Biological Journal of Microorganism 5th Year, Vol. 5, No. 20, Winter 2017 **Received:** August 26, 2015/ **Accepted:** March 2, 2016. **Page**: 71-80

Characterization and evaluation of catechol oxygenases by twelve bacteria, isolated from oil contaminated soils in Malaysia

Arezoo Tavakoli * Assistant professor of Microbiology, Islamic Azad University Eghlid branch, Eghlid, Iran, a_tavakoli2003@yahoo.com Ainon Hamzah Professor of Microbial biotechnology, University Kebangsaan Malaysia, Selangor, Malaysia, ainonh@gmail.com

Abstract

Introduction: Catechol is a common intermediate compound in aromatic degradation process. Some microorganisms have this potentiality to degrade aromatic hydrocarbons by catechol dioxygenases to less toxic compounds with ability of entering the tricarboxylic acid cycle. In the present study, the catechol oxygenase activity was measured for 12 crude oil degrader bacteria.

Materials and methods: Catechol oxygenase activity of two enzymes includes catechol 1, 2 dioxygenase and catechol 2, 3 dioxygenase were determined using spectrophotometer at 260 nm and 375 nm, respectively.

Results: The highest enzyme activity for catechol 1, 2 dioxygenase by *Bacillus cereus* UKMP-6G was (0.07 U/mL) and about catechol 2, 3 dioxygenase was 0.031 U/mL by *Rhodococcus ruber* UKMP-5M during the first minute of incubation. Catechol 1, 2 dioxygenase and catechol 2, 3 dioxygenase followed the *ortho* and *meta* pathway, respectively.

Discussion and conclusion: The enzyme assay results showed that among 12 examined bacteria, only *R. ruber* UKMP-5M has the ability to use *meta* pathway for degradation and produce 2-hydroxymuconic acid. The other isolates use *ortho* pathway and create *cis, cis*-muconic acid.

Key words: Catechol oxygenase, Degradation, Toluene, Enzyme assay

^{*}Corresponding Author

Copyright © 2017, University of Isfahan. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http: //creativecommons.org/licenses/BY-NC-ND/4.0/), which permits others to download this work and share it with others as long as they credit it, but they cannot change it in any way or use it commercially.

Introduction

Many bacteria have the ability to degrade aromatic hydrocarbons of sites. The contaminated soil microorganisms are potential catalytic sources for biodegradation of organic compounds. A number of bacteria are able to biodegrade toxic organic compounds that improved clean-up of different environments including water, soil and wastewater in aerobic and anaerobic conditions (1). Aromatic hydrocarbons could be degraded to less toxic compounds via conversion into dihydroxylated intermediates such as catechol or substituted catechols and then metabolized by intradiol or extradiol dioxygenases (2).

The aerobic degradation of hydrocarbon pathways consist three steps; in the first step, two hydroxyl groups are introduced into aromatic ring and catalyzed by monoor dioxygenase to produce dehydroxy aromatic compounds which are usually catechols. Catechols are substrates for the next step of the catabolism by ring cleavage via catechol 1, 2 dioxygenase and catechol 2, 3 dioxygenase (Fig. 1) (3). Subsequently, catechol oxygenases have an important role in fundamental pathway in the carbon biochemical cycle and а high biotechnological potential in treatment of liquid wastes contaminated with aromatics compounds (4).



Fig. 1- Catechol degradation via *ortho* and *meta* pathway (7)

Catechol 1, 2 dioxygenase (*ortho* pathway) that also known as β -ketoadipate pathway, is Fe³⁺ enzymes and catechol 2, 3 dioxygenase (*meta* pathway) is Fe²⁺ enzymes. During the last step, the ring cleavage products convert to small aliphatic compounds which metabolized to carbon dioxide during tricarboxylic acid cycle (TCA cycle) (5). Catechol 1, 2 dioxygenase

is an intradiol dioxygenase that incorporate two oxygen atoms between the vicinal hydroxyl groups, while an extradiol dioxygenase, such as catechol 2, 3 dioxygenase, cleaves the aromatic ring of the substrate with two hydroxyl groups and produces muconic semialdehyde (6). Catechol 2, 3 dioxygenase has an important role on biodegradation of alkyl substitution aromatic hydrocarbons such as xylenase and is usually encoded by plasmids (7). Catechol oxygenases are the common biomarker for evaluation of hydrocarbon biodegradation by bacteria. The objective of this study was assessment of catechol dioxygenases in cell-free extracts by 12 bacterial isolates in order to find the appropriate candidate for bioremediation in contaminated sites. All the used bacteria in this study were isolated from groundwater and oil contaminates soils samples which were collected from the Petronas crude oil refinery in Terengganu, Malaysia.

Materials and methods

Chemicals: Toluene and catechol were obtained from Merck Company.

Isolates and culture conditions: Twelve isolates had been identified from crude oil contaminated soils Terengganu, in Malaysia. The samples were obtained from culture collection of School of Bioscience and Biotechnology, University Kebangsaan Malaysia (UKM). All the isolates were identified using commercial kit Microbact 24E (Oxoid) according to the manufacture's instruction. The isolates were more confirmed by polymerase chain reaction (PCR) assay targeting 16S rDNA gene. Several isolates (UKMP-5T, UKMP-7T, and UKMP-8T) were also analyzed morphologically and their biochemical and physiological properties were determined (8).

Preparation of standard inoculums: The bacterium was grown in nutrient broth and the culture was incubated at 37°C in an orbital shaker (Infors Ht multitron

Germany) at 150 rpm for 18 hours. The culture was harvested by centrifugation (Eppendorf centrifuge 5810 R) at 4000 rpm for 15 minutes at 4°C. The pellet was washed and resuspended with 50 mM phosphate buffer to obtain an optical density (OD) ~ 0.5 at wavelength 550 nm using spectrophotometer (BiowaveII WPA England) (9). This served as the standard inoculums for the subsequent study.

Sample preparation for catechol oxygenase assay: The catechol oxygenase activity was performed according to Farrell and Quilty method (10). The activity was measured for two enzymes, catechol 1, 2 dioxygenase and catechol 2, 3 dioxygenase. Catechol 1, 2 dioxygenase activity was determined by increasing the absorbance at 260 nm by spectrophotometer due to the cleavage of catechol into cis, cis-muconate (11). Catechol 2, 3 dioxygenase was catalyzed the conversion of catechol to 2hydroxymuconic semi-aldehyde which increased the absorbance at 375 nm. The culture preparation for catechol oxygenase assay was as follows: Ten percent culture from standard inoculums (v/v) and 0.25 mL of 100 mM toluene as an aromatic hydrocarbon source added into 50 mL Minimal salt medium (MSM) which containing (K₂HPO₄ 1.8 g, KH₂PO₄ 1.2 g, NH₄Cl 4.0 g, NaCl 0.1 g, MgSO₄.7H₂O 0.2 g, FeSO₄.7H₂O 0.01 g in 1 L distilled water (9) and pH adjusted to 6.5 in 250 mL conical flask. The culture was incubated at 30°C, with shaking at 150 rpm for 5 days. At the end of the incubation, the bacterial cells were centrifuged at 4000 rpm for 20 minutes at 4°C. The pellet was washed twice and resuspended with 0.33 mM Tris-HCl buffer (pH 7.6). The OD _{550nm} of the cells was adjusted to 0.6. The cells were put in ice and sonicated (Sonics material vibra cell) for 20 seconds for 3 times to break the cells and centrifuged at 4°C, 20000 rpm for 20 minutes. The supernatant (cell free extract) was collected and kept in ice to use for catechol dioxygenase assay (10).

Catechol 1, 2 dioxygenase assay: The activity of catechol 1, 2 dioxygenase was prepared by mixing 2 mL of 50 mM Tris-HCl buffer (pH 8.0), 0.7 mL distilled water, 0.1 mL of 100 mM 2-mercaptoethanol, 0.1 mL cell free extract and 0.1 mL catechol from 1 mM (Sigma) in sterile tube. The sample was read in a quartz cuvette. A control was prepared by replacing the catechol with distilled water. The formation of cis, cis muconic acid from orthocleavage pathway was monitored using spectrophotometer at wavelength 260 nm for 10 minutes at 1 minute interval. The extinction coefficients for catechol at OD_{260nm}=16.800 l/mol/cm (10 and 12).

Catechol 2, 3 dioxygenase assay: For the assay, the mixture was prepared in a cuvette as follows: 2 mL (50 mM) Tris-HCl buffer (pH 8.0), 0.6 mL distilled water, 0.2 mL cell-free extract and 0.2 mL of 100 mM catechol. The formation of 2hydroxymuconic semi-aldehyde from meta cleavage pathway was monitored for 10 minutes at 1 minute interval using spectrophotometer at wavelength 375 nm. The extinction coefficients for catechol at $OD_{375nm} = 36.000 \text{ l/mol/cm} (10, 11).$

The catechol oxygenase activity was calculated based on formula (13) as follows:

Enzyme unit/mL sample = Measured Value × Test Volume × Dilution Factor /

(Time \times Concentration Constant \times Enzyme Volume)

One unit is defined as 1 μ mol product (*cis*, *cis*-muconate or 2-hydroxymuconic semi-aldehyde by catechol 1, 2 dioxygenase or catechol 2, 3 dioxygenase) per minute in one milliliter assay (2 and 14).

Results

In this study, twelve isolates were used to determine the catechol oxygenase activities (Table 1).

No	Specific name	Genus
1	UKMP-5T	Pseudomonas haemolytica
2	UKMP-8T	Pseudomonas aeruginosa
3	UKMP-1M	Pseudomonas multivorans
4	UKMP-14T	Pseudomonas aeruginosa
5	UKMP-5G	Pseudomonas aeruginosa
6	UKMP-7T	Bacillus sp
7	UKMP-10T	Bacillus sp
8	UKMP-6G	Bacillus cereus
9	UKMP-2M	Acinetobacter baumannii
10	UKMP-12T	Acientobacter baumannii
11	UKMP-5M	Rhodococcus ruber
12	UKMP-1G	Exiguobacterium lactigens

Table 1- Bacteria used in this study

Catechol 1, 2 dioxygenase assay: The catechol 1, 2 dioxygenase activity for all 12 isolates was measured at 260 nm over a period of 10 minutes at one minute interval (Fig. 2).



Fig. 2- The catechol 1, 2 dioxygenase activity for all isolates using catechol as substrate

The highest enzyme activity for catechol 1, 2 dioxygenase was from *B. cereus* UKMP-6G (0.07 U/mL) at the first minute while the lowest enzyme activity was elicited in *A. baumannii* UKMP-12T (0.04 U/mL) after 10 minutes reaction. The optimum activity for each one of the isolates was different at range 5 to 9 minutes. For example, the highest catechol 1, 2 dioxygenase activity for *P. aeruginosa* UKMP-14T was 0.07 U/mL after 5 minutes incubation and for *P. haemolytica* UKMP-5T was 0.09 U/mL after 6 minutes. The

highest catechol 1, 2 dioxygenase activity by *Bacillus sp* UKMP-10T was 0.13 U/mL produced for 7 minutes and for *B. cereus* UKMP-6G was 0.14 U/mL after 8 minutes. The catechol 1, 2 dioxygenase activity by *A. baumannii* UKMP-2M was 0.12 U/mL after 9 minutes.

Catechol 2, 3 dioxygenase assay: The catechol 2, 3 dioxygenase activities for all 12 isolates was measured at 375 nm over a period of 10 minutes at one minute interval (Fig. 3).



Fig. 3- The catechol 2, 3 dioxygenase activity for all isolates using catechol as substrate.

The highest catechol 2, 3 dioxygenase activity was by R. ruber UKMP-5M (0.031 U/mL) at the first minute of reaction, while the lowest was by A. baumannii UKMP-12T (0.018 U/mL) after 10 minutes reaction. The optimum activity for each of the isolates was different at ranges 4 to 10 minutes. For example, the highest catechol 2, 3 dioxygenase activity for P. aeruginosa UKMP-8T was 0.05 U/mL after 4 minutes incubation and P. aeruginosa UKMP-14T was 0.05 U/mL after 5 minutes incubation and R. ruber UKMP-5M at 0.08 after 9 minutes. The highest catechol 2. - 3 dioxygenase activity by B. cereus UKMP-6G was 0.03 U/mL produced for 10 minutes.

The catechol 1, 2 dioxygenase and catechol 2, 3 dioxygenase followed the *ortho* and *meta* pathway, respectively.

Discussion and conclusion

Many aromatic hydrocarbons such as BTEX (Benzene, Toluene, Etheylbenzene and Xylenes) are main compounds of petroleum crude oil (15). Aromatic hydrocarbons could be biodegraded to less toxic compounds by several steps which were described in bacteria with different pathways. Biodegradation using microbes applicable method to reduce is an hydrocarbons in polluted environments. Most of the microorganism were isolated from petroleum contaminated sites (water or soil) have shown abilities for oil and hydrocarbon degradation (8) Using microbial enzyme is a simple and benefit method for bioremediation. Toluene as a common hydrocarbon was converted to catechols via toluene oxygenase in upper Catechol is pathway. an important intermediate compound which is degraded into Krebs cycle products during the lower pathway (12). The catechol oxygenase was determined in terms of catechol 1, 2 dioxygenase and catechol 2, 3 dioxygenase. The activity of catechol 1, 2 dioxygenase was determined by monitoring the formation of cis, cis-muconic acid from catechol through ortho cleavage. The Catechol 2, 3 dioxygenase activity was determined by monitoring the formation of 2-hydroxymuconic acid semialdehyde from catechol by meta cleavage and further conversion to form, acetaldehyde and pyruvate. The final products of both pathways are molecules which can enter the tricarboxylic acid (TCA) cycle. It is believed that ortho pathway catalyze completed degradation of hydrocarbons. The role of ortho pathway is more efficient than meta pathway for carbon conversion to cell mass (growth yield) (12). In contrast, meta pathway is known for incomplete metabolism due to production of dead-end or suicide-metabolites. Nevertheless, meta pathway is more capable than ortho pathway to degrade alkyl-compounds (10). In general, chromosomal genes encode the ortho pathway while plasmids such as TOL plasmids encode the meta pathway (16).

The highest catechol 1, 2 dioxygenase activity produced by *B. cereus* UKMP-6G

was 0.07 U/mL which this value is nearby catechol 1, 2 dioxygenase activity by P. putida PsU-0 0.083 U/mL and PsU-08 0.077 U/mL (17), but the enzyme activity was lower compared with catechol 1, 2 dioxygenase activity by Stenotrophomonas maltophilia strain KB2 that was 1.03 U/mL (18). The catechol 1, 2 dioxygenase activity for Rhodococcus sp. NCIM 2891 was 0.6 U/mL (19), Rhodococcus erythpolis AN-13 (0.54 U/mL) (20), P. putida GJ31 (4 U/mg) (21) and 22 U/mL was determined by Pseudomonas sp. JL1 (22). The highest catechol 2, 3 dioxygenase activity determined by R. ruber UKMP-5M was 0.031 U/mL which was nearby catechol 2, 3 dioxygenase activity by P. putida PsU-0 (0.024 U/mL) and PsU-E1 (0.028 U/mL) However, (17).the catechol 2. 3 dioxygenase activity from R. ruber UKMP-5M was much lower than catechol 2, 3 dioxygenase activity by S. maltophilia strain KB2 which was 0.37 U/mL (18), Alcaligenes eutrophus (0.64 U/mg) and 4/8 U/mL reported by P. putida GJ31 (21).

Several factors were involved in catechol oxygenase activities. The product of enzyme can be changed based on used substrate. for example, in S. aromaticivorans strain F199, catechol 1, 2 dioxygenase with ortho pathway was favored while the cells were utilized toluene and benzoate as substrate (23). Rhodococcus strain YU6 (24) and strain 19070 (25) were used benzene to induce the catechol 1, 2 dioxygenase (ortho pathway) with higher activity than catechol 2, 3 dioxygenase. In contrast, the bacteria follow the *meta* pathway using xylene (24) or toluate (25). The growth of *P. putida* in methylcatechols leads to *meta* pathway induction but, the presence of benzoate or catechol induced the *ortho* pathway (17).

Generally chlorocatechols are mineralized via *ortho* pathways while methyl aromatics are commonly mineralized via *meta* pathways (21). Catechol oxygenase activities are decreased to a low level in anaerobic condition (26).

Many bacteria have been used *meta* pathway for hydrocarbons biodegradation such as *Gordonia polyisoprenivorans* (1), *Rhodococcus sp.* strain DK17 (27), *Rhodococcus* sp. strain YU6 (24), *Bacillus* S-5 (28), *P. putida* F1 (29) and *A. calcoaceticus* (30) that were degrade catechol and derivatives by catechol 2, 3 dioxygenase. In contrast, in *Rhodococcus* sp NCIM 2891 (19), *Acientobacter* strain DF4 and W-17 (28), and *P. putida* mt-2 strain paW8 (31) *ortho* pathway was favored.

In most cases, the induction of one catechol oxygenase has an influence to another catechol oxygenase. The catechol metabolism by meta pathway are to be active if the ortho pathway enzymes are unable prevent intracellular to its accumulation (17). The reverse effect for catechol oxygenase activates showed by S. *maltophilia* (18). The *ortho* pathway enzymes are produced in most strains when the *meta* pathway enzymes have not been induced and then catechol has been accumulated (32). In this study, both catechol 1, 2 dioxygenase and catechol 2, 3 dioxygenase were produced simultaneously but the catechol 1, 2 dioxygenase (ortho

pathway) showed higher activity for 11 isolates. In contrast, Bayly and Mckenzie study showed the catechol induction of catechol 2, 3 dioxygenase by *P. aeruginosa* lead to elicit of 1, 2 dioxygenase activity (17).

The maximum catechol oxygenase activity for most of 12 isolates was at 5 to 7 minutes of incubation. In a similar study, the activity of catechol 1, 2 dioxygenase and catechol 2, 3 dioxygenase by Gordonia polyisoprenivorans (1) and Mycobacterium fortuitum (4) were reduced after 10 minutes of incubation. Study done by Zaki showed catechol 1, 2 dioxygenase activity was the highest after 10 minutes of incubation (28). But the higher time for catechol 2, 3 dioxygenase activity, was reported by P. fluorescens was 12 hours (20). In summary, the present study clearly indicates that the described catechol dioxygenases make it useful for bioremediation applications.

Acknowledgment

The work was supported by the grant number STGL-003-2007.

References

- (1) Silva AS., Camargo FAdO., Andreazza R., Jacques RJS., Baldoni DB., Bento FM. Enzymatic activity of catechol 1, 2-dioxygenase and catechol 2, 3-dioxygenase produced by *Gordonia polyisoprenivorans. Quimica Nova* 2012; 35 (8): 1587-1592.
- (2) Dokic L., Narancic T., Nikodinovic-Runic J., Bajkic S., Vasiljevic B. Four *Bacillus* sp. soil isolates capable of degrading phenol, toluene, biphenyl, naphthalene and other aromatic compounds exhibit different aromatic catabolic potentials. *Archives of Biological Sciences* 2011; 63 (4): 1057-1067.

- (3) Kitayama A., Achioku T., Yanagawa T., Kanou K., Kikuchi M., Ueda H., et al. Cloning and characterization of extradiol aromatic ring-cleavage dioxygenases of *Pseudomonas aeruginosa* JI104. *Journal of Fermentation and Bioengineering* 1996; 2 (3): 217-23.
- (4) Silva ASd., Jacques RJS., Andreazza R., Bento FM., Roesch LFW., Camargo FAdO. Properties of catechol 1, 2-dioxygenase in the cell free extract and immobilized extract of *Mycobacterium fortuitum. Brazilian Journal of Microbiology* 2013; 44 (1): 291-297.
- (5) Williams PA., Sayers JR. The evolution of pathways for aromatic hydrocarbon oxidation in *Pseudomonas. Biodegradation* 1994; 5 (3-4): 195-217.
- (6) Haddad S., Eby DM., Neidle EL. Cloning and expression of the benzoate dioxygenase genes from *Rhodococcus* sp. strain 19070. *Applied and Environmental Microbiology* 2001; 67 (6): 2507-2514.
- (7) Zeyaullah M., Abdelkafe AS., Zabya WB., Ali A. Biodegradation of catechols by microorganisms - A short review. *African Journal of Biotechnology* 2009; 8 (13): 2916-22.
- (8) Hamzah A., Phan CW., Abu Bakar NF., Wong K K. Biodegradation of crude oil by constructed bacterial consortia and the constituent single bacteria isolated from Malaysia. *Bioremediation Journal* 2013; 17 (1): 1-10.
- (9) Hamzah A., Tavakoli A., Rabu A. Detection of toluene degradation in bacteria isolated from oil contaminated soils. *Sains Malays* 2011; 40: 1231-1235.
- (10) Farrell A., Quilty B. Degradation of monochlorophenols by a mixed microbial community via a *meta*- cleavage pathway. *Biodegradation* 1999; 10 (5): 353-362.
- (11) Briganti F., Pessione E., Giunta C., Scozzafava A. Purification, biochemical properties and substrate specificity of a catechol 1, 2-dioxygenase from a phenol degrading *Acinetobacter radioresistens. FEBS Letters* 1997; 416 (1): 61-4.
- (12) Veenagayathri K., Vasudevan N. Ortho and meta cleavage dioxygenases detected during the

degradation of phenolic compounds by a moderately halophilic bacterial consortium. *Research Journal of Microbiology* 2011; 2: 406-414.

- (13) Bisswanger H. *Practical Enzymology* 2rd ed. New York. Wiley -Black Well; 2004.
- (14) Kilpi S., Backström V., Korhola M. Degradation of catechol, methylcatechols and chlorocatechols by *Pseudomonas* sp. HV3. *FEMS Microbiology Letters* 1983;18 (1): 1-5.
- (15) Ghsemi SM., Hosseini Abari A., Emtiazi G. Biodegradation of petrochemical industry compounds by cytochrome P450-producing toluene-degradaing bacterium. *Biological Journal of Microorganism* 2012; 1 (3): 61-70.
- (16) Zhao K., Guo X., Gong J. A novel benzoatedegrading *Rhodococcus* strain contains three *cat*A genes with one being transcriptionally active during the growth on benzoate 2013; 34: 401-407.
- (17) Bayly R., McKenzie D. Catechol oxygenases of *Pseudomonas putida* mutant strains. *Journal of Bacteriology* 1976; 127 (3): 1098-1107.
- (18) Urszula G., Izabela G., Danuta W., Sylwia L. Isolation and characterization of a novel strain of *Stenotrophomonas maltophilia* possessing various dioxygenases for monocyclic hydrocarbon degradation. *Brazilian Journal of Microbiology* 2009; 40: 285-91.
- (19) Nadaf NH., Ghosh JS. Purification and characterization of catechol 1, 2-dioxygenase from *Rhodocococcus* sp. NCIM 2891. *Research Journal of Environmental and Earth Sciences* 2011; 3: 608-613.
- (20) Aoki K., Konohana T., Shinke R., Nishira H.
 Purification and Characterization of Catechol 1,
 2-Dioxygenase from Aniline-assimilating *Rhodococcus erythropolis* AN-13. Agricultural and Biological Chemistry.1984; 48 (8): 2087-2095
- (21) Mars AE., Kasberg T., Kaschabek SR., Van Agteren M., Janssen DB., Reineke W. Microbial degradation of chloroaromatics: use of the *meta*cleavage pathway for mineralization of chlorobenzene. *Journal of Bacteriology* 1997;179 (14): 4530-4537.

- (22) Latorre J., Reineke W., Knackmuss H-J. Microbial metabolism of chloroanilines: enhanced evolution by natural genetic exchange. *Archives of microbiology* 1984;140(2-3): 159-65.
- (23) Wojcieszynska D., Guzik U., Gren I., Perkosz M., Hupert-Kocurek K. Induction of aromatic ring: cleavage dioxygenases in *Stenotrophomonas maltophilia* strain KB2 in cometabolic systems. *World Journal of Microbiology and Biotechnology* 2011; 27 (4): 805-11.
- (24) Jang JY., Kim D., Bae HW., Choi KY., Chae J-C., Zylstra GJ., et al. Isolation and characterization of a *Rhodococcus* species strain able to grow on ortho-and para-xylene. *Journal of Microbiology* 2005; 43 (4): 325-30.
- (25) Haddad S., Eby DM., Neidle EL. Cloning and expression of the benzoate dioxygenase genes from *Rhodococcus* sp. strain 19070. *Applied and Environmental Microbiology* 2001;67 (6): 2507-2514.
- (26) Cinar O. Stability of aerobic benzoatedegrading capability of *Pseudomonas aeruginosa* 142. *Process Biochemistry* 2005;40 (3): 1409-1413.
- (27) Martinkova L., Uhnakova B., Patek M., Nesvera J., Kren V. Biodegradation potential of the genus *Rhodococcus*. *Environment International* 2009; 35 (1): 162-177.
- (28) Zaki S. Detection of *meta-* and *ortho*cleavage dioxygenases in bacterial phenoldegraders. *Journal Applied Science Environmental and Manengment* 2006;10 (3): 75-81.
- (29) Okoh AI. Standard review biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnology and Molecular Biology Review* 2006; 1 (2): 38-50.
- (30) Gillooly DJ., Fewson CA. Production of a perillyl alcohol dehydrogenase by site-directed mutagenesis of a benzyl alcohol dehydrogenase. *Biotechnology Letters* 1998; 20 (3): 325-7.
- (31) Williams PA., Murray K. Metaboilsm of benzoate and methylbenzoate by *Pseudomonas putida* (*arvilla*) mt-2: Evidence for the existance

of a TOL plasmid. *Journal of Bacteriology* 1974; 120 (1): 416-423

(32) Ilori MO., Amund DI. Degradation of anthracene by bacteria isolated from oil polluted tropical soils. Zeitschrift für Naturforschung C 2000; 55 (11-12): 890-897.

ویژگیها و ارزیابی کاتکول اکسیژناز در دوازده باکتری جدا شده از خاکهای آلوده به نفت در مالزی

آرزو تــــوكلى: استاديارميكروبيولوژى، دانشگاه آزاد اسلامى، واحد اقليد، اقليد، ايران، a_tavakoli2003@yahoo.com **اينـــون حمـــزه:** پروفسور بيوتكنولورى ميكروبى، دانشگاه ملى مالزى، سلانگور، مالزى، ainonh@gmail.com

چکیدہ

مقدمه: کاتکول یک ترکیب حد واسط در مسیر تجزیه ترکیبات آروماتیک است. در برخی از میکروار گانیسمها، کاتکول اکسیژناز هیدرو کربن های حلقوی را به ترکیبات کم خطرتر تجزیه کرده و محصولات ایجاد شده در مسیر چرخه کربس قرار می گیرند. در این بررسی، میزان فعالیت کاتکول اکسیژناز برای ۱۲ باکتری تجزیه کننده نفت خام ارزیابی شد.

مواد و روش ها: فعالیت کاتکول اکسیژناز با ارزیابی دو آنزیم کاتکول ۱،۲ دی اکسیژناز و کاتکول ۲،۳ دی اکسیژناز با استفاده از اسپکتروفتومتر در طول موج ۲۶۰ و ۳۷۵ نانومتر تعیین شد.

نتایج: بیشترین فعالیت کاتکول ۲،۱ دی اکسیژناز در *باسیلوس سرئوس* UKMP-6G (۰/۰۷ U/mL) و کاتکول ۳،۲ دی اکسیژناز در *رودوکوکوس روبر* UKMP-5M (۰/۰۳۱ U/mL) در نخستین دقیقه بررسی شد. این دو آنزیم به ترتیب بیانگر مسیرهای ارتو و متا هستند.

بحـث و نتیجـه گیـری: نتـایج سـنجش آنزیمـی نشـان داد کـه در میـان ۱۲ بـاکتری ارزیـابی شـده فقـط بـاکتری *رودو کو کوس روبر* UKMP-5M از مسیر متا تجزیه را انجام میدهد که ۲- هیدرو کسی میکونیک اسید تولید می شود. سایر باکتریهای جدا شده با استفاده از مسیر ارتو ماده cis,cis - میکونیک اسید ایجاد میکنند.

واژەھاي كليدي: كاتكول اكسيژناز، تجزيه، تولوئن، سنجش آنزيمي

^{*} نویسنده مسؤول مکاتبات تاریخ دریافت: ۱۳۹۴/۰۶/۰۴ – تاریخ پذیرش: ۱۳۹۴/۱۲/۱۲