ISSN 0026-2617, Microbiology, 2015, Vol. 84, No. 4, pp. 531–537. © Pleiades Publishing, Ltd., 2015.

EXPERIMENTAL ARTICLES

Molecular Identification and Resistance Investigation of Atrazine Degrading Bacteria in the Sediments of Karun River, Ahvaz, Iran[1]

Farshid Kafilzadeh[2] and Niloufar Farhadi

Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran

Received July 10, 2014

Abstract—Nowadays, the use of pesticides as plant protection products has become widely prevalent, leading to the entry of large amounts of pesticides into soil and water resources, and subsequently, a threat to the environment. The objective of this study was molecular identification and resistance investigation of atrazine degrading bacteria in the sediments of Karun River, Ahvaz, Iran. Nine samples were collected in both summer (Jul) and autumn (Nov) year 2012 from a depth of 3 to 5 cm of the sediments. Atrazine degrading bacteria were enriched in a culture containing atrazine with initial concentration of 30 mg/L. Identification of isolated bacteria was performed by morphological and biochemical test and molecular analysis based on 16S rDNA sequencing. The atrazine biodegradation rate was obtained by high-performance liquid chromatography (HPLC). Six strains was identified including *Achromobacter insolitus* strain F-N3, *Delftia tsuruhatensis* strain F-N4, *Klebsiella pneumonia* F-N1, *Enterobacter ludwigii* strain F-N5, *Serratia marcescens* strain F-N6 in both summer and autumn, and *Exiguobacterium profundum* strain F-N2 only in the summer. The minimum inhibitory concentration (MIC) of atrazine showed that the most resistant species belonged to *E. ludwigii* F-N5 and *D. tsuruhatensis* F-N4 in the both seasons. The atrazine degradation rates of the two strains reached 90 and 85%, respectively after 7 days culture. Result showed that indigenous bacteria in the Karun River can degrade the atrazine effectively.

Keywords: biodegradation, atrazine herbicide, Karun River sediments, MIC, PCR

DOI: 10.1134/S0026261715040098

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a systematic selective triazine herbicide that is broadly used for annual control of broad leaf and grass weeds in farms such as corn, sugar cane, cereals and cornelian cherry. Although this substance is banned for use in many European countries, it is still commonly used in many countries like China, India, and United States, because of its effectiveness in reducing the losses and destruction of farmlands [1]. Atrazine is generally a serious pollutant of drinking water due to its high fluidity in underground water [2]. It is also probably a human carcinogen, nevertheless in 1991, the International Agency for Research on Cancer (IARC) concluded that inadequate evidence in humans and limited evidence in laboratory animals has been provided for human carcinogenesis [3]. A number of methods have been developed for the removal of atrazine from environment such as adsorption, incineration, reduction-oxidation, dechlorination, photolysis, reverse osmosis, chemical degradation, etc. However, these methods are costly and produce many other toxic intermediates. Bioremediation is the promising technology for the treatment of the contaminated sites since it is cost-effective and will lead to complete mineralization. This method is considered as the most effective way to destroy pesticides in the environment [4]. Because of the diversity of biochemical characteristics and the strong ability to adapt to the environment, the bacteria play an important role in atrazine degradation. Many studies have shown that microorganisms in water and soil have the ability to degrade atrazine. Therefore, it is important to isolate strains with high degradation efficiency [5]. Presently, the isolated atrazine-degrading strains include bacteria, fungi, algae, and actinomycetes such as *Rhodococcus* [6], *Pseudomonas, Agrobacterium* [7], *Acinetobacter* [8], *Rhizopus, Aspergillus, Aspergillususstus, Fusarium, Penicillium, Trichoolerma*, White rot fungi, and so on [9, 10]. An atrazine-degrading bacterial culture was isolated from an agricultural soil previously impacted by herbicide spills. The organism was capable of using atrazine under aerobic conditions as the sole source of C and N [11]. A high efficiency atrazine-degrading bacterium, strain AD1, which was capable of utilizing atrazine as a sole nitrogen source for growth, was isolated from industrial wastewater. 16S rDNA sequencing identified AD1 as an *Arthrobacter* sp. [12]. Atrazine is slowly degraded in the water and soil, yielding metabolites such as desethylatrazine, desisopropylatrazine, desethyl-desisopropylatrazine,

[1] The article is published in the original.

[2] Corresponding author; e-mail: Kafilzadeh@jia.ac.ir

Table 1. Geographical coordinates of the sampling stations

Station	North, N	East, E
A	31°19′04.62″	48°39′55.84″
B	31°20′08.72″	48°41′12.87″
C	31°20′07.68″	48°41′12.43″

and hydroxyatrazine, which can be further, degraded [13].

Khuzestan—Iranian province—having a large number of sugarcane plantations, is faced with the danger of agricultural pollution, including herbicide entering into the Karun River. In spite of this, the lack of sufficient study on atrazine biodegradation in the contaminated river sediments is apparent. In this study, atrazine degrading strains were isolated from the sediments of Karun River and identified by physiological and biochemical tests and 16S rDNA gene sequencing. HPLC analysis and MIC test were also used to determine atrazine biodegradation rate and the most resistant isolated strains, respectively.

MATERIALS AND METHODS

Sampling. The sampling was conducted in both summer (Jul) and autumn (Nov) year 2012 at three stations (A to C) from sediments at the depth of 3 to 5 cm in the Karun River. Each station was sampled three times, and sediment samples were carried to the laboratory by ice-protected sterile containers. Geographical coordinates of sampling locations, which are shown in Table 1, were determined by GPS devices.

Counting bacteria. The bacteria were counted based on viable plate count method. In order to count the bacteria, 10^{-1} to 10^{-9} dilutions were prepared by physiological serum from collected sediment samples. The diluted samples were then cultured on nutrient agar by surface plate method, both with atrazine (30 mg/L; 99.5% purity) and without it. The cultured plates were incubated at 30°C and the colonies were eventually counted after 24 h [14].

Enrichment and isolation of bacteria. Enrichment was performed using a basal salt medium (BSM) containing (per liter) $MgSO_4 \cdot 7H_2O$ (0.5 g), $(NH_4)_2SO_4$ (0.5 g), K_2HPO_4 (0.5 g), $FeCl_3 \cdot H_2O$ (10 mg), $CaCl_2 \cdot H_2O$ (10 mg), $MnCl_2$ (0.1 mg), $ZnSO_4$ (0.01 mg) and sodium citrate (0.01 mg). 95 mL of BSM containing 30 mg/L of atrazine was mixed with 10 g of sediment sample. The pH of the medium was adjusted to 7.0. Cultures were incubated aerobically on a reciprocal shaker (100 rpm) at room temperature (23–25°C) in the dark to preclude photolysis reactions. All enrichment cultures were subcultured on the same medium at a one week interval. From a one week-old culture, 10 mL was transferred to 90 mL of freshly prepared atrazine medium. Repeat the transfer process said above every week. After two months enrichment, the liquid medium was plated on solid culture medium and cultured at 30°C. Morphologically distinct colonies were selected for plate clearance assay [3].

Identification of atrazine-degrading bacteria. The isolated strains were identified by colony morphology, Gram staining, microscopic form, and some biochemical tests including oxidase, catalase, TSI, urease, citrate, motility, indole which are shown in Table 2.

Molecular identification. In order to confirm the results obtained from the biochemical and morphological tests molecular identification was done by of 16S rDNA gene sequencing. DNA extraction was done with the CinnaGen company kit program (DNP kit, CinnaGen, Iran). For PCR amplification, two universal primers for the Domain Bacteria, (27F 5'-AGAGTTTGATCMTGGCT CAG-3') and (338R 5'-GCTGCCTCCCGTAGGAGT-3') were used as sense and antisense primers, respectively. The reaction mixture was prepared to a total volume of 25 μL containing: 1 μL dNTP, 10 μL $MgCl_2$, 10× PCR buffer, 5 μL of each primers, 2 μL template DNA, 2 μL *Taq* polymerase. The reaction mixture was incubated in a thermal cycler with an initial denaturation step at 96°C for 3 min, followed by 35 cycles of denaturation at 96°C for 30 s, annealing at 59°C for 30 s and extension at 65°C for 40 s and a final extension step at 65°C for 10 min.

Electrophoresis. The electrophoresis gel was used on the 0.1% agarose (w/v), with the voltage of 70 V for one hour in order to see the band pattern of the PCR product. The TAE buffer was applied for electrophoresis. The 1 kb ladder (Fermentase Gen Ruller SM 0373) was also used to determine the size of PCR products.

The 16S rDNA gene sequencing. The multiplied 16S rDNA genes obtained by PCR were sent to South Korea for sequencing, with the cooperation of Tiba Biotechnology Company, Fars, Iran. DNA sequences of the cloned 16S rDNA fragments compared using BLAST at http://www.ncbi.nlm.nih.gov/BLAST/ (National Center of Biotechnology Information, NCBI).

Biodegradation testing. Atrazine degradation experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of BS medium. Atrazine was added to BS liquid media to final concentration of 30 mg/L. Bacteria strains (10^6 cells/mL) was incubated overnight (~14 h) in 5 mL nutrient broth at 30°C. After incubation, cultures were centrifuged at 6000 rpm for 15 min and cells collected. These were washed three times with BS liquid medium and resuspended in a small volume (5 mL) of the same medium. Inocula for all experiments were prepared by diluting the recovered cultures with desired media to give a spectrophotometric reading of 0.05 at 600 nm. Cell suspension (500 μL) was inoculated into a 100 mL BS. The cultures were incubated on orbital shaker at 150 rpm at 30°C for 7 days. At 24 h interval, 20 mL of each culture were sampled for chromatographic anal-

Table 2. The results of biochemical tests

Bacteria	Gram staining	Motility	Oxidase	Catalase	Urease	Citrate	Indole	MR	VP	TSI
A. insolitus strain F-N3	Negative	+	+	+	–	+	–	–	–	ALK/ALK
D. tsuruhatensis strain F-N4	Negative	+	+	+	–	–	–	–	–	A/A
K. pneumonia strain F-N1	Negative	–	–	+	+	+	–	–	+	ALK/ALK
E. ludwigii strain F-N5	Negative	+	–	–	–	+	–	+	+	A/A
S. marcescens strain F-N6	Negative	+	–	+	–	+	–	–	+	A/A
E. profundum strain F-N2	Positive	+	–	+	–	–	–	+	–	A/A

yses. Controls involving the use of uninoculated flasks were included and each experiment was performed in triplicate [15].

Extraction and determination of atrazine. Two mL of methanol were added to 5 mL BS medium in screw cap glass tubes that were than shaken several times. One mL of the upper phases (methanol) were transferred to clean tubes and evaporated on a rotary evaporator. The remaining pellets were dissolved in 2 mL of HPLC mobile phase and stored at 4°C for HPLC analysis. Ten μL of each sample were injected into a Shimadzu HPLC system equipped with UV detector at a fixed wavelength 254 nm. The analysis was carried out by using a C18 column. Methanol : water (80 : 20 v/v) was used as a mobile phase at a flow rate of 0.5 mL/min [15].

Determination of minimum inhibitory concentrations (MIC). MIC test was used to determine the most resistant isolated bacteria to atrazine which is the minimum concentration of atrazine that inhibit the growth of bacteria. Nine tubes, containing LB broth were used for each bacterium. One milliliter of atrazine solution (concentration of 1500 mg/L) was added to the first tube using a sterile sampler, and dilution was performed to the final tube. The amount of 0.1 mL bacterial suspension was added to the tubes (according to 0.5 McFarland). Finally, the tubes were examined for turbidity after 24 h. The lowest concentration that inhibits the growth represents the MIC.

Statistical analysis. Comparisons were made between the different seasons and stations of the atrazine-degrading bacteria in sediment samples, and the results were analyzed using SPSS statistical software and the analysis of variance (ANOVA).

RESULTS

Counting bacteria. Logarithmic mean of the numbers of bacteria in a medium containing atrazine in summer 4.652 ± 0.022 (CFU/g) was significantly lower than a medium without atrazine 5.16 ± 5.025 (CFU/g) in the same season ($P < 0.01$). In autumn season in a medium containing atrazine, logarithmic mean bacterial count 4.112 ± 0.032 (CFU/g) was significantly lower than logarithmic bacterial count in a medium without atrazine 4.751 ± 0.036 (CFU/g) in a mentioned season ($P < 0.01$) (Fig. 1).

Maximum number of bacteria was seen in the summer 5.467 (CFU/g) in a medium without atrazine while the lowest number was in the autumn 4.103 (CFU/g) in a medium containing atrazine. A significant difference of mean count of bacteria was observed between these two seasons in medium containing atrazine and without atrazine ($P < 0.01$).

Identification of atrazine degrading bacteria. Bacteria were identified by various biochemical tests and Gram staining which is shown in Table 2. Different bacterial species were isolated from the Karun River sediments which all grow well on the salt medium. Six aerobic strains was identified including *Achromobacter insolitus, Delftia tsuruhatensis, Klebsiella pneumonia, Enterobacter ludwigii, Serratia marcescens* in both summer and autumn, and *Exiguobacterium profundum* only in the summer.

The sequences of 16S rDNA of isolated bacteria were compared with sequence of registered bacteria from the GenBank. The homology between sequences obtained from 16s rDNA and gene bank showed *K. pneumonia* with 85% similarity, *E. profundum* with 97% similarity, *A. insolitus* with 96% similarity, *D. tsuruhatensis* with 99% similarity, *E. ludwigii* with 97% similarity and *S. marcescens* with 92% similarity.

The 16S rDNA gene for these bacteria were registered in the NCBI GenBank, and their accession numbers were as *K. pneumonia* strain F-N1 Accession: KF366299, *E. profundum* strain F-N2 Accession: KF366300, *A. insolitus* strain F-N3 Acces-

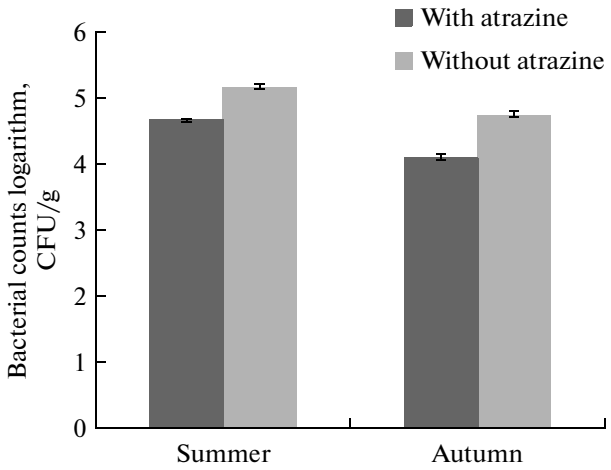

Fig. 1. Logarithmic mean of the numbers of bacteria in two seasons, with and without atrazine.

sion: KF366301, *D. tsuruhatensis* strain F-N4 Accession: KF366302, *E. ludwigii* strain F-N5 Accession: KF366303 and *S. marcescens* strain F-N6 Accession: KF366304.

MIC test. The MIC test results of the various atrazine concentrations (i.e., 1.5, 1.25, 1, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015, and 0.0075 g/L) indicated that the most resistant bacteria belonged to *D. tsuruhatensis* strain F-N4, *E. ludwigii* strain F-N5, and *E. profundum* strain F-N2 in the summer (Table 3), as well as *D. tsuruhatensis* strain F-N4 and *E. ludwigii* strain F-N5 in autumn (Table 4), with MIC 1 g/L. In both seasons, *S. marcescens* strain F-N6 was determined as the less resistant bacterium with MIC 0.5 g/L.

Biodegradation rate. Results from HPLC analysis indicated that atrazine was considerably degraded by the bacterial isolates. The highest atrazine degradation rate (90%) was observed by *E. ludwigii* strain F-N5 followed by *D. tsuruhatensis* strain F-N4 (85%), *E. profundum* strain F-N2 (82%), *A. insolitus* strain F-N3 (68%), *K. pneumonia* strain F-N1 (65%) and *S. marcescens* strain F-N6 (54%). Results are graphically presented in Fig. 2.

DISCUSSION

Atrazine herbicide is extremely toxic to freshwater and river organisms [16], but fortunately, this herbicide could be eliminated by biodegradation process [17]. In the present work, the sediments of Karun River were sampled in summer and autumn. The colonies of bacteria appeared in the atrazine agar medium within 3 to 5 days which indicates their resistance to atrazine. The logarithmic mean of bacterial counts in the summer was 4.908 (CFU/g), which is more than the logarithmic mean of bacterial counts in autumn which was 4.431 (CFU/g). Like every microbial activity, biodegradation of atrazine is influenced by various environmental factors such as temperature. The logarithmic mean of bacterial count in the presence of atrazine was 4.382 (CFU/g) and in the absence of atrazine was 4.958 (CFU/g). This result obtained as atrazine is a toxic substance for bacteria which inhibits their growth.

Among gram negative bacteria, complete degradation of atrazine has been limited to the genera, e.g., *Pseudomonas, Agrobacterium, Pseudaminobacter, Chelatobacter, Delftia*, and unidentified genera. Atrazine-degrading gram-positive bacteria are distributed within the limited genera, e.g., *Rhodococcus, Arthrobacter*, and *Nocardioides* [4]. In the current study, atrazine degrading bacteria were identified via usual biochemical tests and PCR method with 16S rDNA

Table 3. The results of MIC test in the summer

Atrazine initial concentration, g/L	Bacterial strains					
	E. profundum strain F-N2	*A. insolitus* strain F-N3	*D. tsuruhatensis* strain F-N4	*K. pneumonia* strain F-N1	*E. ludwigii* strain F-N5	*S. marcescens* strain F-N6
1.5	–	–	–	–	–	–
1.25	–	–	–	–	–	–
1	+	–	+	–	+	–
0.5	+	+	+	+	+	+
0.25	+	+	+	+	+	+
0.125	+	+	+	+	+	+
0.06	+	+	+	+	+	+
0.03	+	+	+	+	+	+
0.015	+	+	+	+	+	+
0.0075	+	+	+	+	+	+
Control and bacteria	+	+	+	+	+	+
Control and atrazine	–	–	–	–	–	–

Table 4. The results of MIC test in the autumn

Atrazine initial concentration, g/L	Bacterial strains				
	A. insolitus strain F-N3	*D. tsuruhatensis* strain F-N4	*K. pneumonia* strain F-N1	*E. ludwigii* strain F-N5	*S. marcescens* strain F-N6
1.5	–	–	–	–	–
1.25	–	–	–	–	–
1	–	+	–	+	–
0.5	+	+	+	+	–
0.25	+	+	+	+	+
0.125	+	+	+	+	+
0.06	+	+	+	+	+
0.03	+	+	+	+	+
0.015	+	+	+	+	+
0.0075	+	+	+	+	+
Control and bacteria	+	+	+	+	+
Control and atrazine	–	–	–	–	–

sequence. Li et al. (2008) isolated a bacterial strain (AD26) capable of utilizing atrazine as a sole carbon and nitrogen source for growth from an industrial wastewater sample by enrichment culture. The 16S rRNA gene sequencing identified AD26 as an *Arthrobacter* sp. [18]. In the present research, this bacterium was also isolated in both summer and autumn seasons.

In the present study, different bacteria were used for atrazine degradation with varying degrading ability. It was found that the highest degradation rate (90%) was shown by *E. ludwigii* F-N5 which may be due to favorable environmental conditions as well as high production of enzymes responsible for atrazine biodegradation. Ngigi et al. (2012) identified strain ISL 14 as *Enterobacter cloacae* which was capable of degrading 82% of atrazine [19]. Zhang et al. (2009) isolated two strains *Microbacterium* sp. and *Arthrobacter* sp. from black earth in a cold area. The atrazine degradation rates of the two strains reached 77.7 and 65.6%, respectively after 14 days culture in a liquid medium with an atrazine concentration of 100 mg/L [5]. No *Micobacterium* and *Arthrobacter* were found in the current research. Vargha et al. (2005) isolated a bacterium member of *Delftia* genus capable of degrading atrazine as a sole source of carbon and nitrogen [20]. In the present research *D. tsuruhatensis* F-N4 could degrade 82% of atrazine.

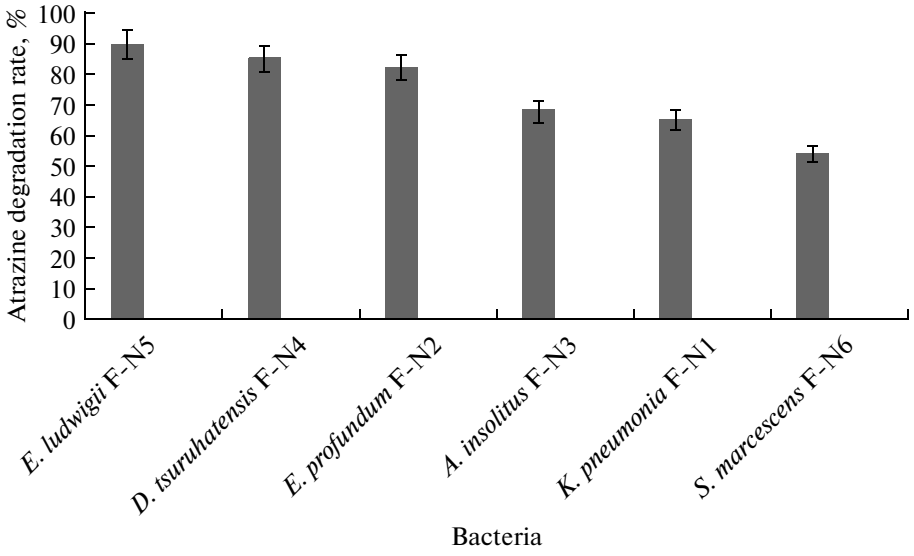

Fig. 2. Amount of degraded atrazine (%) by bacteria.

Atrazine is converted by bacteria to cyanuric acid through a series of hydrolytic reactions starting with dechlorination and then the sequential removal of the two alkylamino chains [21]. The intermediates of atrazine biodegradation through the formation of cyanuric acid are believed to be common, but the sequence of pathway steps varies among the known degrader [22].

In the current study MIC test was used to determine the most resistant isolated bacteria to atrazine. The results indicated that the most resistant bacteria belonged to *E. ludwigii* F-N5 and *D. tsuruhatensis* F-N4 in both summer and autumn season. Moreover no growth could be observed at the concentrations greater than 1 g/L. It can be concluded that high concentrations of atrazine inhibits the growth of bacteria. Khan et al. (2006) used the MIC test for atrazine resistant *Bradyrhizobium* sp. which its value obtained 3200 μg/L [23]. Therefore, the tolerance rate of this bacterium to atrazine was less than isolated bacteria in the current study. Bacteria have several mechanisms that allow them to be tolerant or resistant to toxic compounds. Bacteria may utilize efflux pumps, which remove toxic compounds from the cell [24]. Bacteria may alter surface receptor sites to block entry into the cell or alter the chemical by mechanisms such as methylation. Bacteria may also produce degrading enzymes that metabolize the compound [25]. The latter mechanism is employed in atrazine degradation.

The results show with MIC increase, also increases biodegradation rate. So that *E. ludwigii* F-N5 and *D. tsuruhatensis* F-N4 have the most degradation rate with the highest MIC. But *S. marcescens* has the least degradation rate with the lowest MIC.

CONCLUSIONS

In this work, we have reported the isolation and molecular identification of atrazine degrading bacteria in the sediments of Karun River. According to MIC tests and HPLC analysis, novel strains of *E. ludwigii* F-N5 and *D. tsuruhatensis* F-N4 are good choices to apply for degradation and elimination of atrazine in the environment.

ACKNOWLEDGMENTS

The authors are grateful for all the staff of the Islamic Azad University, Jahrom Branch, Iran, who sincerely cooperated in performing this research.

REFERENCES

1. Ma, W.T., Fu, K.K., Cai, Z., and Jiang, G.B., Gas chromatography/mass spectrometry applied for the analysis of triazine herbicides in environmental waters, *Chemosphere*, 2003, vol. 52, no. 9, pp. 1627–1632.
2. Seiber, J.N., Glotfelty, D.E., Lucas, A.D., McChesney, M.M., Sagebiel, J.C., and Wehner, T.A., A multiresidue method by high performance liquid chromatography-based fractionation and gas chromatography determination of trace levels of pesticides in air and water, *Arch. Environ. Contamin. Toxicol.*, 1990, vol. 19, no. 4, pp. 583–592.
3. Dehghani, M., Nasseri, S., Amin, S., Naddafee, K., Taghavi, M., and Yunesian, M., Isolation and identification of atrazine-degradating bacteria from corn field in Fars province of Iran, *Pakistan J. Biol. Sci.*, 2007, vol. 10, no. 1, pp. 84–89.
4. Abigail, M, E.A. and Das, N., Microbial degradation of atrazine, commonly used herbicide, *Int. J. Adv. Biol. Res.*, 2012, vol. 2, no. 1, pp. 16–23.
5. Zhang, Y., Ning, Z., Zhao, J., Xinran, P., Shuyan, M., and Miao, H., Isolation of two atrazine-degrading strains and their degradation characteristics, *Int. J. Agric. Biol. Eng.*, 2009, vol. 2, no. 3, pp. 27–33.
6. Behlci, R.M. and Khan, S., Degradation of atrazine propazine and simazine by *Rhodococcus* strain B-30, *J. Agric. Food. Chem.,* 1994, vol. 42, pp. 123–124.
7. Garcia-Gonzalez, V., Govantes, F., Porrua, O., and Santero, E., Regulation of the *Pseudomonas* sp. strain ADP cyanuric acid degradation operon, *J. Bacteriol.*, 2005, vol. 187, pp. 155–167.
8. Struthers, J.K., Jayachandran, K., and Moorman, T.B., Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil, *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 9, pp. 3368–3375.
9. Bouquard, C., Ouazzani, J., Prome, J., Michel-Briand, Y., and Plesiat, P., Dechlorination of atrazine by a *Rhizobium* sp. isolate, *Appl. Environ. Microbiol.*, 1997, vol. 63, no. 3, pp. 862–866.
10. Mougin, C., Laugero, C., Asther, M., Dubroca, J., Frasse, P., and Asther, M., Biotransformation of the herbicide atrazine by the white rot fungus *Phanerochaete chrysosporium, Appl. Environ. Microbiol.*, 1994, vol. 60, pp. 705–708.
11. Radosevich, M., Traina, S.J., Hao, Y.L., and Tuovinen, O.H., Degradation and mineralization of atrazine by a soil bacterial isolate, *Appl. Environ. Microbiol.*, 1995, vol. 61, no. 1, pp. 297–302.
12. Cai, B., Han, Y., Liu, B., Ren, Y., and Jiang, S., Isolation and characterization of an atrazine-degrading bacterium from industrial wastewater in China, *Lett. Appl. Microbiol.*, 2003, vol. 36, no. 5, pp. 272–276.
13. Baluch, H.U., Somasundaram, L.R., Kanwar, S., and Coats, J.R., Fate of major degradation products of atrazine in Iowa soils, *J. Environ. Sci. Heal. A,* 1993, vol. 28, pp. 127–149.
14. Kafilzadeh, F., Amiri, P., Rezaeian, J.A., and Mojoodi, N., Isolation and molecular identification of fluoranthene degrading bacteria from the mangrove sediments in South of Iran, *Int. J. Biosci.,* 2013, vol. 3, no. 5, pp. 60–66.
15. Coral, G. and Karagoz, S., Isolation and characterization of phenanthrene degrading bacteria from a petroleum refinery soil, *Ann. Microbiol.*, 2005, vol. 55, no. 4, pp. 255–259.
16. Haghnia, G.H. and Razavi, E., *Remediation of Soil and Water Contaminated with Pesticides*, 1st ed., Tehran: Environmental Protection Agency, 2008.

17. Sene, L., Converti, A., Secchi, G.A.R., and Simao, R.C.G., New aspects on atrazine biodegradation, *Braz. Arch. Biol. Technol.*, 2010, vol. 53, no. 2, pp. 487–496.

18. Li, Q., Li, Y., Zhu, X., and Cai, B., Isolation and characterization of atrazine-degrading *Arthrobacter* sp. AD26 and use of this strain in bioremediation of contaminated soil, *J. Environ. Sci.*, 2008, vol. 20, no. 10, pp. 1226–1230.

19. Ngigi, AN., Getenga, Z.M., Boga, H.I., and Ndalut, PK., Biodegradation of s-triazine herbicide atrazine by *Enterobacter cloacae* and *Burkholderia cepacia* sp. from long-term treated sugarcane-cultivated soils in Kenya, *J. Environ. Sci. Health. B*, 2012, vol. 47, no. 8, pp. 769–778.

20. Vargha, M., Takats, Z., and Marialigeti, K., Degradation of atrazine in a laboratory scale model system with Danube River sediment, *Water Res.*, 2005, vol. 39, no. 8, pp. 1560–1568.

21. Boundy-Mills, K., de Souza, M.L., Mandelbaum, R.M., Wackett, L.P., and Sadowsky, M.J., The *atzB* gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway, *Appl. Environ. Microbiol.*, 1997, vol. 63, no. 3, pp. 916–923.

22. Ostrofsky, E.B., Robinson, J.B., Traina, S.J., and Tuovinen, O.H., Analysis of atrazine-degrading microbial communities in soils using most-probable-number enumeration, DNA hybridization, and inhibitors, *Soil Biol. Biochem.*, 2002, vol. 34, pp. 1449–1459.

23. Khan, M.S., Chaudhry, P., Wani, P.A., and Zaidi, A., Biotoxic effects of the herbicides on growth, seed yield, and grain protein of greengram, *J. Appl. Sci. Environ. Manage.*, 2006, vol. 10, no. 3, pp. 141–146.

24. Silver, S. and Phung, L.T., Bacterial heavy metal resistance: new surprises, *Annu. Rev. Microbiol.*, 1996, vol. 50, pp. 753–89.

25. Talaro, K.P., *Foundations in Microbiology, Basic Principles*, 7th ed., New York: McGraw-Hill, 2008.