African Journal of Biotechnology Vol. 12(8), pp. 791-797, 20 February, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.576
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Identification and genetic characterization of phenol-degrading bacterium isolated from oil contaminated soil

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Accepted 28 May, 2012

A phenol-degrading novel monosodium glutamate was isolated from oil contaminated soil in India. This strain was found capable to utilize and tolerate up to 9.5 mM of phenol. Based on the results of phylogenetic similarity of 16S recombinant ribonucleic acid (rRNA) gene sequences and fatty acid analysis, strain MSG8 was identified as a novel *Acinetobacter* sp. The sequence of the partial largest subunit of multicomponent phenol hydroxylase (*LmPH*) gene of this isolated strain was identified and compared with *LmPH* gene of *Pseudomonas* sp.CF600. Reversed phase high performance liquid chromatography showed that the isolate can degrade phenol through catechol ortho fission pathway. In this paper, we reported about the new strain of *Acinetobacter* sp. capable of degrading phenol (9.5 mM of phenol concentration) under normal conditions. Therefore, strain MSG8 can potentially be used in bioremediation of phenol. The given data demonstrates that the isolated strain exhibits the characteristics of an efficient phenol-degrading microorganism.

Key words: Phenol, degradation, monosodium glutamate, bioremediation, *Acinetobacter* sp.

INTRODUCTION

Phenol and its derivatives are widely used in a variety of chemical synthesis (Neumann et al., 2004) and as pesticides (Nadavala et al., 2009) and as a result, these compounds are widespread in the environment. The problem is compounded by the fact that phenol is toxic, carcinogenic, mutagenic and teratogenic (Autenrieth et al., 1991) even at low concentrations. It has been included in the list of priority pollutants of U.S. Environmental Protection Agency. Therefore, efforts have been made to remove/degrade phenol using various physical (for example, solvent extraction, activated carbon adsorption) (Suidan et al., 1981, 1983) and chemical (Lin and Juang, 2009) methods. However these methods are not used extensively because of their expensive nature and also because the chemicals used in these treatment processes may harm the surrounding environment (Jiang et al., 2002). To overcome the problems related with use of physical and chemical treatment process, biological treatment processes are preferred for treating the wastewaters containing phenol (Moussavi et al., 2009). Large numbers of phenol degrading microorganisms have been isolated for biological treatment of such wastewater. These include *Acinetobacter* (Abd-El-Haleem et al., 2002), *Bacillus* (Arutchelvan et al., 2005), *Burkholderia* (El-sayed et al., 2003), *Pseudomonas* (Arutchelvan et al., 2005; Whiteley et al., 2001*), Valivorax* (Watanbe et al., 1998), mesophilic

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Abbreviations: MSM, Minimal salts medium; **HPLC,** high-performance liquid chromatography.

and thermophilic methanogens (Chen et al. 2008) and the yeast *Candidatropicalis* (Jiang et al., 2005). Phenol-degrading bacteria have also been isolated from plant leaves (Sandhu et al., 2009), roots (Abd-El-Haleem et al., 2002; Wang et al., 2007), root nodules (Wei et al., 2008), rivers (Heinaru et al., 2000), and marine ecosystems (Shashirekha et al., 1997). The enzyme phenol hydroxylase (PH), has a key role in the phenol degradation pathway, has been used as a molecular tool to identify phenol degradation capability in different bacteria which are present in environmental samples contaminated with phenol (Dong et al., 2008). PHs occurs as both single- and multi-component variants in nature. Most commonly, the sequence of the largest subunit of the multicomponent phenol hydroxylase (LmPH) has been used to evaluate ability to degrade phenol (Dong et al., 2008). In the present study, we report a new strain of *Acinetobacter* (a Gram negative bacterium) isolated from oil contaminated soil, which can tolerate phenol at a concentration as high as 9.5 mM. The phenol biodegradation ability of this bacterium was analyzed using high performance liquid chromatography with or without supplementing the growth medium with glucose in the presence of phenol.

MATERIALS AND METHODS

Culture conditions

The minimal salts medium (MSM) contained (gL^{-1}): KH_2PO_4 (34.8), K_2HPO_4 (27), $MgSO_4.7H_2O$ (24.50), EDTA (Na salt) (67), $ZnSO_4.7H_2O$ (5.8), $MnSO_4.4H_2O$ (33.8), $CUSO_4.7H_2O$ (5.0), $FeSO_4.7H_2O$ (55.6), $Na_4MoO_4.2H_2O$ (2.28), $(NH_4)_2SO_4$ (26). Luria bertani (LB) Medium (HiMedia, India) (Bertani G, 1951) contained (gl^{-1}): tryptone (10.0), NaCl (10.0) and yeast extract (5.0) glucose (0.5% w/v) and phenol 1 to 9.5 mM.

Isolation of phenol-degrading microorganisms

Soil samples for the isolation of phenol-degrading microorganisms were collected from an oil refinery in India. 2.5 g soil was suspended in 50 ml sterilized water, after vortexing and shortly sonicating, it was appropriately diluted. The diluted suspensions were spread on solid MSM plates with 9.5 mM phenol as the sole source of carbon. After two days of incubation at 37°C, all the colonies that appeared on the plate were picked out and restreaked on MSM-phenol plates to obtain pure cultures. On the basis of phenol degradation, isolate MSG8 was selected and maintained in MSM slants with 9.5 mM phenol for further study.

Growth patterns of MSG8

Growth pattern of strain monosodium glutamate was studied on MSM medium containing 1 to 9.5 mM phenol with or without addition of 0.5% Glucose. Growth was estimated by reading absorbance of culture broth at 600 nm using ultra-violet visible (UV-Vis) spectrophotometer (Shimadzu). Growth curves of MSG8 strain in MSM with 0.5 % glucose, cresol and phenol (1 to 9.5 mM) are shown in Figure 2, polymerase chain reaction (PCR) amplification of 16S recombinant deoxyribonucleic acid (rDNA) and the partial *LmPH* gene. The 16S rDNA was amplified using the primers; (5′-CAGCAGCCGCGGTAATAC-3′) (5′-TACGGCTACCTTGTTACG-3′) as reverse primer. PCR amplification was performed under the following conditions; 3 min at 95°C; 30 cycles of 1 min at 95°C, 1 min at 65°C and 1 min 30 s at 72°C, plus an additional 7 min cycle at 72°C. The PCR product was gel extracted using gel extraction kit (Qiagen). The sequencing was done using DNA sequence (Applied Bio Systems 3730). The resultant sequence was aligned in FASTA(Mackey et al., 2000; Pearson and Lipman, 1988) followed by search of the GenBank nucleotide library for similar sequences was performed using Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) through the National Center for Biotechnology Information Web site (http://www.ncbi.nlm.nih.gov/BLAST).

The Genbank access numbers for the bacterium is FJ848381. The closest relatives of the seguence were aligned using Clustalw (Larkin et al., 2007). Phylogeny was analyzed by Treeconw software (Van and De, 1994). Distances were calculated using the Kimura two-parameter distance model (Kimura, 1980). Rooted trees were built by the neighbour-joining method (Kumar and Gadagkar, 2000). The correctness of the phylogenetic tree was estimated by bootstrap analysis (Holmes, 2003) with 1000 resamplings. The partial largest subunit of multicomponent phenol hydroxylase (*LmPH*) gene of both *Acinetobacter* sp. MSG8 and *Pseudomonas sp*. CF600, was amplified with primers Lph1 (5′-AGG CAT CAA GAT CAC CGACTG-3′) and Lph2 (5′-CGC CAG AAC CAT TTA TCG ATC-3′) as described previously by Xu et al. (2001, 2003). Plasmid isolation of both bacteria (*Acinetobacter sp.* MSG8 and *Pseudomonas* CF600) was done using mini prep kit (Invitrogen). The plasmid was used as the template for the reaction. The PCR reaction conditions were same as 16S rDNA amplification. The PCR products were sequenced and analyzed with the same methods that of 16S rDNA.

Phenol degradation by isolated bacterium

This strain was cultured in Luria Bertani medium in a shaking incubator at 37°C and 200 rpm in shaking incubator. After 3 to 4 h (late-exponential phase), the culture broth was centrifuged at 5000 rpm for 4 min at room temperature. Pellet was washed with potassium phosphate buffer (pH 7.5) and suspended in the same buffer to obtain as 1mM absorbance of 0.8 to 0.9 at 600 nm. This suspension (5% v/v) was used to inoculate and incubated at 30 to 37°C level into 50 ml liquid MSM containing phenol (9.5 mM) and incubated at 30 to 37°C and 200 rpm. The culture was collected periodically for determination of $OD_{600\ (nm)}$ and phenol concentration was determined with a direct colorimetric method using 4-aminoantipyrene (APHA, 1989).

Culture preparation for high-performance liquid chromatography (HPLC)

Minimal salt medium (MSM) was prepared to culture the *Acinetobacter sp* (The phenol degrading strain), was pre cultured in LB medium in a rotary shaker at 30 to 37°C with 180 to 200 rpm till the later-exponential phase, and the cells were collected by centrifugation at 5000 rpm at room temperature. The cell pellets were washed twice with sterilized 0.2 M potassium phosphate buffer (pH 7.5), and adjusted to an OD_{600} of 1.0 for phenol degradation experiments. The cells were inoculated at 3 % (v/v) level into 100 ml liquid MSM plus phenol (1 mM) medium and then incubated at 30 to 37°C with 180 to 200 rpm. This culture sample was used for high-performance liquid chromatography (HPLC) after filtration.

HPLC analysis of phenol

The study was carried out at initial concentration of phenol around

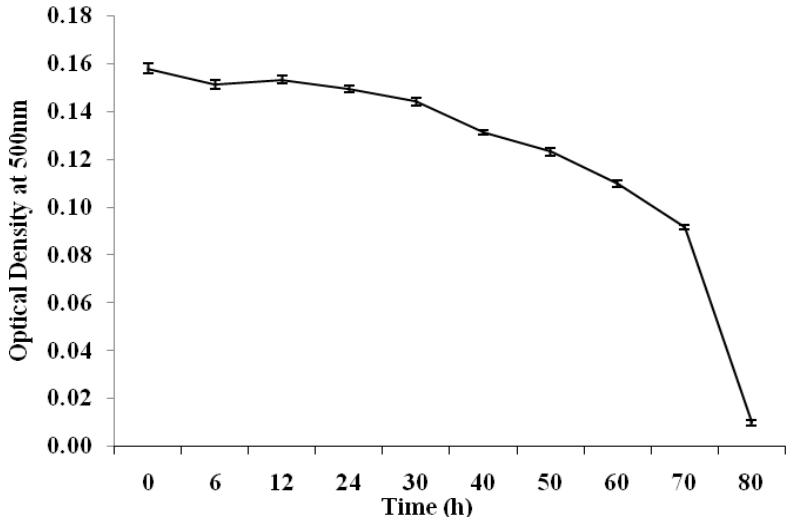

Figure 1. Time-course of phenol degradation of MSG8 in liquid MSM containing 9.5 mM phenol initially. The results are shown as average of six independent experiments and the bars indicate the standard deviation less than 5%.

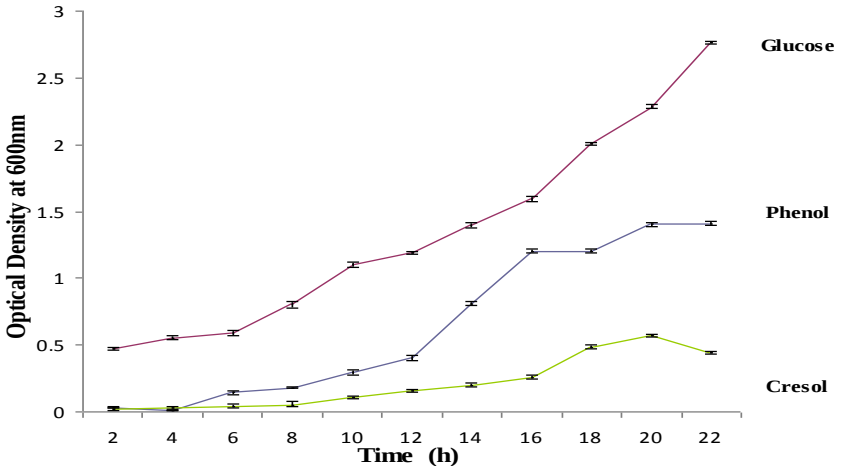

Figure 2. Growth patterns of MSG8 strain in MSM suspended with only glucose, phenol and cresol. The results are shown as average of five independent experiments and the bars indicate the standard deviation <5%.

1.0 mM using reversed phase (RP)-HPLC. For the estimation of phenol degradation RP-HPLC method was used. 20 µl of the sample was injected on 4.0 x 250 mm ODS C18 column. A mixture of methanol /de ionized water /glacial acetic acid (60:38:2, v/v/v) was used as the solvent and the flow rate was maintained at 0.5 ml/min. The analysis was carried out at 274 nm. The retention time of standard phenol solution (1 mM) under the above conditions was found to be 9.80 min.

Nucleotide sequence accession number

The nucleotide sequence of the 16S rRNA gene of strain MSG8 has been deposited in the GenBank database under accession no. FJ848381.

RESULTS AND DISCUSSION

Phenol-degrading bacteria exist widely in the environments and they are usually isolated from phenol-contaminated sites. In this study, we isolated one degrading strain, MSG8; which was from the genus of Acinetobacter. This strain was isolated from the oil refinery soil in India. The bacterial isolate was morphologically and biochemically typified and properties were listed in Table 1. The colorimetric assay data presented in the curve showed the capability of strain MSG8 for degrading 9.5 mM of phenol within 80 h (Figure 1).

Table 1. Morphological and biochemical characteristics of the bacterial strain MSG8 that is able to grow in a medium where phenol is the sole carbon source.

Characteristic	MSG8
Starch hydrolysis	-
Casein hydrolysis	-
Citrate utilization	+
Gelatin liquefaction	+
H_2S production	-
MR	+
VP	-
Nitrate reduction	-
Indole	-
Acid production from	
Arabinose	+
Galactose	+
Glucose	+
Mannitol	+
Raffinose	-
Acid production from	
Arabinose	+
Galactose	+
Glucose	+
Mannitol	+
Raffinose	-
Gram staining	-
Spore staining	-
Motility	-
Catalase	+
Oxidase	-
Acid production from	
Urea	-
Salicin	-
Xylose	+
Sucrose	-
Rhamnose	-
meso-inositol	-
Fructose	-
Growth at different Temperature (°C)	
At 15	+
At 25	+
At 37	+
At 42	+
Growth at different pH	
At pH 5.2	+
At pH 8.0	+
Growth at pH 9.0	+
Growth at pH 10.0	+
Growth at different concentration of NaCl (%)	
On NaCl 2	+
On NaCl 5	-
On NaCl 7	-
On NaCl 10	-

MSG8 strain showed tolerance up to 9.5 mM and has high phenol-degrading ability (98%) with phenol. Degradation rate is 0.118 mMh^{-1}. The phenol tolerance of the genera Acinetobacter, Pseudomonas and Vibrio were reported to be 7 to 10 mM (Dong et al., 2008). A strain of *Pseudomonas putida* (MTCC 1194) could tolerate phenol up to 10 mM (Bandhopadhyay et al., 1998). *Burkholderia* cepacia PW3 and *Pseudomonas aeruginosa* AT2 are the highest phenol tolerant bacteria which were reported at 30 mM (El-Sayed et al., 2003). The growth patterns in different carbon sources (for example, glucose/phenol/cresol) (Figure 2) indicate that the presence of glucose as well as phenol in MSM help MSG8 in adaptation to medium, however in case of cresol in MSM, MSG8 was not able to grow as glucose and phenol, as a sole source of carbon. In case of phenol, growth was observed very fast in first two days after which growth was found to be constant. The partial *LmPH* gene was taken as molecular marker for phenol-degrading ability (Watanabe et al., 1998, 2002) as this gene encodes phenol degrading enzyme phenol hydroxylase, which converts phenol to catechol. Specific bands of 684 bp of *LmPH* gene sequence were amplified (Figure 3) and program BLAST confirmed that this had high similarities with *LmPH* gene of *Pseudomonas* sp.CF600.

In HPLC analysis, concentration of phenol was estimated with the peak area, which was found to decrease after 2 h from the initial value. This trend continued for 30, 40, 50 and 60 h. After 70 h of incubation (Figure 4), the peak disappeared completely indicating almost complete degradation of phenol. A compound with a retention time of 7.62 was found to accumulate simultaneously with the phenol degradation. The retention time of this compound matches with that of 1 mM standard solution of catechol (7.67). No degradation was observed in case of un-inoculated medium and medium inoculated with negative control even after 7 h of incubation. It may be due to production of catechol as a result of ortho ring cleavage of phenol.

The results of the chemotaxonomic analysis of MSG8 are shown in Table 2. The main non-polar fatty acids detected were octadecenoic acid ($C_{18:1}$) and hexadecenoic acid ($C_{16:1}$). The hydroxyl fatty acids detected were 3-hydroxydodecanoic acid ($C_{12:0}$3-OH) and 2-hydroxydodecanoic acid ($C_{12:0}$2-OH).

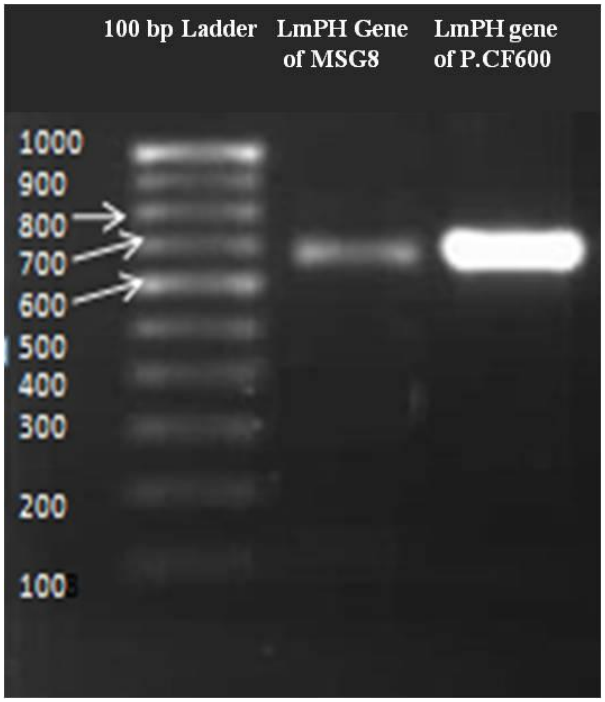

Figure 3. The existence of genes encoding LmPH was investigated in strain MSG8. PCR amplification showed that MSG8 strain and *Pseudomonas* sp.CF600 had the DNA fragment of 684 bp in length.

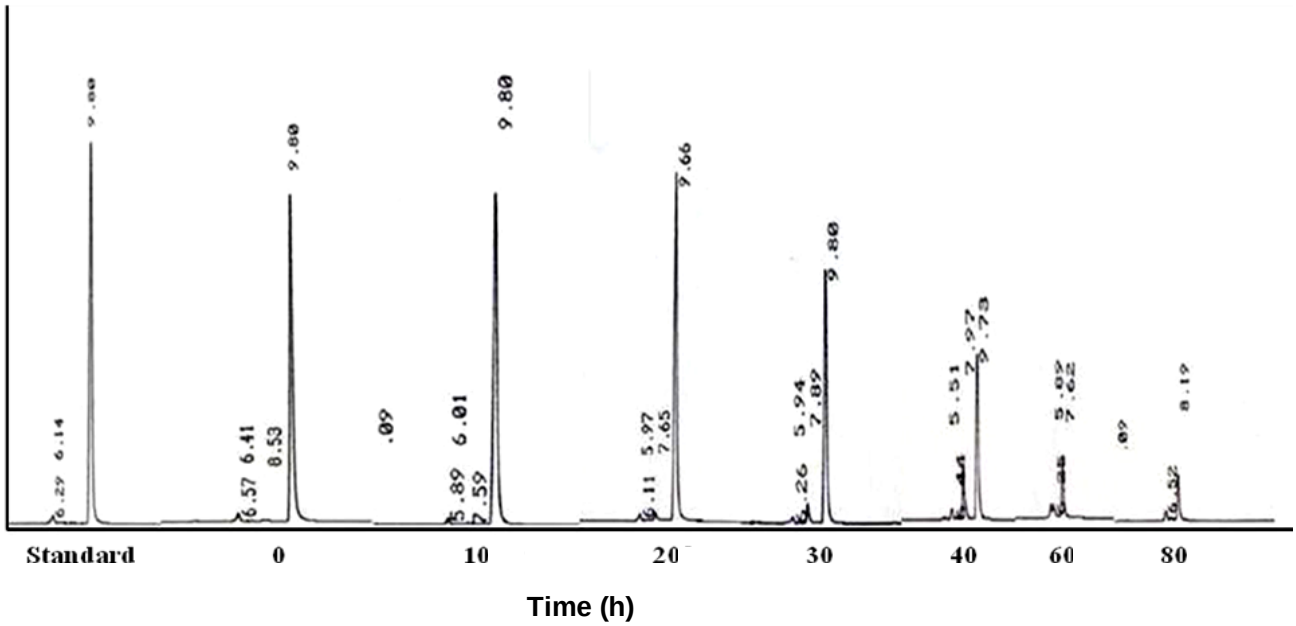

Figure 4. HPLC analysis of phenol by strain MSG8 at different time interval.

Gas chromatography-fatty acid methyl ester (GC-FAME) analysis (Lu and Harrington, 2010) of MSG8 showed highest similarity with *Acinetobacter baumannii* in the index (0.843) which has <97% similarity with MSG8. Phylogenetic tree showed the relationship of strain MSG8 with GenBank database (Figure 5). The isolated strain shared high identity (99%) with *Acinetobacter calcoaceticus.* In the present study, we have shown the

Table 2. Percentage cellular fatty acid composition of MSG8.

Fatty acid	Percentage (%)
$C_{12:0}$	4.15
$C_{12:0}$2-OH	2.06
$C_{12:0}$3-OH	3.36
$C_{14:0}$3-OH	1.92
$C_{16:1}$	14.03
$C_{16:0}$	21.11
$C_{17:1}$	7.96
$C_{17:0}$	2.82
$C_{18:1}$	42.57

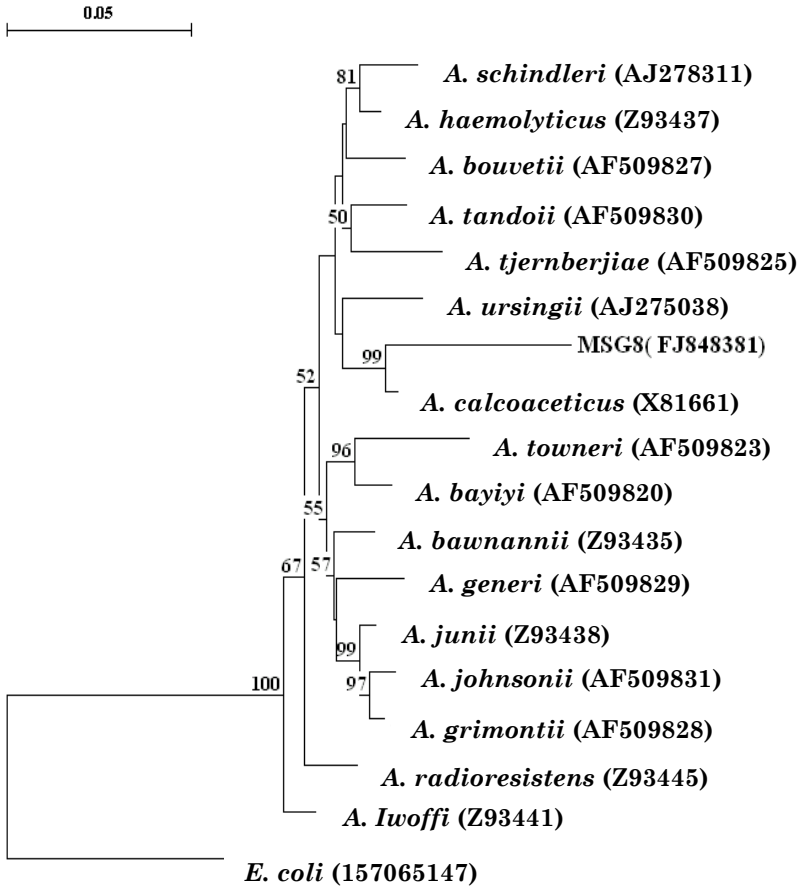

Figure 5. Phylogenetic tree based on 16s rRNA gene sequences showing the relationship between *Acinetobacter sp.*MSG8 and species from genus *Acinetobacter* with validly published names. Neighbour-joining model is employed for the tree construction and bootstrap values were obtained after 1000 resamplings. The scale bars represent 0.05 substitution/site. *E.coli* was used to root the tree.

growth and phylogenetic analysis of the isolated strain of *Acinetobacter* which is highest phenol tolerant bacterium among all the strains of *Acinetobacter* genus, based on 16S rDNA sequences which is a conserved sequence in prokaryotes (Weisberg et al. 1991). Phylogenetic tree showed the relationship of strain MSG8 with GenBank database (Figure 5). The isolated strain shared high identity (99%) with *A. calcoaceticus.*

Conclusion

In the continuation of isolation new and efficient microbial strain for phenol degradation, we report here a novel bacterial strain, which is quite capable to utilize phenol as sole source of carbon and energy. The degradation ability of isolate was checked up to 9.5 mM of phenol concentration. GC-FAME and 16 rDNA analysis prove it as a novel strain. HPLC analysis shows the degradation capability of this strain. This work has provided a useful guideline in evaluating potential phenol biodegraders isolated from environment.

REFERENCES

Abd-El-Haleem D, Moawad H, Zaki EA, Zaki S (2002). Molecular characterization of phenol-degrading bacteria isolated from different Egyptian ecosystems. Microb. Ecol. 43:217-224.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. J. Mol. Biol. 215:403-410.

APHA (1989). Standard methods for the examination of water and wastewater, American Public Health Association, Washington, DC.

Arutchelvan V, Kanakasabai V, Nagarajan S, Muralikrishnan V (2005). Isolation and identification of novel high strength phenol degrading bacterial strains from phenol-formaldehyde resin manufacturing industrial wastewater. J. Hazard. Mater. 127:238-243.

Autenrieth RL, Bonner JS, Akgerman A, Okaygun M, McCreary EM (1991). Biodegradation of phenolic wastes. J. Hazard. Mater. 28:29-53.

Bandyopadhyay K, Das D, Maiti BR (1998). Kinetics of phenol degradation using *Pseudomonas putida* MTCC 1194. Bioproc. Biosyst. Eng. 18:373-377.

Bertani G (1951). Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. 62:293-300.

Chen CL, Wu JH, Liu WT (2008) Identification of important microbial populations in the mesophilic and thermophilic phenol degrading methanogenic consortia. Water Res. 42:1963-1976.

Dong X, Hong Q, He L, Jiang X, Li S (2008). Characterization of phenol-degrading bacterial strains isolated from natural soil. Int. Biodeterior. Biodegr. 62:257-262.

El-Sayed WS, Ibrahim MK, Abu-Shady M, El-Beih F, Ohmura N, Saiki H, Ando A (2003). Isolation and characterization of phenol catabolising bacteria from a cooking plant. Biosci. Biotechnol. Biochem. 67:2026-2029.

Heinaru E, Truu J, Stottmeister U, Heinaru A (2000). Three types of phenol and p-cresol catabolism in phenol- and p-cresol degrading bacteria isolated from river water continuously polluted with phenolic compounds. FEMS Microbiol. Ecol. 31:195-205.

Holmes S (2003) Bootstrapping Phylogenetic Trees: Theory and Methods. Statist. Sci. 18:241-255.

Jiang HL, Tay JH, Tay STL (2002). Aggregation of immobilized activated sludge cells into aerobically grown microbial granules for the aerobic biodegradation of phenol. Lett. Appl. Microbiol. 35:439-445.

Jiang Y, Wen JP, Li HM, Yang SL, Hu ZD (2005). The biodegradation of phenol at high initial concentration by the yeast *Candida tropicalis*. Biochem. Eng. J. 24:243-247.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111-120.

Kumar S, Gadagkar SR (2000). Efficiency of the Neighbour-Joining Method in Reconstructing Deep and Shallow Evolutionary Relationships in Large Phylogenies. J. Mol. Evol. 51:544-553.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007). ClustalW and ClustalX version 2.

Lin SH, Juang RS (2009). Adsorption of phenol and its derivatives from water using synthetic resins and low-cost natural adsorbent. J. Environ. Manag. 90:1336-1349.

Lu Y, Harrington PB (2010). Classification of bacteria by simultaneous methylation–solid phase microextraction and gas chromatography/mass spectrometry analysis of fatty acid methyl esters. Anal. Bioanal. Chem. 397:2959-2966.

Mackey AJ, Haystead TA, Pearson WR (2002). Getting more from less: algorithms for rapid protein identification with multiple short peptide sequences. Mol. Cell. Proteomics. 1(2):139-147.

Moussavi G, Mahmoudi M, Barikbin B (2009). Biological removal of phenol from strong wastewaters using a novel MSBR. Water Res. 43:1295-1302.

Nadavala SK, Swayampakul K, Bodd VM, Abburi K (2009). Biosorption of phenol and o-chlorophenol from aqueous solutions on to chitosan–calcium alginate blended beads. J. Hazard. Mater. 162:482-489.

Neumann G, Teras R, Manson L, Kivisaar M, Schayer F, Heipieper HJ (2004). Simultaneous degradation of atrazine and phenol by Pseudomonas sp. strain ADP: effects of toxicity and adaptation. Appl. Environ. Microb. 70:1907-1912.

Pearson WR, Lipman DJ (1988). Improved Tools for Biological Sequence Comparison. Proc. Natl. Acad. Sci. 85:2444-2448.

Sandhu A, Larry JH, Gwyn AB (2009). Identification and Genetic Characterization of Phenol-Degrading Bacteria from Leaf Microbial Communities. Microb. Ecol. 57:276-285.

Shashirekha S, Uma L, Subramanian G (1997). Phenol degradation by the marine cyanobacterium *Phormidium valderianum* BDU 30501. J. Ind. Microbiol. Biotechnol. 19:130-133.

Suidan MT, Cross WH, Fong M, Calvert JW (1981). Anaerobic carbon filter for degradation of phenols. J Environ Eng. Div. ASCE, 107:563-579.

Suidan MT, Siekerka GL, Kao SW, Pfeffer JT (1983). Anaerobic filters for the treatment of coal gasification wastewater. Biotechnol. Bioeng. 25:1263-1270.

Van de Peer Y, De Wachter R (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Appl. Biosci. 10:569-570.

Wang YJ, Xiao M, Geng XL, Liu JY, Chen J (2007). Horizontal transfer of genetic determinants for degradation of phenol between the bacteria living in plant and its rhizosphere. Appl. Microbiol. Biotechnol. 77:733-739.

Watanabe K, Futamata H, Harayama S (2002). Understanding the diversity in catabolic potential of microorganisms for the development of bioremediation strategies. Anton. Leeuw. Int .J. G. 81:655-663.

Watanabe K, Teramoto M, Futamata H, Harayama S (1998). Molecular detection, isolation, and physiological characterization of functionally dominant phenol-degrading bacteria in activated sludge. Appl. Environ. Microbiol. 64:4396-4402.

Wei GH, Yu JF, Zhu YH, Chen WM, Wang L (2008). Characterization of phenol degradation by Rhizobium sp CCNWTB 701 isolated from *Astragalus chrysopteru* in mining tailing region. J. Hazard. Mater. 151:111-117.

Weisberg WG, Barns SM, Pelletier DA, Lane DJ (1991). 16S Ribosomal DNA Amplification for Phylogenetic Study. J. Bacteriol. 173:697-703.

Whiteley AS, Wiles S, Lilley AK, Philp J, Bailey MJ (2001). Ecological and physiological analyses of Pseudomonad species within a phenol remediation system. J. Microbiol. Meth. 44:79-88.

Xu YQ, Chen M, Zhang W, Lin M (2003). Genetic organization of genes encoding phenol hydroxylase, benzoate 1,2-dioxygenase alpha subunit and its regulatory proteins in *Acinetobacter calcoaceticus* PHEA-2. Curr. Microbiol. 46:235-240.

Xu YQ, Fang XJ, Chen M, Zhang W, Li JM, Lin M (2001). The detection of phenol degrading strain in environment with specific primer of phenol hydroxylase gene. Acta. Microbiologica. Sinica. 41:298-303.