinities ranging from 5 to 20% were determined. **Fig. (6)** showed that *Stenotrophomonas* TS48exhibited better growth and consequently toluene degradation in MSM containing NaCl concentrations ranging between2.5 to 10% with an optimal NaCl concentration at 5%.The highest biodegradation rate was obtained after 24h at all concentrations. The cell growth and consequently toluene biodegradation was gradually decreased at high salt concentration (≥ 15%). Therefore, *Stenotrophomonas* TS48 can be classified as moderately halophilic bacterium as described by **Oren (2002), Ventosa and Nieto (1995)** who classified halophilic bacteria according to their salt adaptation into slightly halophilic (1-3%,*w/v*), moderately halophilic (3-15%,*w/v*) and extremely halophilic (>15 %,*w/v*).

Our findings is superior to that obtained by **Lee et al., (2002)** who report that *Stenotrophomonas maltophilia* T3-cable to grow in a mineral salt medium containing each one of toluene, benzene, or ethylbenzene as the sole source of carbon in absence of sodium chloride. This indicates the potentiality of isolated strain, *Stenotrophomonas* sp. TS48, for BETX biodegradation in saline soil containing 2.5-5% salts compared to other reported species belonging to same genus.

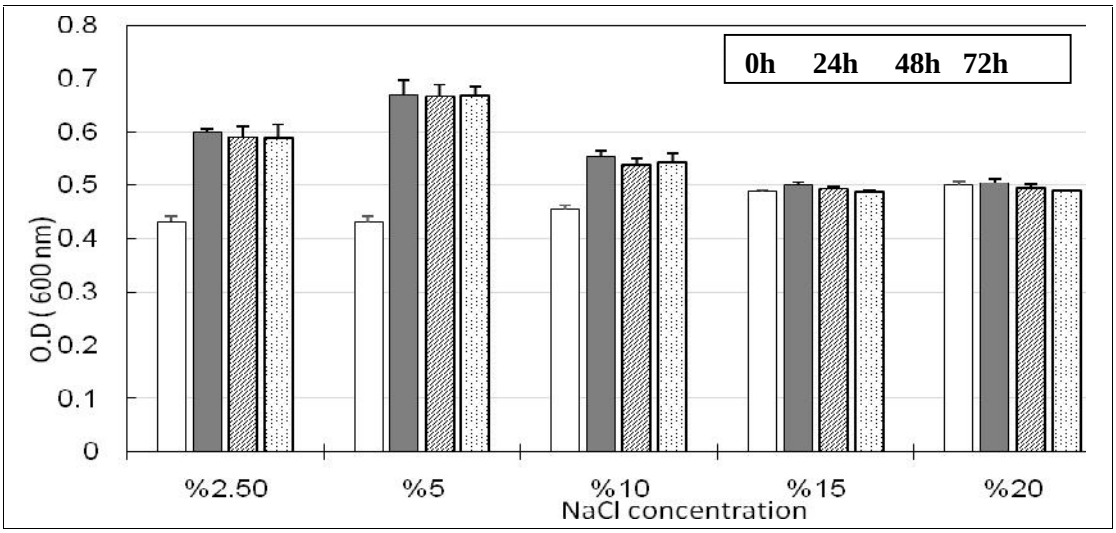

Fig. 6: Effect of different sodium chloride concentration on the growth of *Stenotrophomonas* TS48 in MSM supplemented with (~245 µmol) of toluene as a sole carbon source at varying incubation periods (0-72 h).

Biodegradation of BTX under the optimal condition

The degradation ratio of BTX compounds by *Stenotrophomonas* TS48was determined after 24 at previously mentioned optimum conditions (pH 7; temperature 35 ºC; NaCl 5%) by using Purge-Trap GC-ELCDMS using under the sub-lethal BTX concentration (~450 µmol) as showed in **Fig. 7, 8, 9**. *Stenotrophomonas* TS48 strain exhibited different degradation ratio toward BTX compounds, in which 24.5 %, 31.2% and 40.0 %of each one of toluene, xylene or benzene, respectively were utilized after 24 h of incubation (Table 3). Comparable data was recently observed by Desouky *et al.* (2015). They reported that strain *Planococcus* sp. TS1 which isolated from alkaline soil in Al- Hamra Lake, Wadi An Natrun, Egypt utilize 25.3%, 46.7% of toluene and xylenes, respectively.

The high efficacy of *Stenotrophomonas* TS48 to utilize each one of BTX compounds within 24 h is superior to other reported strains in literature. **Lee *et al.*, (2002**) have reported that *Stenotrophomonas maltophilia*T3-c can utilize toluene and benzene with specific degradation rates at 2.38 and 4.25µmol/g-DCW/h, respectively. Moreover, that isolate *Stenotrophomonas* cannot utilize xylene as a growth substrate while experimental isolated strain *Stenotrophomonas* TS48 showed the efficiency to utilized xylenes. Furthermore, studying strain was superior than *Alcanivorax* sp. HA03 isolated by Hassan *et al.* (2012) from soda lakes in Wadi El Natrun that require almost 4 weeks for utilized toluene

as well as for *Marinobacter hydrocarbonoclasticus* exhibited that degraded 10% of benzene, 20% of toluene in 7 days as the sole sources of carbon (**Berlendis *et al.*, 2010**; **Hassan *et al.*, 2012**). Further studies are recommended for *Stenotrophomonas* TS48 including the capacity to degrade other aromatic compounds as well as determination of the catabolic end products and the metabolic pathway for BTX degradation.

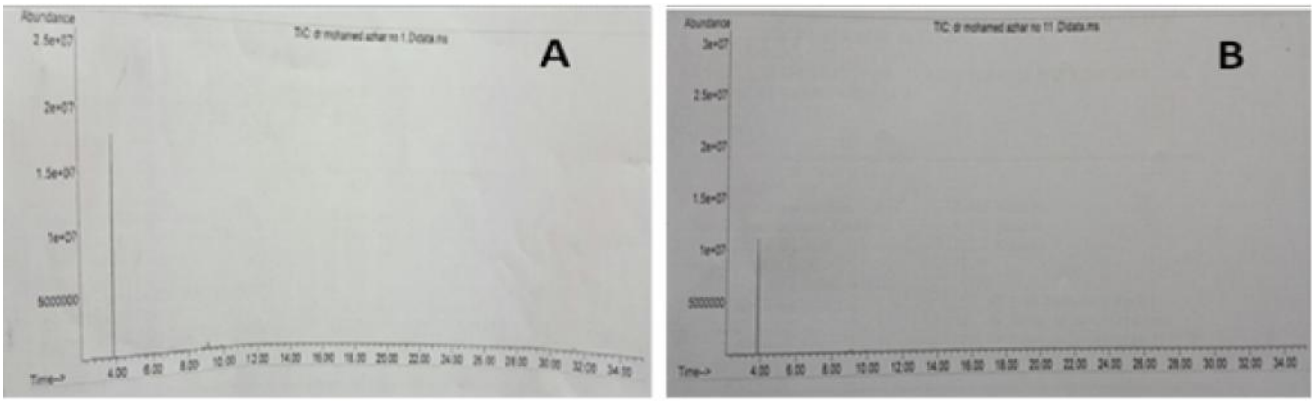

Fig. 7: Purge-Trap GC-MS analysis for biodegradation of benzene by isolate *Stenotrophomonas* TS48 at retention time (3.83) in which (A) Control: at abundance 1.5 to 2 x 10^7 (B) treated sample: at abundance ~1 x 10^7.

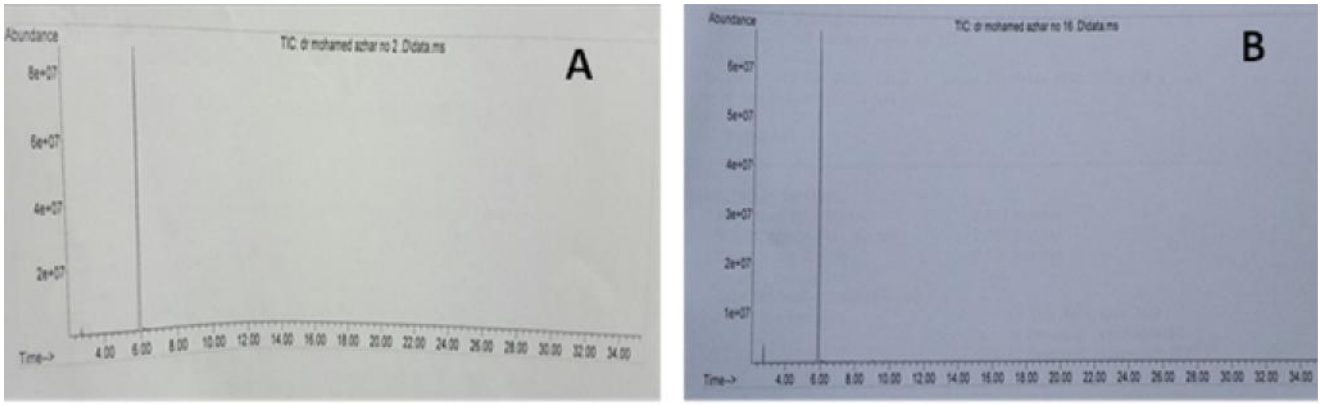

Fig. 8: Purge-Trap GC-MS analysis for biodegradation of toluene by isolate *Stenotrophomonas* TS48 at retention time (5.87) in which (A) Control: at abundance ~8 x 10^7 (B) treated sample: at abundance ~6 x 10^7.

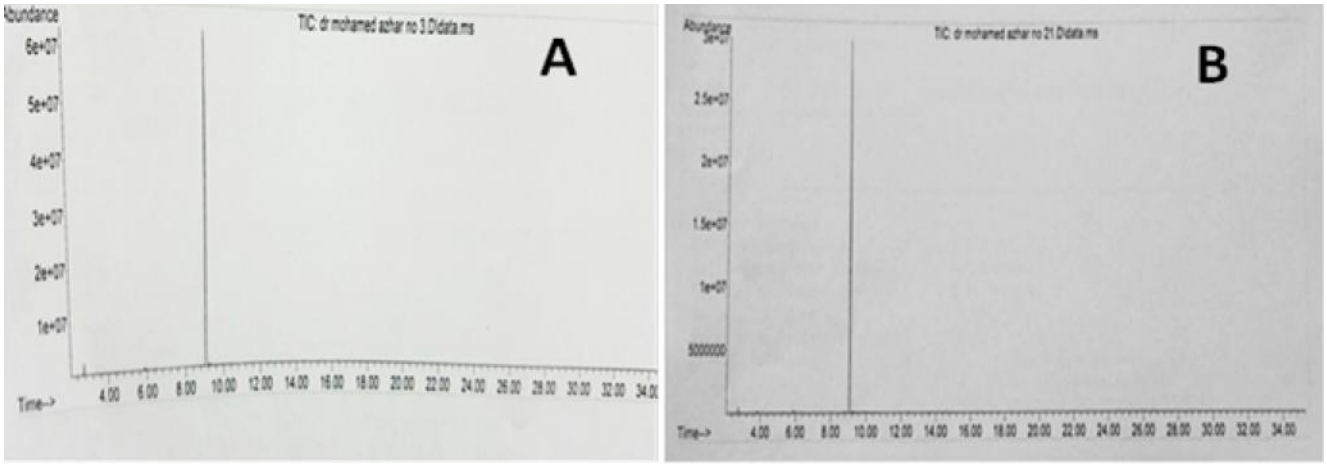

Fig. 9: Purge-Trap GC-MS analysis for biodegradation of xylene by isolate *Stenotrophomonas* TS48 at retention time (9.06) in which (A) Control: at abundance ~6 x 10^7 (B) treated sample: at abundance ~3 x 10^7.

Table 3: Biodegradation ratio of each BTX compound by *Stenotrophomonas* sp. TS48 under optimized conditions.

BTX compounds (~450 µmol)	Biodegradation ratio / day
Toluene	24.46%
Xylene	31.15%
Benzene	40%

Conclusion

Bioremediation which involves the use of microorganisms such as bacteria to detoxify and degrade pollutants had been considered as an effective biotechnological approach to clean up polluted environments. In the present work, thirty nine halophilic bacteria were isolated from alkaline soil of Al- Hamra Lake, Wadi ElNatrun, Egypt able to utilize BTX as a sole sources of carbon and energy under salt condition. Among these isolates,isolateTS48which identified *as Stenotrophomonas* TS48was able to utilize each of toluene, o, m- xylenes and benzene as the only sources of carbon and energy at an optimal salt concentration of 5% at pH 7.0 and 35^{o}C. The strain is considered a good bioremediation tool to remove monoaromatic compounds from BTEX-contaminated soil and groundwater.

References

Amany, G.T., 1999. Inland saline lakes of Wadi El Natrun depression, Egypt. *Int. J. Salt Lake Res.*, 8: 149-169.

Assih, E.A., A.S. Ouattara, S. Thierry, J. Cayol, M. Labat and H. Macarie, 2002.*Stenotrophomonas acidaminiphila* sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) Reactor. *Int. J. SystEvol. Microbiol.*,52: 559–568.

Baraniecki, C.A., J. Aislabie and J.M. Foght, 2002. Characterization of *Sphingomonas* sp. Ant 17, an aromatic hydrocarbon-degrading bacterium isolatedfrom Antarctic soil. *Microbiol. Ecol.*, 43: 44–54.

Barrow, G.I., R.K.A. Feltham, 1993. Cowan and Steel's. Manual for the identification of medical bacteria, 3rd ed., Cambridge, Cambridge University Press.

Bayoumi, R.A. and A.T. Abul-Hamd, 2010. Optimization of Bacterial Biodegradation of Toluene and Phenol Under Different Nutritional and Environmental Conditions. *J. Appl. Sci. Res.*, 6(8): 1086-1095.

Beller, H.R., A.M. Spormann, P.K. Sharma, J.R. Cole and M. Reinhard, 1996. Isolation and Characterization of a Novel Toluene-Degrading, Sulfate-Reducing Bacterium. *Appl. Environ. Microbiol.*, 62: 1188-1196.

Berlendis, S., J.L. Cayol, F. Verhe, S. Laveau, J.C., Tholozan and B. Ollivier, 2010. First evidence of aerobic biodegradation of BTEX compounds by pure cultures of *Marinobacter*. *Appl. Biochem. Biotechnol.*, 160: 1992–1999.

Brenner, D.J., N.R. Krieg and J.T. Staley, 2005. (Eds): Bergey's Manual of Systematic Bacteriology (Second edition) (Volume two). *Springer*. (pp) 107-115.

Budavari, S., 1996. The Merck index: cited from an encyclopedia of chemicals, drugs and biologicals. Whitehouse Station, NJ, Merck, USA.

Coozzarelli, I., R.P. Eganhous and M.J. Baedecker, 1990. Transformation of Monoaromatic Hydrocarbons to Organic Acids in an Anoxic Groundwater Environment. *Environ. Geol. Water Sci.*, 16: 135-141.

Deeb, R.A.and L. Alvarez-Cohen, 1999. Temperature Effects and Substrate Interactions during the Aerobic Biotransformation of BTEX Mixtures by Toluene-Enriched Consortia and *Rhodococcus rhodochrous*. *Biotechnol. Bioeng*.,62: 526-536.

Desouky, S.E., M.S. El-Gamal, M.G. Barghoth, 2015. Aerobic Biodegradation of BTX by Halophilic *Planococcus* sp. strain TS1 Isolated from Egypt. *J. Adv. Biol. Biotech*., 4 (4): 1-13.

Eriksson, M., A. Swartling and G. Dalhammar, 1998. Biological degradation of diesel fuel in water and soil monitored with solid-phase microextraction and GC-MS. *Appl. Microbiol. Biotechnol.*, 50: 124-129.

Fathepure, B.Z., 2014. Recent studies in microbial degradation of petroleum hydrocarbons in hypersaline environments. *Front. Microbiol*., 5: (pp)173

Hassan, H.A., N.M.H. Rizk, M.A. Hefnawy and A.M. Awad, 2012. Isolation and characterization of halophilic aromatic and chloroaromatic degrader from Wadi El-Natrun Soda lakes. *Life Sci. J.*, 9: 1565–1570.

Holt, J.G., N.R. Krieg, P.H. Sneath, J.T. Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology, 9th Edn. Williams and Wilkins, Baltimore, MD.

Irwin, R.E.A.. 1997. Fuel oil number 2 heating oil entry. Environmental Contaminants Encyclopedia. Fort Collins, CO, National Park Service, R.J. Irwin (Ed.), 80525.

Lee, E.Y., Y.S. Jun, K. Cho and H.W. Ryu, 2002.Degradation Characteristics of Toluene, Benzene, Ethyl benzene, and Xylene by *Stenotrophomonas maltophilia* T3-C. *Air Waste Manage. Assoc.*, 52: 400-406.

Li, L.H., Y.H. Luo, N. Zhang, X.Y. Luan and T.G. Hu, 2006.Biodegradation of benzene and its derivatives by a psychrotolerant and moderately haloalkaliphilic *Planococcus* sp. Strain ZD22.*Res. Microbiol.*, 157: 629-636.

Liu, Z., C. Yang and C. Qiao, 2007. Biodegradation of p -nitrophenol and 4-chlorophenol by *Stenotrophomonas* sp. FEMS *Microbiol. Lett.*, 277:150–156.

Lovley, D.R. and J.R. Lloyd, 2000. Microbes with a mettle for bioremediation. *Nat. Biotechnol.*, 18: 600–601.

Lu, C., M.R. Lin and C. Chu, 1999. Temperature Effects of Trickle-Bed Biofilter for Treating BTEX Vapors. *J. Environ. Eng*., 125:775-779.

Margesin, R. and F. Schinner, 2001. Biodegradation and bioremediation of hydrocarbons in extreme environment. *Appl. Microbiol. Biotechnol*., 56: 650-663.

Medina-BellverJ., P. Marin,A. Delgado, E.R. Rodriguez-sanches, J.L.Ramosand S. Marques, 2005.Evidence for in situ crude oil biodegradation after the Prestige oil spill. *Environ. Microbiol*., 7(6): 773–779.

Nicholson, C.A. and B.Z. Fathepure, 2004. Biodegradation of benzene by halophilic and halotolerant bacteria under aerobic conditions. *Appl. Environ. Microbiol.*, 70: 1222-1225.

Nicholson, C.A. and B.Z. Fathepure, 2005. Aerobic biodegradation of benzene and toluene under hypersaline conditions at the Great Salt Plains, Oklahoma. *FEMS. Microbiol. Lett*., 245: 257-262.

Oren, A., 2002. Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. *J. Ind. Microbiol. Biotechnol*.,28: 56-63.

Palleroni, N.J. and J.F. Bradbury, 1993. *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings *et al*. *Int. J. Syst. Bacteriol.*,43(3):606-9.

Patzelt, H., 2005. Hydrocarbon degradation under hypersaline conditions some facts, some experiments and many open questions. pp 105–122. *In* Gunde-Cimerman N, Oren A.

Sorokin, D.Y., A.J.H. Janssen and G. Muyzer, 2011. Biodegradation potential of halo(alkali)philic prokaryotes. *Crit. Rev. Environ. Sci. Technol.*, 42: 811–856.

Taiwo, A.M.,2011. Compostingasasustainable waste management technique in developing countries. *J. Environ. Sci. Technol*., 4:93-102.

Vauterin, L., B. Hoste, K. Kersters, and J. Swings, 1995.Reclassication of *Xanthomonas*. *Int. J. Syst. Bacteriol.*, 45: 472-489.

Ventosa, A. andJ. Nieto, 1995. Biotechnological applications and potentialities of halophilic microorganism.*World J. Microbiol. Biotechnol*., 11: 85-94.

Wolfgang, F., K. Altendorf, E. Stackebrandt and A.Lipski, 2000. Characterization of N_2O-producing *Xanthomonas*-like isolates from biofilters as *Stenotrophomonas nitritireducens* sp. nov., *Luteimonas mephitis* gen. nov., sp. nov. and *Pseudoxanthomonas broegbernensis* gen. nov., sp. nov. *Int. J.l. Syst. Evol. Microbiol.*, 50:273–282

Yang, C., N. Liu, X.M. Guo and C.L. Qiao, 2006. Cloning of mpd gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil. *FEMS Microbiol. Lett*., 265: 118-125.

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