BIODEGRADATION OF PHENOL BY *STENOTROPHOMONAS* SP. AND *STAPHYLOCOCCUS* SP. ISOLATED FROM CONTAMINATED SITES

AHMAD, N.[1] – AHMED, I.[1,2*] – IQBAL, M.[1] – KHALID, N.[3, 4] – ABBAS, S.[1] – MEHBOOB, F.[1,5] – AHAD, K.[5]

[1]*PARC Institute of Advanced Studies in Agriculture, National Agricultural Research Centre (NARC), Park Road, Islamabad-45500, Pakistan*
[2]*Institute of Microbial Culture Collection of Pakistan (IMCCP), National Agricultural Research Centre (NARC), Park Road, Islamabad-45500, Pakistan*
[3]*School of Food and Agricultural Sciences, University of Management and Technology, Lahore 54000, Pakistan*
[4]*Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Victoria 3216, Australia*
[5]*Ecotoxicology Research Institute (ERI), National Agricultural Research Centre (NARC), Park Road, Islamabad-45500, Pakistan*

**Corresponding author*
e-mail: iftikharnarc@hotmail.com
(phone: +92-51-9073 3729; fax: +92-51-9255034)

(Received 27th Apr 2016; accepted 22nd Jul 2016)

Abstract. Phenol as environmental pollutant is detrimental to living organisms and needed to be eliminated for environmental safety. Among the various practiced approaches for its removal, bacterial utilization gets attraction due to its eco-friendly and cost effective nature. For this purpose, bacterial strains were isolated from bioremediation site and industrial waste through enrichment in phenol (250 mg L^{-1}) for 3 days at 28°C. After enrichment, morphologically distinct colonies were purified on phenol (200 mg L^{-1}) agar plates and the strains were identified through 16S rRNA gene sequence. Total of eight strains were identified, among them two strains, NCCP-310 and NCCP-405 had the best potential of phenol degradation which were identified as the members of the genera *Stenotrophomonas* and *Staphylococcus*. NCCP-310 and NCCP- 405 showed 98.85 and 98.9% sequence identity with *Stenotrophomonas maltophilia and Staphylococcus equorum* subsp. *equorum*, respectively. Both strains have ability to tolerate 1000 mg L^{-1} phenol. The isolated strains degraded 750 mg L^{-1} of phenol at pH 7 and 28+2°C. NCCP-310 and NCCP-405 showed degradation of such amount in 65 and 85 h with the average rate of 15.65 and 11.64 mg L^{-1} h^{-1}. Our work suggests that these strains are efficient in phenol removal and could be used for bioremediation.
Keywords: *phenol, biodegradation, Stenotrophomonas, Staphylococcus, bioremediation*

Introduction

The foremost challenge of environmental concern is the elimination of pollutant that is liberated at alarming rate in our ecosystem and food chain due to rapid industrialization. Untreated industrial wastes containing xenobiotics drained up and contaminate water resources which are used for domestic as well as in agricultural practices (Kwon and Yeom, 2009; Ahmed et al., 2012). Phenol is used most widely in many industries such as pharmaceutical, plastic, ceramics, oil refinery, resin manufacturing, coke plant, textile and steel industries etc. (Han et al., 2010; Zhu et al., 2012) and due to its toxicity, phenol is in the priority list of US Environmental Protection Agency (US EPA, 2007).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

Phenol ranging from 10 to 17500 mg L^{-1} is detected in industrial effluent (Carbajo et al., 2010) while, only 0.5 mg L^{-1} is permitted by the Environmental Protection Agencies in the effluent based on production, exposure and biological effects (Giti et al., 2005). The contamination of water by phenol generates polychlorinated phenols which at low concentration (2.0 μg L^{-1}) cause unpleasant smell in drinking water (Arutchelvan et al., 2006). Phenol is lethal for all form of life includes humans, animals, plants, aquatic life and microorganisms (Rocha et al., 2007). Proper hygienic techniques are obligatory to dispose bulk phenol containing effluents. To cope with this situation, many physical and chemical methods (adsorption, solvent extraction, activated carbon adsorption, chemical oxidation) are practiced which are no more desirable owing to high cost of production, hazardous to workers and nearby population (Idris and Saed, 2002). Therefore, biodegradation is the plausible approach for phenol removal because of low cost and eco-friendly nature (Saravanan et al., 2008).

Diverse groups of microorganisms including fungi, algae and bacteria are naturally endowed with the property of phenol degradation (Godjevargova et al., 2003; Fialova et al., 2004; Quan et al., 2004) and many bacterial isolates with high phenol degrading potential are evaluated belonging to various genera including *Rhodococcus* (Larkin et al., 2005), *Stenotrophomonas* (Urszula et al., 2009), *Pseudomonas* (Ahmad et al., 2014), etc.

The objectives of the current study were the isolation, identification of phenol degrading bacteria based on 16S rRNA gene and to determine their phenol degrading potential. We reported phenol degrading potential of *Stenotrophomonas* sp. NCCP-310 and *Staphylococcus* sp. NCCP-405 which was isolated from sludge of bioremediation site and industrial waste respectively.

Materials and methods

Isolation and enrichment

Samples (waste) were collected from two sites i.e. Bioremediation Garden, NARC and industrial area I-9 Islamabad, Pakistan. The enrichment of samples was conducted at ambient temperature for 3 days at 120 rpm in mineral salt medium (MSM) containing phenol (250 mg L^{-1}). Two to three drops of the enriched samples were spread on MSM plates containing only phenol (200 mg L^{-1}) for carbon need. Plates were placed in incubator at 28 ^{o}C till growth. Morphologically distinct colonies appeared was sub-cultured and purified on phenol (200 mg L^{-1}) containing agar plates again. Subculturing was perfumed many times to get pure culture. The isolated strains were preserved at -80°C in 70 % glycerol solution.

Identification of bacterial strains

Identification of the isolated strains was performed on the bases of 16S rRNA gene followed by the method of Ahmed et al. (2007). For this purpose, pure culture of each strain was obtained by growing on Tryptic Soy Agar (TSA) plates incubated at 28°C. After purification, single colony of each strain was dipped and stirred in TE buffer (20 μL) in PCR strips, homogenized and kept in thermal cycler at 95°C for 10 min for extraction of DNA. Strips were then removed and centrifuged at 12000 rpm for 5 min.
Genes of 16S rRNA was amplified using 1 μL template bacterial DNA contained in supernatant. 49 μL master mix was prepared for each strain by mixing TAKARA pre-

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

mix (25 µL), 2 µL of each following primers: 9F (5'-GAGTTTGATCCTGGCTCAG-3') and 1510R (5'-GGCTACCTTGTTACGA-3') and 20 µL PCR water. The final reaction volume was made to 50µL in PCR tube by adding the prepared 49 µL of master mix to 1 µL of the template DNA which was already added to each PCR tube. In centrifuge machine the samples were short spin for a min or two for homogenization. PCR strips were then placed in thermal cycler (Applied Biosystems, Veriti, USA), PCR program was set as described by Ahmed et al. (2007) to amplify the said gene. Amplified products were confirmed by gel electrophoresis using 0.8% agarose in which, bromo phenol blue and ethidium bromide were used as loading dye and staining dye, respectively. Images of the gel were taken on gel documentation system (UVIPro Platinum, England). Purification of the Amplified products were done following the manufacturer's protocol (Invitrogen, USA). The amplified 16S rRNA gene products were sequenced using universal forward 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse 1492R (5'-ACCTTGTTACGACTT-3') primers.

The obtained sequences were refined with BioEdit software and to retrieve closest matches, BLAST search was performed on Ez-Taxon Server. On the basis of maximum identity score sequences were selected which were aligned in Clustal W (V. 1.6) (Thompson et al., 1994). Phylogenetic trees were generated in MEGA-6 software using Neighbor-Joining algorithms (Tamura et al., 2011).

Biochemical characterization

Consumption of different carbon sources by the isolates were determined using API 20E kit (bioMerieux, France). Few pure colonies (16 to 18 h) of each strain were added in 0.85% saline solution and the microtubes of API 20E kit were filled with prepared inoculums. The kits were then placed in incubator at 28°C for 24-48 h and after then, the results were recorded according to color change.

Phenol tolerance

Phenol resistance of isolated strains was determined by introducing the pre-culture of each strain in MS broth augmented with 0, 250, 500, 750 and 1000 mg L^{-1} phenol in 100 mL flasks. Flask were placed on shaker within incubator at 28°C and incubated for 3-4 days. Blank without inoculum of each concentration was prepared in parallel. At different time gap growth was checked with the help of spectrophotometer (IMPLEN, Germany) at 600 nm wavelength. The growth of each strain at a given phenol concentration was determined with corresponding blank.

Phenol degrading potential and analysis

Phenol degrading efficiency of isolated strains was determined by adding the preculture of each strain in MSM broth augmented with 750 mg L^{-1} of phenol for 2-4 days at 200 rpm. Samples of the culture were collected at specific intervals for 3-5 days depending upon the growth of strain. Optical density (OD) of the samples was determined at 600 nm with spectrophotometer (IMPLEN, Germany) to observe the growth of cells over time.

One mL sample was taken from each flask at different time intervals and centrifuged at 12000 rpm for 7-10 min. Then 0.5 mL of centrifuged sample was diluted with an equal amount of acetonitrile. High Performance Liquid Chromatography (HPLC) (PerkinElmer, USA) consisting of C-18 column together with LC 295 UV/V detector.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

Mobile phase was comprised of acetonitrile and water at the rate of 60:40% (v/v) with flow rate of 0.8 mL/min. Detector wavelength was set to 280 nm (Ahmad et al., 2014). Identification of phenol was done on the basis of retention time and quantification on the basis of 6 points external standards calibration curve. The data obtained from bacterial growth versus time and phenol degradation versus time was analyze using regression analysis.

Results and discussion

Isolation and identification

Eight bacterial isolates were isolated through enrichment in 250 mg L^{-1} phenol but, here we focus on two strains i.e. NCCP-310 and NCCP-405 which showed high phenol degrading potential. Enrichment of culture was often practiced to isolate the desired microorganisms among the diverse microbial populations (Dunbar et al., 1997). After purification, the strains were characterized morphologically. Colony of the NCCP-310 was pale yellow in color with round shape and entire margin. The elevation was convex. Shape of NCCP-405 was circular with white color having smooth surface. Margin of the colony was entire with flat elevation.

Isolated strains were identified using 16S rRNA gene to avoid problems raised in identification merely on morphological bases (Roohi et al., 2012). Refined sequences (16S rRNA gene) of NCCP-310 and NCCP-405 were deposited to DNA Data Bank of Japan (DDBJ) with the accession numbers AB983341and AB983342, respectively. 16S rRNA gene sequence comparison of NCCP-310 showed that this strain shared 98.85% similarity with *Stenotrophomonas maltophilia* (AB008509) which was isolated by Hugh (1981) and assigned as *Pseudomonas maltophilia* but later on due to biochemical characterizations and 16S rRNA gene sequence affiliation, the strain was assigned as *Stenotrophomonas maltophilia* (Palleroni and Bradbury, 1993). This strain shared 98.85, 98.75 and 98.66% sequence identity with *Pseudomonas geniculata* (AB021404), *Pseudomonas hibiscicola* (AB021405) and *Pseudomonas beteli* (AB021406), respectively. Phylogenetic analysis confirmed the affiliation of NCCP-310 (*Fig. 1*) with the above said strains but Anzai et al. (2000) performed phylogenetic analysis of γ-β subclasses of the *Proteobacteria* and reclassified the strains as the members of the genus *Stenotrophomonas*. However, we suggest the DNA-DNA hybridization of NCCP-310 with the closely related strains to determine the exact taxonomic position. NCCP-405 exhibit 98.9, 98.8 and 97.79% identity with *Staphylococcus equorum* subsp. *Equorum* (AB009939), *Staphylococcus equorum* subsp. *Linens* (AF527483) and *Staphylococcus xylosus* (D83374), respectively. Phylogenetic analysis (*Fig. 2*) affirmed the association of NCCP-405 with the genus *Staphylococcus*.

Biochemical characterizations

Using API 20E kit, the isolated strains were tested for various organic substrates utilization. *Table 1* shows biochemical characterizations of *Stenotrophomonas* sp. NCCP-310 and *Staphylococcus* sp. NCCP-405. Both strains showed positive results for β-galactosidase and NO_2 production. In addition, *Stenotrophomonas* sp. NCCP-310 showed positive results for lysine decarboxylase, citrate utilization, gelatinase while negative for all the other substrates used. Similarly, *Staphylococcus* sp. NCCP-405 showed positive results for urease while negative for all other substrates tested.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

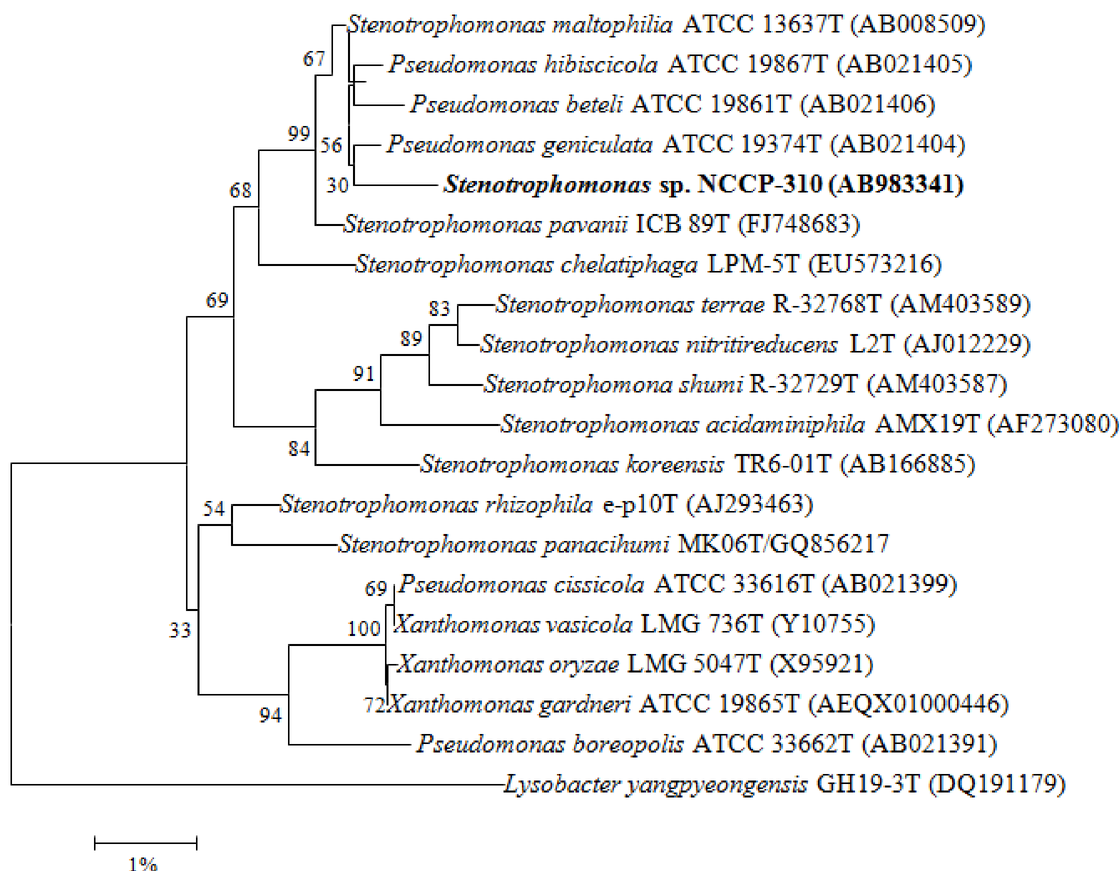


Figure 1. *Phylogenetic tree constructed in MEGA-5 with NJ method, showing the interelation of NCCP-310 with other closest matches using Lysobacter yangpyeongensis (DQ191179) as an out group.*

Table 1. *Biochemical characterization of Stenotrophomonas sp. NCCP-310 and Staphylococcus sp. NCCP-405.*

Biochemical tests	***Stenotrophomonas* sp. NCCP-310**	***Staphylococcus* sp. NCCP-405**
Arginine dihydrolase	–	–
Citrate utilization	+	–
Gelatinase	+	–
H_2S production	–	–
Indole production	–	–
Lysine decarboxylase	+	–
Ornithine dacarboxylase	–	–
Sodium pyruvate	–	–
Tryptophane deaminase	–	–
Urease	–	+
β-galactosidase	+	+
Fermentation/oxidation of:		
Amygdalin	–	–
Arabinose	–	–

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

Glucose	–	–
Inositol	–	–
Mannitol	–	–
Melibiose	–	–
Rhamnose	–	–
Sacharose	–	–
Sorbitol	–	–
NO_2 production	+	+
Reduction to N_2 gas	–	–

+, positive reaction; –, negative reaction. These results are obtained after 48-72 h of incubation at 28°C.

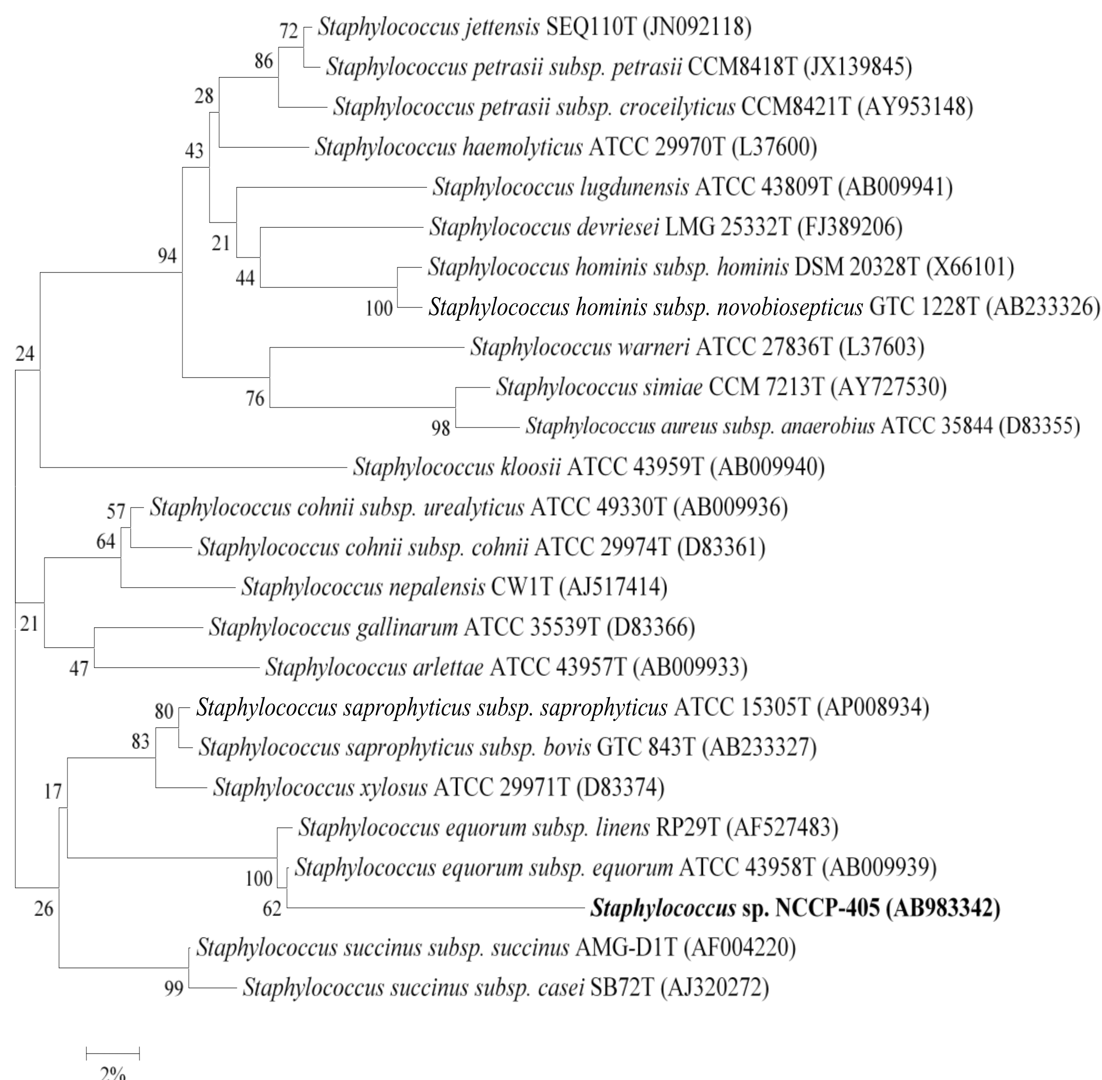


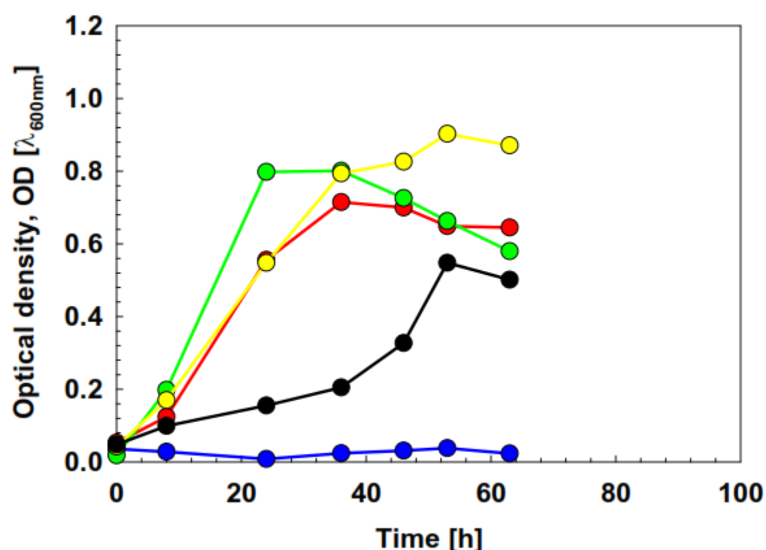
Figure 2. *Phylogenetic tree showing the inter-relationships of strain NCCP-405 with the most closely related type species inferred from sequences of 16S rRNA gene. The tree was generated using the NJ method. Bootstrap values were expressed as a percentage of 1000 replications. The bar shows 2% sequence divergence.*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

Phenol tolerance

Both stains were tested for their growth in MSM broth containing 0, 250, 500, 750 and 1000 mg L^{-1} phenol. The stain *Stenotrophomonas* sp. NCCP-310 was incubated for 63 h and growth was observed at all specified concentrations except 0 mg L^{-1}. Maximum growth was observed at 750 mg L^{-1} phenol after 53 h of incubation (*Fig. 3a*). Similarly, *Staphylococcus* sp. NCCP-405 was incubated for 86 h and showed growth at all concentrations except 0 mg L^{-1}. At all the given concentrations, the stain showed a very slow growth up to 12 h. Results indicated the extreme growth of NCCP-405 at 1000 mg L^{-1} phenol after 72 h of incubation (*Fig. 3b*).

(a)



(b)

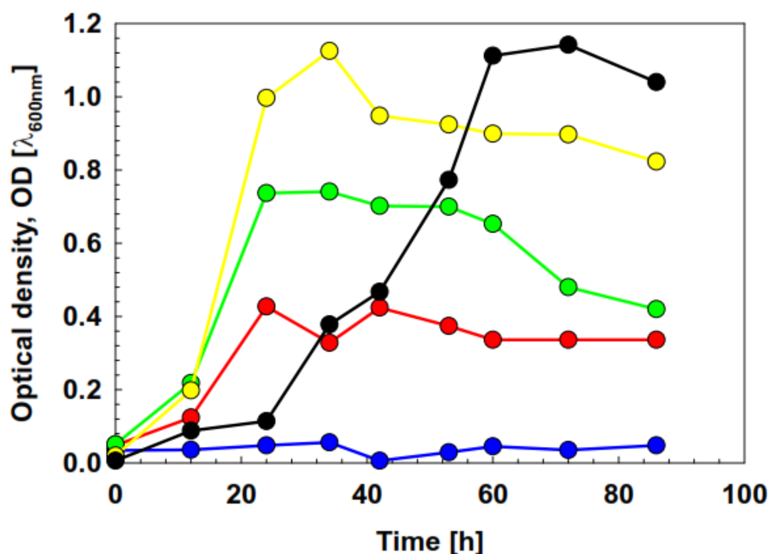


Figure 3. *Tolerance of phenol at different concentrations grown at 28°C in relation to time scale.* ***(a)*** *Stenotrophomonas sp. NCCP-310 and (b) Staphylococcus sp. NCCP-405. ( ) denote 0 mg L^{-1}, ( )250 mg L^{-1}, ( )500 mg L^{-1}, ( )750 mg L^{-1}, and ( ) denote 1000 mg L^{-1}.*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

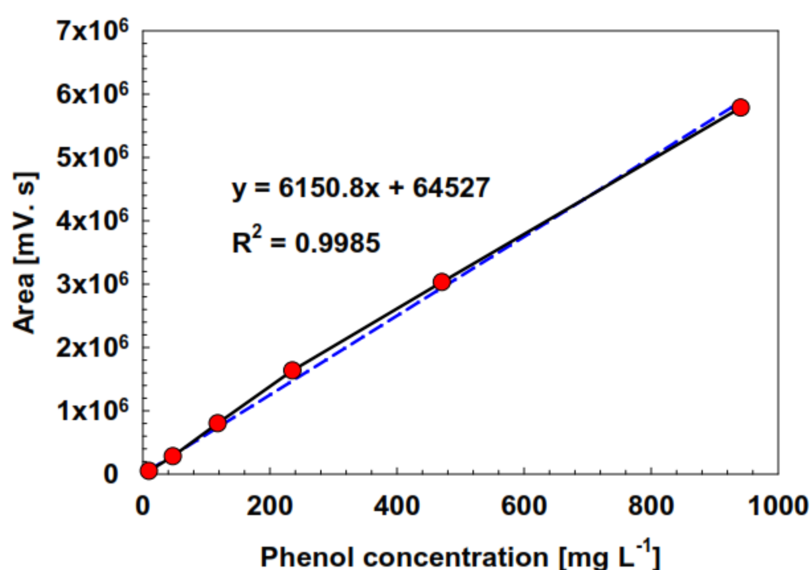
Tolerance of bacteria toward high concentration of phenol may be natural or due to some genetic changes particularly mutation in plasmid carrying gene for phenol degradation (Ajaz et al., 2004) or horizontal gene transfer. Tolerance of bacteria to a particular concentration of phenol is not related to the degradation of such amount. Nagamani et al. (2009) reported the tolerance of *Xanthobacter flavus* to 1100 mg L^{-1} of phenol but this strain could not degrade such amount and showed the ability to degrade only 650 mg L^{-1} phenol. This was because bacteria acquire different mechanism to withstand high concentration of phenol like increase in saturation of lipid membrane like in increase in fatty acid amount (Keweloh et al., 1991) and change in protein expression associated with efflux of phenol from cell (Randall et al., 2007).

Phenol degrading potential and analysis

Remaining phenol in cultural supernatant was quantified using the equation obtained from the regression analysis of external standards (*Fig. 4a and b*). The coefficient of determination (R^2) and adjusted R^2 values of standards were calculated as 99.09 and 98.9%, respectively. The closeness of both values indicates the accuracy of the model. Two types of controls were used for comparison of phenol degraded by the isolated strains, one control containing 750 mg L^{-1} phenol in MSM broth (uninoculated) and the other control with no phenol in MSM broth (inoculated). In both controls no growth observed nor observed any degradation of phenol. Similarly, no phenol degradation was detected in control without inoculation.

Stenotrophomonas sp. NCCP-310 was incubated for 74 h at 28$\pm$2°C in MSM broth augmented with 750 mg L^{-1} phenol. The strain degraded such amount in 65 h with average degradation rate of 15.65 mg L^{-1} h^{-1} for which the approximate doubling time was determined as 11.7 h^{-1}. After 49 h of incubation the strain showed maximum growth (*Fig. 5a*) as indicated by OD (0.95). Percentage removal of phenol from MSM by this strain was presented in *Fig. 5b*.

(a)



http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

(b)

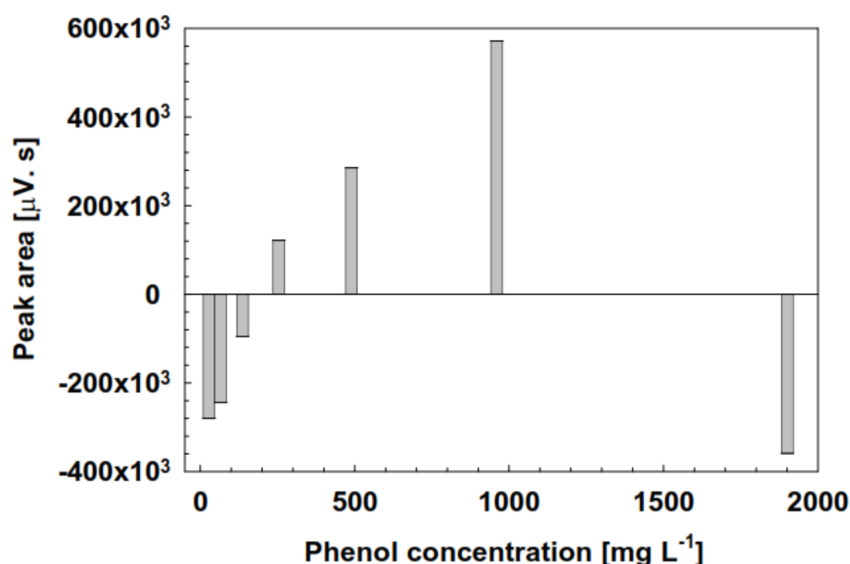


***Figure 4**. (a) Standard curve and regression analysis presenting peak area and phenol concentration. (b) Residual plot of the external standards used in this study.*

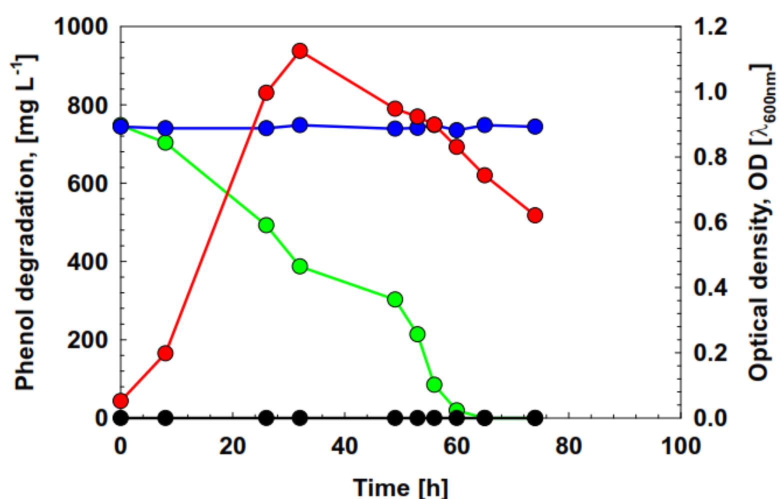
The phylogenetic neighbor of *Stenotrophomonas* sp. NCCP-310 was *Stenotrophomonas maltophilia* (Palleroni and Bradbury, 1993). This strain was well reported for phenol biodegradation (Gunasundari and Muthukumar, 2013). Phenol degradation pattern of *Stenotrophomonas* sp. NCCP-310 was found nearly similar to *Stenotrophomonas maltophilia* K279a (Han et al., 2010) which was able to degrade 805 mg L^{-1} of phenol and found that this strain degraded such amount in 48 h. Similarly, Urszula et al. (2009) reported the isolation of phenol degrading *Stenotrophomonas maltophilia* KB2 which degraded 12 mM phenol. Stains of this genus comprises a range of activities including plant growth promoting activity, human pathogenicity, role in nitrogen and sulphur cycles, production of secondary metabolites, metal tolerance and biodegradation of pollutants etc. (Ryan et al., 2009). Degradation of various organic compounds including p-nitrophenol and 4‑chlorophenol (Liu et al., 2007), polycyclic aromatic hydrocarbons (Juhasz et al., 2000), benzene, toluene (Lee et al., 2002), EDTA (Kaparullina et al., 2009) validates the natural biodegradation potential of the genus *Stenotrophomonas*.

Staphylococcus sp. NCCP-405 was incubated for 92 h at 28 ± 22°C in MSM broth supplemented with 750 mg L^{-1} of phenol. The strain degraded such amount in 85 h with the average degradation rate of 11.64 mg L^{-1} h^{-1} for which the approximate doubling time was recorded as 12.95 h^{-1}. After 53 h of incubation the strain showed maximum growth (*Fig. 6a*) as indicated by OD (0.924). Percentage removal of phenol from MSM by this strain was presented in *Fig. 6b***.** Among the phylogenetic neighbors of *Staphylococcus* sp. NCCP-405, no strain is reported for phenol degradation. However, few strains of this genus are documented for bioremediation of phenol. Naresh et al. (2012) reported the isolation of *Staphylococcus aureus* from effluent which degraded 1000 mg L^{-1} phenol in 7 days. Prasanna et al. (2008) reported the degradation of 43.94% of 100 mg L^{-1} phenol in 120 h. The difference of time to degrade phenol by members of a genus is common and acceptable (Larkin et al., 2005). Similarly, few more strains showed the phenol degrading potential belonging to this genus. Phenol

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

degrading pattern of *Staphylococcus* sp. NCCP-405 is somewhat similar to that of *Xanthobacter flavus* which was isolated from the soil near dye industry and showed complete degradation of 600 mg L^{-1} phenol in 120 h. In their work more than 97% of available phenol was degraded after 80 h of incubation but after that the process was prolonged (Nagamani et al., 2009). Comparatively, *Stenotrophomonas* sp. NCCP-310 degraded 750 mg L^{-1} phenol faster than *Staphylococcus* sp. NCCP-405. The presence of phenol degradation potential of phylogenetically diverse bacteria indicates the wide distribution of this trait. However, toxicity of phenol at high concentration showed growth with phenol by the strain *Staphylococcus* sp. NCCP-405 whose closely related strains don't have phenol degradation capacity indicates that horizontal gene transfer might have an important role in widely distribution of this trait.

(a)



(b)

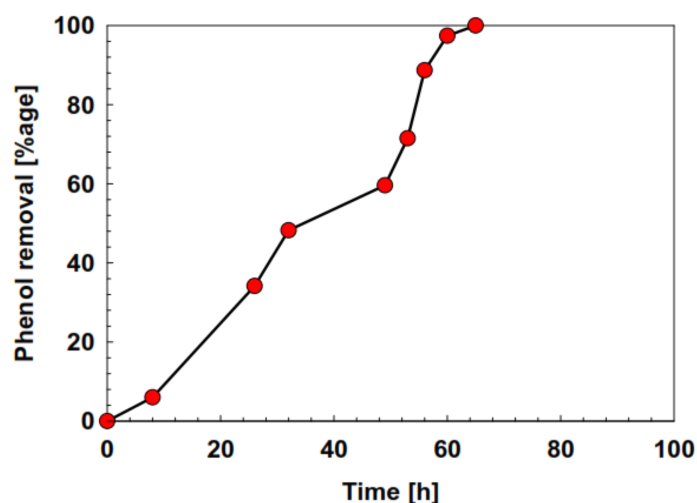
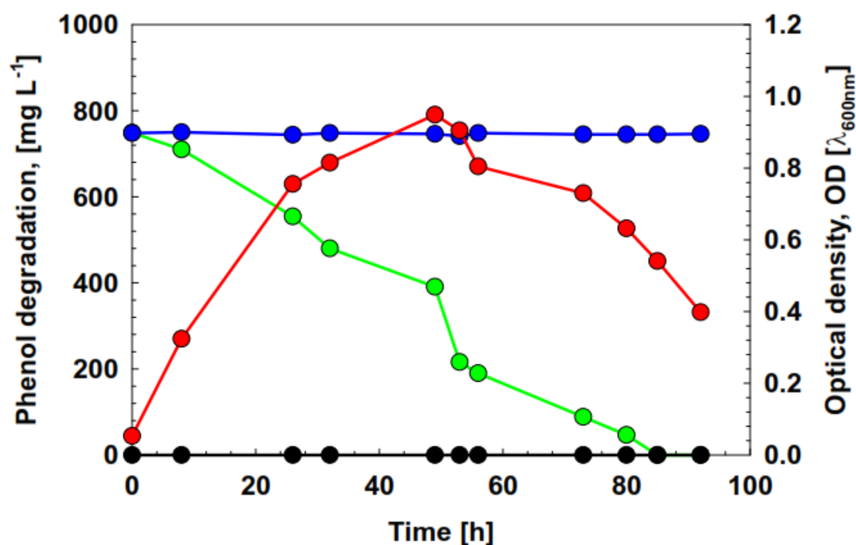


Figure 5. *(a) Degradation of phenol (750 mg L^{-1}) and growth as measured by optical density at 600 nm of NCCP-310. (—●—) denote phenol concentration in control (without inoculum), (—●—) denote phenol concentration in inoculum, (—●—) present observed OD in inoculum and (—●—) present OD in control. (b) Percentage phenol removal from MSM broth by NCCP-310.*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

(a)



(b)

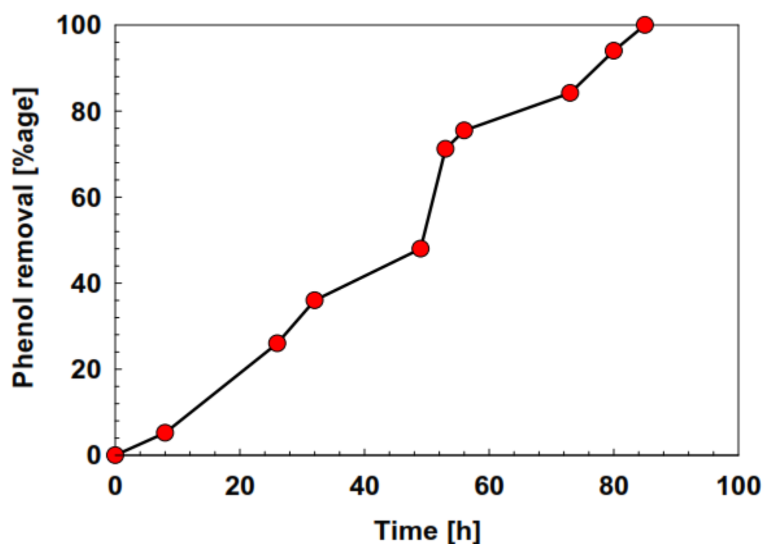


Figure 6. *(a) Degradation of phenol (750 mg L^{-1}) and growth as measured by optical density at 600 nm of NCCP-405. (blue) denote phenol concentration in control (without inoculum), (green) denote phenol concentration in inoculum, (red) present observed OD in inoculum and (black) present OD in control. (b) Percentage phenol removal from MSM broth by NCCP-405.*

Conclusion

Present study delivers a useful guideline for identification of bacteria based on sequence analysis of 16S rRNA gene. In this study two bacteria strains NCCP-310 and NCCP-405 were isolated through enrichment process from two different contaminated sites. These strains showed growth up to 1000 mg L^{-1} of phenol. The isolated strains were found to degrade 750 mg L^{-1} phenol, given as sole source of carbon and energy. These degrade phenol in 65 and 85 h respectively.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

Acknowledgements. This work was supported by financial assistance from PSDP funded Project Research for Agricultural Development Project under a sub-project (Grant No. CS-55/RADP/PARC to Iftikhar Ahmed) entitled "Establishment of Microbial Bio-Resource Laboratories: National Culture Collection of Pakistan (NCCP)" from Pakistan Agricultural Research Council, Islamabad, Pakistan.

REFERENCES

[1] Ahmad, N., Ahmed, I., Shahzad, A., Khalid, N., Mehboob, F., Ahad, K. Ali, G. M. (2014): Molecular identification and characterization of *Pseudomonas* sp. NCCP-407 for phenol degradation isolated from industrial waste. – Journal of the Korean Society for Applied Biological Chemistry 57: 341-346.

[2] Ahmed, I., Yokota, A., Fujiwara, T. (2007): A novel highly boron tolerant bacterium, *Bacillus boroniphilus* sp. nov., isolated from soil, that requires boron for its growth. – Extremophiles 11, 217-224.

[3] Ahmed, W., Ahmed, A., Ahmad, A., Randhawa, M. A., Ahmad, R. Khalid, N. (2012): Heavy Metal Contamination in Vegetables Grown in Rawalpindi, Pakistan. – Journal of the Chemical Society of Pakistan 34: 914-919.

[4] Ajaz, M., Noor, N., Rasool, S. A. Khan, S. A. (2004): Phenol resistant bacteria from soil: identification-characterization and genetical studies. – Pakistan Journal of Botany 36: 415-424.

[5] Anzai, Y., Kim, H., Park, J.Y., Wakabayashi, H. Oyaizu, H. (2000): Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. –International Journal of Systematic and Evolutionary Microbiology 50: 1563-1589.

[6] Arutchelvan, V., Kanakasabai, V., Elangovan, R., Nagarajan, S. Muralikrishnan, V. (2006): Kinetics of high strength phenol degradation using *Bacillus brevis*. –Journal of Hazardous Materials 129: 216-222.

[7] Carbajo, J. B., Boltes, K. Leton, P. (2010): Treatment of phenol in an anaerobic fluidized bed reactor (AFBR): continuous and batch regime. –Biodegradation 21: 603-613.

[8] Dunbar, J., White, S. Forney, L. (1997): Genetic diversity through the looking glass: effect of enrichment bias. –Applied and Environmental Microbiology 63: 1326-1331.

[9] Fialova, A., Boschke, E. Bley, T. (2004): Rapid monitoring of the biodegradation of phenol-like compounds by the yeast Candida maltosa using BOD measurements. – International biodeterioration & biodegradation 54: 69-76.

[10] Giti, E., Mehdi, H. Nasser, G. (2005): Development of a microtitre plate method for determination of phenol utilization, biofilm formation and respiratory activity by environmental bacterial isolates.–International Journal of biodegradation and biodeterioration 56: 231-235.

[11] Godjevargova, T., Ivanova, D., Alexieva, Z. Dimova, N. (2003): Biodegradation of toxic organic components from industrial phenol production waste waters by free and immobilized *Trichosporon cutaneum* R57. – Process Biochemistry 38: 915-920.

[12] Gunasundari, D. Muthukumar, K. (2013): Simultaneous Cr (VI) reduction and phenol degradation using *Stenotrophomonas* sp. isolated from tannery effluent contaminated soil. –Environmental Science and Pollution Research 20: 6563-6573.

[13] Han, H.J., Fang, F., Ma, W.C., Xun, C.Y., Wang, W., Li, H.Q. (2010): Phenoloxidase activity of phenol-degrading bacteria. –Bioinformatics and Biomedical Technology, International Conference, IEEE, pp. 23-26.

[14] Hugh, R. (1981): Note: Pseudomonas maltophilia sp. nov., nom. rev. – International Journal of Systematic Bacteriology 31: 195-195.

[15] Idris, A. Saed, K. (2002): Degradation of phenol in wastewater using anolyte produced from electrochemical generation of brine solution. – Global Nest 4: 139-44.

[16] Juhasz, A. L., Stanley, G. A. Britz, M. L. (2000): Microbial degradation and detoxification of high molecular weight polycyclic aromatic hydrocarbons by

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

Stenotrophomonas maltophilia strain VUN 10,003. – Letters in Applied Microbiology 30: 396-401.

[17] Kaparullina, E., Doronina, N., Chistyakova, T. Trotsenko, Y. (2009): *Stenotrophomonas chelatiphaga* sp. nov., a new aerobic EDTA-degrading bacterium. –Systematic and Applied Microbiology 32: 157-162.

[18] Keweloh, H., Diefenbach, R. Rehm, H.J. (1991): Increase of phenol tolerance of Escherichia coli by alterations of the fatty acid composition of the membrane lipids. – Archives of Microbiology 157: 49-53.

[19] Kwon, K. H. Yeom, S. H. (2009): Optimal microbial adaptation routes for the rapid degradation of high concentration of phenol. –Bioprocess and Biosystems Engineering 32: 435-442.

[20] Larkin, M. J., Kulakov, L. A. Allen, C. C. (2005): Biodegradation and *Rhodococcus*– masters of catabolic versatility. –Current Opinion in Biotechnology 16: 282-290.

[21] Lee, E. Y., Jun, Y. S., Cho, K.-S. & Ryu, H. W. (2002): Degradation characteristics of toluene, benzene, ethylbenzene, and xylene by *Stenotrophomonas maltophilia* T3-c. – Journal of the Air & Waste Management Association 52: 400-406.

[22] Liu, Z., Yang, C. & Qiao, C. (2007). Biodegradation of p-nitrophenol and 4-chlorophenol by *Stenotrophomonas* sp. –FEMS Microbiology Letters 277: 150-156.

[23] Nagamani, A., Soligalla, R. Lowry, M. (2009): Isolation and characterization of phenol degrading *Xanthobacter flavus*. –African Journal of Biotechnology 8: 5449-5453.

[24] Naresh, B., Honey, P. Vaishali, S. (2012): Biodegradation of phenol by a bacterial strain isolated from a phenol contaminated site in India. –Research Journal of Environment Sciences1: 46-49.

[25] Palleroni, N. J. Bradbury, J. F. (1993): *Stenotrophomonas*, a new bacterial genus for Xanthomonas maltophilia (Hugh 1980) Swings et al. 1983. –International Journal of Systematic Bacteriology 43: 606-609.

[26] Prasanna, N., Saravanan, N., Geetha, P., Shanmugaprakash, M., Rajasekaran, P. (2008): Biodegradation of Phenol and Toluene by *Bacillu*s sp., *Pseudomonas* sp., and *Staphylococcus* sp. Isolated from Pharmaceutical Industrial Effluent. 7: 20-24.

[27] Quan, X., Shi, H., Zhang, Y., Wang, J. Qian, Y. (2004). Biodegradation of 2, 4-dichlorophenol and phenol in an airlift inner-loop bioreactor immobilized with *Achromobacter* sp. –Separation and Purification Technology 34: 97-103.

[28] Randall, L., Cooles, S., Coldham, N., Penuela, E., Mott, A., Woodward, M., Piddock, L. Webber, M. (2007): Commonly used farm disinfectants can select for mutant Salmonella enterica serovar Typhimurium with decreased susceptibility to biocides and antibiotics without compromising virulence. –Journal of Antimicrobial Chemotherapy 60: 1273-1280.

[29] Rocha, L. L., de Aguiar Cordeiro, R., Cavalcante, R. M., do Nascimento, R. F., Martins, S. C. S., Santaella, S. T. Melo, V. M. M. (2007): Isolation and characterization of phenol-degrading yeasts from an oil refinery wastewater in Brazil. –Mycopathologia 164: 183-188.

[30] Roohi, A., Ahmed, I., Iqbal, M. Jamil, M. (2012): Preliminary isolation and characterization of halotolerant and halophilic bacteria from salt mines of Karak, Pakistan. –Pakistan Jounal of Botany 44: 365-370.

[31] Ryan, R. P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M. B., Berg, G., van der Lelie, D. Dow, J. M. (2009): The versatility and adaptation of bacteria from the genus Stenotrophomonas. –Nature Reviews Microbiology 7: 514-525.

[32] Saravanan, P., Pakshirajan, K. Saha, P. (2008): Kinetics of phenol and m-cresol biodegradation by an indigenous mixed microbial culture isolated from a sewage treatment plant. –Journal of Environmental Sciences 20: 1508-1513.

[33] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. Kumar, S. (2011): MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary

distance, and maximum parsimony methods. –Molecular Biology and Evolution 28: 2731-2739.

[34] Thompson, J. D., Higgins, D. G. Gibson, T. J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. –Nucleic acids research 22: 4673-4680.

[35] Urszula, G., Izabela, G., Danuta, W. Sylwia, Ł. (2009): Isolation and characterization of a novel strain of Stenotrophomonas maltophilia possessing various dioxygenases for monocyclic hydrocarbon degradation. –Brazilian Journal of Microbiology 40: 285-291.

[36] US EPA (2007): National Recommended Water Quality Criteria. Washington, USA: U.S. Environmental Protection Agency Office of Water

[37] Zhu, X., Tian, J. Chen, L. (2012) : Phenol degradation by isolated bacterial strains: kinetics study and application in coking wastewater treatment. –Journal of Chemical Technology and Biotechnology 87: 123-129.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1405_107120
© 2016, ALÖKI Kft., Budapest, Hungary