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Short Genome Communications

# Complete genome sequence of *Arthrobacter* sp. ZXY-2 associated with effective atrazine degradation and salt adaptation

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ABSTRACT

An atrazine-degrading strain *Arthrobacter* sp. ZXY-2 was originally isolated from Jilin Pesticide Plant (China). Strain ZXY-2 demonstrated excellent atrazine degradation performance and saline tolerance. Here we report the complete genome sequence of strain ZXY-2 contained a circular chromosome and five circular plasmids encoding for the mechanism of salt adaptation and pollutant degradation.



Atrazine has been widely used as a broad-leaf weed control herbicide. Due to low biodegradability, a proliferation of environmental concerns arises (Bhardwaj et al., 2015; Fazlurrahman et al., 2009; Singh and Singh, 2016). Further, long-term exposing herbicide would generate endocrine-disrupting influences (Vanraes et al., 2015). The fate of atrazine in contaminated sites largely depends on the metabolism by microorganisms (Wang et al., 2014). However, the increasing salinity in some contaminated water resource has impeded the biodegradation of atrazine (Shir et al., 2016). This necessitates the isolation and identification of atrazine-degrading microorganisms with salt toleration. Various atrazine-degrading microorganisms belonging to diverse genera have been reported, e.g., *Pseudomonas*, *Acinetobacter*, *Rhodococcus*, *Arthrobacter*, *Bacillus*, and *Variovorax* (El Sebaï et al., 2011; Fazlurrahman et al., 2009; Mandelbaum et al., 1995; Singh et al., 2004; Wang et al., 2014; Wang and Xie, 2012; Zhao et al., 2016). These strains could degrade atrazine effectively, with the highest degradation efficiency of 19.03 mg $L^{-1}$ $h^{-1}$ (Zhao et al., 2016). However, no salt-toleration capacity was revealed. Up to date, although *Pseudomonas* sp. ADP and *Ochrobactrum oryzae* are reported to be capable of degrading atrazine with salt toleration (4% *w/w* and 1% *w/w*), they revealed the limited atrazine-degrading efficiencies with 1.04 mg $L^{-1}$ $h^{-1}$ and 0.04 mg $L^{-1}$ $h^{-1}$, respectively (Shapir et al., 1998; Shir et al., 2016).

In this study, a gram-positive strain *Arthrobacter* sp. ZXY-2 (belongs to *Micrococcaceae* family, *Actinobacteria* class) was isolated from Jilin Pesticide Plant (China) at January 2015. As demonstrated, strain ZXY-2 could effectively degrade atrazine and exhibit high salinity tolerance. Fig. 1 shows a maximum likelihood phylogenetic tree based on the 16 s rRNA gene analysis.

Fig. 2a displays the growth of ZXY-2 inoculated with a series of salinities, i.e., 0%, 1%, 3%, 5%, and 10%. When salt concentration was below 3%, the duration of the lag phases presented no distinction (less than 1.5 h). In contrast, the lag phases last for 10 h when ZXY-2 grew in 10% NaCl solution. Similarly, NaCl concentration influenced atrazine degradation efficiencies. As shown in Fig. 2b, atrazine level dropped from 100 to 0 mg/L within 15 h when NaCl concentration was lower than 3%. The corresponding rate represents 7.14 mg $L^{-1}$ $h^{-1}$. Interestingly, even though the concentration of NaCl reached 10%, strain ZXY-2 could degrade up to 50% after 30 h. According to previous studies, atrazine degradation efficiencies by *Arthrobacter* species were relatively lower (El Sebaï et al., 2011; Li et al., 2008; Zhang et al., 2011). Therefore, these findings demonstrated that strain ZXY-2 displayed enhanced degradation capacity in comparison with reported *Arthrobacter* strains.

Given that *Arthrobacter* sp. ZXY-2 could degrade atrazine efficiently and resist salinity, this strain was chosen to further analyze its complete genomic sequence to gain more insights into the genetic bases. The genomic DNA was extracted by using the Qiagen™QIAamp DNA Kit and sequenced using the Pacific Biosciences (Pacbio) RS II sequencing platform (Pacific Biosciences, Menlo Park, CA, USA) (Eid et al., 2009). In total, 176,767 high-quality

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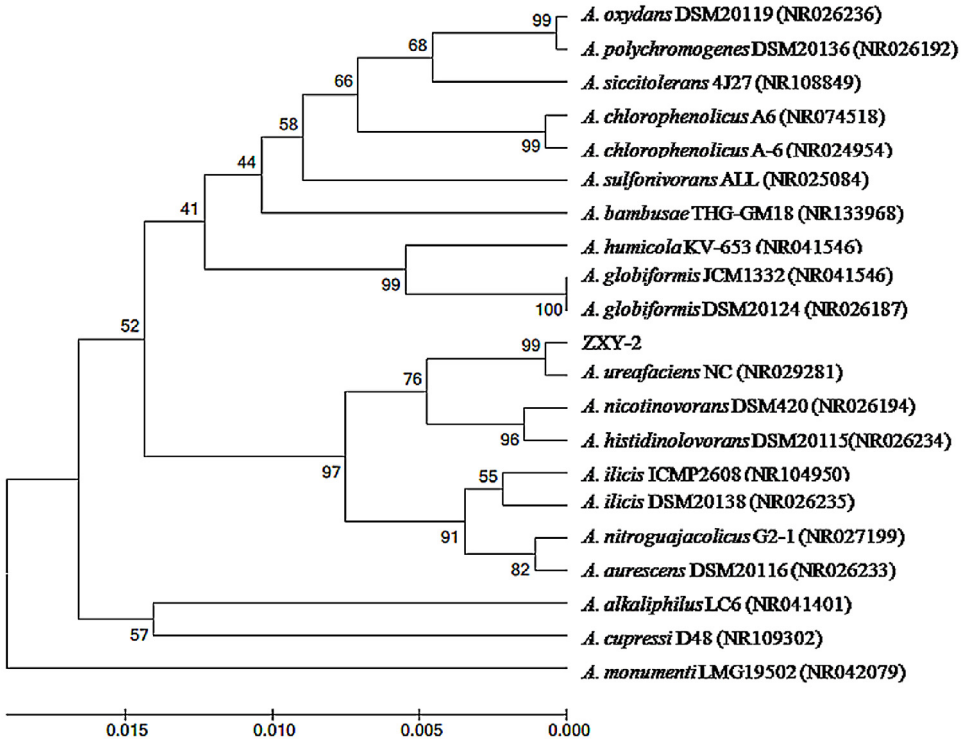


**Fig. 1.** The phylogenetic tree using 16S rRNA gene sequences showing the relationship between *Arthrobacter* sp. ZXY-2 and other *Arthrobacter* species. Numbers at the nodes indicated bootstrap values from the neighborhood-joining analysis of 1000 replications. Scale bar indicated nucleotide substitutions per nucleotide position.

**Table 1**
General features of *Arthrobacter* sp. ZXY-2 genome.

| Features | Genome size (bp) | GC content (%) | r RNAs | t RNAs | Total predicted CDSs |
|---|---|---|---|---|---|
| Chromosome | 4,495,402 | 63.5 | 18 | 54 | 4252 |
| Plasmid pZXY21 | 288,370 | 61.9 | – | – | 349 |
| Plasmid pZXY22 | 116,799 | 60.9 | – | – | 127 |
| Plasmid pZXY23 | 65,137 | 61.5 | – | – | 79 |
| Plasmid pZXY24 | 64,244 | 59.8 | – | – | 62 |
| Plasmid pZXY25 | 28,756 | 61.5 | – | – | 39 |

reads with 1,382,146,368 nucleotides were generated. Reads were then *de novo* assembled using the hierarchical genome assembly process workflow in the single molecule real-time portal.

The genetic equipment of ZXY-2 included one circular chromosome (4,495,402 bp) and five circular plasmids including pZXY21 (288,370 bp), pZXY22 (116,799 bp), pZXY23 (65,137 bp), pZXY24 (64,244 bp), and pZXY25 (28,756 bp), respectively. The chromosome harbors 4252 predicted CDSs, 18 rRNA and 54 tRNA genes. The plasmids were found to contain 656 predicted CDSs in total. The general features of the chromosome and the five plasmids are summarized in Table 1 and Fig. 3.

The genes (*trzN*, *atzB*, *atzC*) responsible for degradation of atrazine are found on the chromosome. In particular, ZXY-2 has three copies of *atzB* genes, implying that the increase in the copy number of catabolic genes might accelerate atrazine degradation due to a gene dosage effect. In addition, the relative abundance of energy metabolism genes (7.16% of energy production and conversion genes, 11.20% of amino acid transport and metabolism genes, and 11.41% of carbohydrate transport and metabolism genes) were significantly higher ($P < 0.05$) compared to two complete sequenced atrazine-degrading strains *Arthrobacter* sp. FB24 and TC1 (Mongodin et al., 2006; Nakatsu et al., 2013). These differences could contribute to the excellent degradation capacity of isolated strain ZXY-2.

The genome of ZXY-2 encoded for cation/$H^+$ antiporter proteins *mrp* (ORF00067, ORF00068, ORF00069, ORF00070, ORF00071, ORF00072), which were essential in terms of maintaining cell pH and sodium ions ($Na^+$) homeostasis (Krulwich et al., 2011). Additionally, many genes associated with salinity resistance were predicted in ZXY-2 genome (Table 2) (Csonka, 1989; Nagler et al., 2016; Saum and Muller, 2007). For example, *trkA* genes (ORF02170, ORF02169, ORF00621, ORF00391) were found on the chromosome, which enabled to export $Na^+$ and enhance salt-resistant ability (Hamamoto et al., 2015). *nhaA* genes (ORF02626, ORF03662) were also of interest in improving salinity tolerance (Carmel et al., 1997).

Although 213 unannotated functional genes are still required to be studied, the fundamental genomic information sheds light on atrazine bioremediation under salinity conditions in agriculture and industrial fields.

## Nucleotide sequence accession number

The complete chromosome genome sequence and five plasmid genome sequences of *Arthrobacter* sp. ZXY-2 have been deposited to GenBank under the accession number CP017421-CP017426. This strain has been stored at China General Microbiological Culture Collection Center (CGMCC) under the accession number CGMCC NO. 10937.

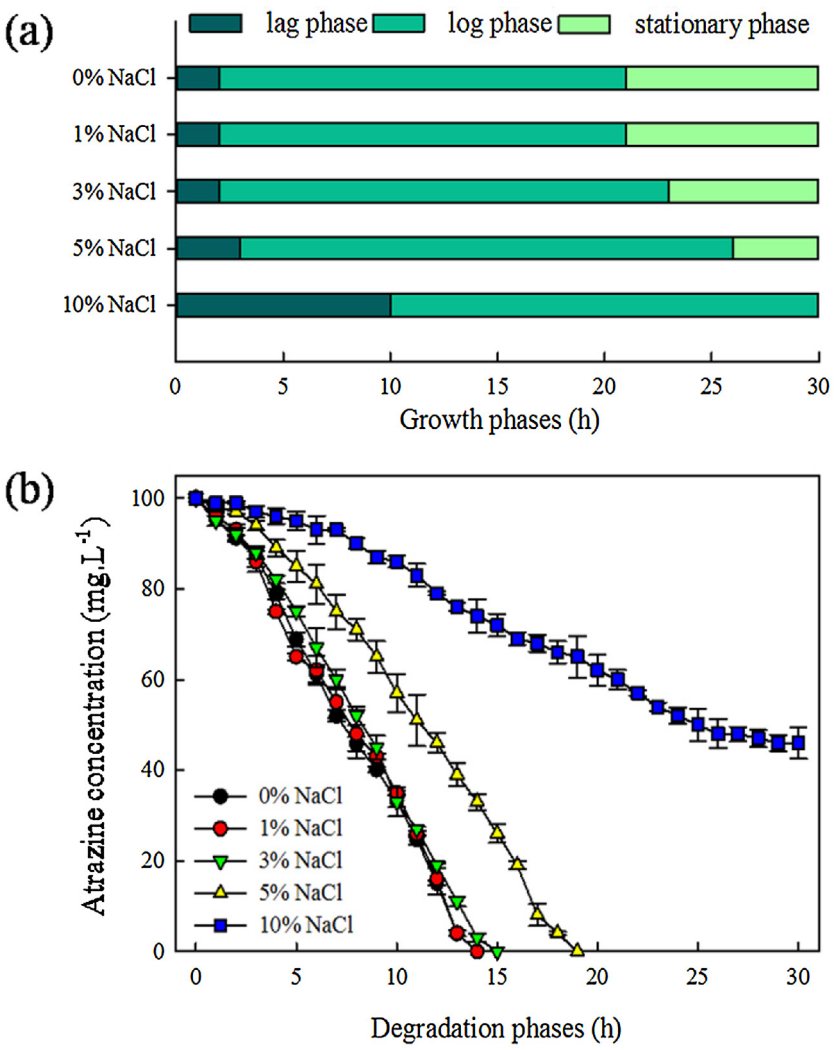


**Fig. 2.** Effect of salinity on the growth (a) and atazine degradation (b) by strain ZXY-2. The error bars indicated standard deviations of the means.

**Table 2**
Genes involved in salinity tolerance mechanism in *Arthrobacter* sp. ZXY-2.

| Gene ID | Gene name | Gene ID | Gene name |
|---|---|---|---|
| **Cation/H$^+$ antiporter protein** | | **Malto-oligosyltrehalosetrehalohydrolase** | |
| ORF00067 | *mrpA* | ORF00772 | *treY* |
| ORF00068 | *mrpC* | ORF00773 | *treZ* |
| ORF00069 | *mrpD* | **Trehalose phosphatase** | |
| ORF 00070 | *mrpE* | ORF03053 | *otsA* |
| ORF00071 | *mrpF* | ORF03054 | *otsB* |
| ORF00072 | *mrpG* | ORF00030 | *otsB* |
| ORF02626 | *nhaA* | **Trehalose synthase** | |
| ORF03662 | *nhaA* | ORF03068 | *treS* |
| ORF00064 | *kef* | ORF03917 | *treS* |
| **Ion transport protein** | | **Glycine/betaine ABC transporter ATPase** | |
| ORF02169 | *trkA* | ORF00837 | *opuA* |
| ORF02170 | *trkA* | ORF00838 | *opuBD* |
| ORF00391 | *TrkA* | ORF00839 | *opuBD* |
| ORF00621 | *TrkA* | ORF00840 | *opuC* |
| **Betaine-aldehyde dehydrogenase** | | **Others** | |
| ORF03100 | *BetB* | ORF03670 | *kdpD* |
| **Proline-betaine transporter protein** | | ORF03671 | *kdpE* |
| ORF04199 | *ProP* | | |
| ORF00134 | *ProP* | | |
| ORF03572 | *ProP* | | |

Note: gene located on chromosome.

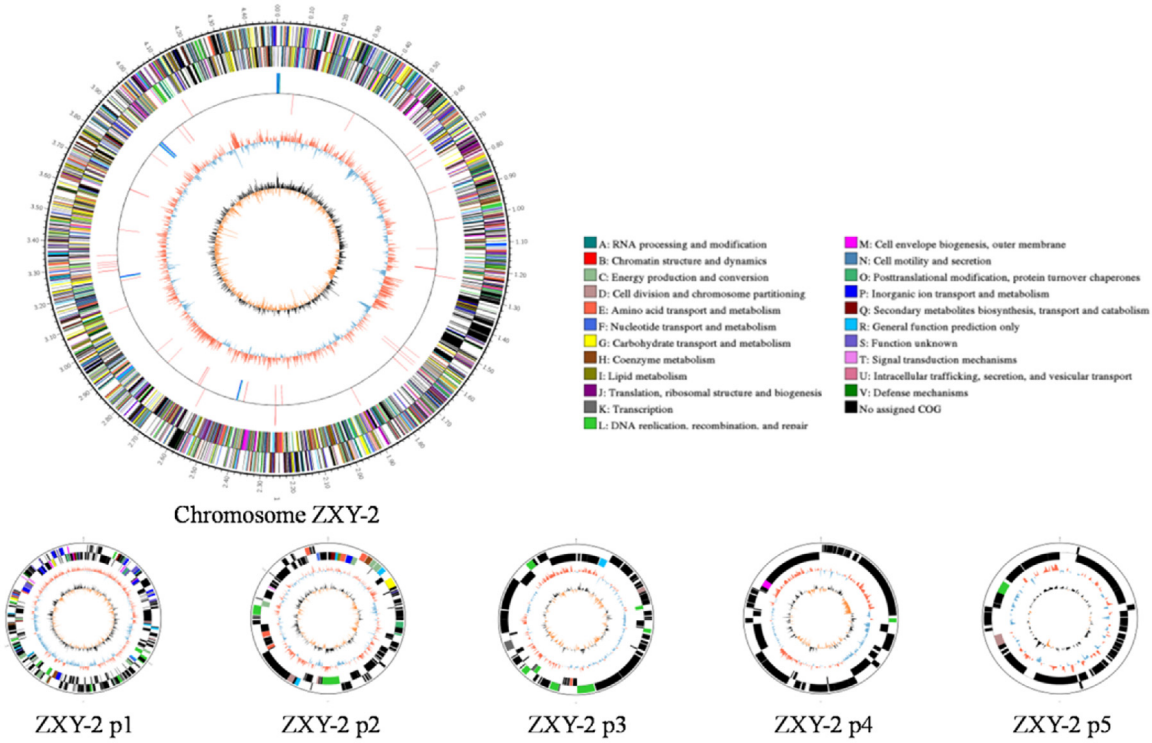


**Fig. 3.** Circular genome map of *Arthrobacter* sp. ZXY-2. From the outermost circle to the innermost circle, the outer black circle shows the scale line in Mbps, circle (1) and circle (2) represent the predicted coding sequences on the forward and backward strands, colored by the COG functional categories. Circle (3) represents tRNA(red) and rRNA (blue). Circle (4) and circle (5) represent the G + C content and G + C skew. The G + C content and G + C skew were calculated using a sliding window, as the deviation from the average G + C content or G + C skew of the entire sequence. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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## References

Bhardwaj, P., Sharma, A., Sagarkar, S., Kapley, A., 2015. Mapping atrazine and phenol degradation genes in *Pseudomonas* sp. EGD-AKN5. Biochem. Eng. J. 102, 125–134.

Carmel, O., RahavManor, O., Dover, N., Shaanan, R., Padan, E., 1997. The $Na^+$-specific interaction between the LysR-type regulator, *nhaR*, and the *nhaA* gene encoding the $Na^+/H^+$ antiporter of Escherichia coli. Embo J. 16, 5922–5929.

Csonka, L.N., 1989. Physiological and genetic responses of bacteria to osmotic-stress. Microbiol. Rev. 53, 121–147.

Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., Peluso, P., Rank, D., Baybayan, P., Bettman, B., Bibillo, A., Bjornson, K., Chaudhuri, B., Christians, F., Cicero, R., Clark, S., Dalal, R., Dewinter, A., Dixon, J., Foquet, M., Gaertner, A., Hardenbol, P., Heiner, C., Hester, K., Holden, D., Kearns, G., Kong, X., Kuse, R., Lacroix, Y., Lin, S., Lundquist, P., Ma, C., Marks, P., Maxham, M., Murphy, D., Park, I., Pham, T., Phillips, M., Roy, J., Sebra, R., Shen, G., Sorenson, J., Tomaney, A., Travers, K., Trulson, M., Vieceli, J., Wegener, J., Wu, D., Yang, A., Zaccarin, D., Zhao, P., Zhong, F., Korlach, J., Turner, S., 2009. Real-time DNA sequencing from single polymerase molecules. Science 323, 133–138.

El Sebaï, T., Devers-Lamrani, M., Changey, F., Rouard, N., Martin-Laurent, F., 2011. Evidence of atrazine mineralization in a soil from the Nile Delta: Isolation of *Arthrobacter* sp TES6, an atrazine-degrading strain. Int. Biodeterior. Biodegrad. 65, 1249–1255.

Fazlurrahman, Batra, M., Pandey, J., Suri, C.R., Jain, R.K., 2009. Isolation and characterization of an atrazine-degrading *Rhodococcus* sp. strain MB-P1 from contaminated soil. Lett. Appl. Microbiol. 49, 721–729.

Hamamoto, S., Horie, T., Hauser, F., Deinlein, U., Schroeder, J.I., Uozumi, N., 2015. HKT transporters mediate salt stress resistance in plants: from structure and function to the field. Curr. Opin. Biotechnol. 32, 113–120.

Krulwich, T.A., Sachs, G., Padan, E., 2011. Molecular aspects of bacterial pH sensing and homeostasis. Nat. Rev. Microbiol. 9, 330–343.

Li, Q., Li, Y., Zhu, X., Cai, B., 2008. Isolation and characterization of atrazine-degrading *Arthrobacter* sp: AD26 and use of this strain in bioremediation of contaminated soil. J. Environ. Sci. 20, 1226–1230.

Mandelbaum, R.T., Allan, D.L., Wackett, L.P., 1995. Isolation and characterization of a *Pseudomonas* sp. that mineralizes the s-triazine herbicide atrazine. Appl. Environ. Microbiol. 61, 1451–1457.

Mongodin, E.F., Shapir, N., Daugherty, S.C., DeBoy, R.T., Emerson, J.B., Shvartzbeyn, A., Radune, D., Vamathevan, J., Riggs, F., Grinberg, V., Khouri, H., Wackett, L.P., Nelson, K.E., Sadowsky, M.J., 2006. Secrets of soil survival revealed by the genome sequence of *Arthrobacter aurescens* TC1. PLoS Genet. 2, e214.

Nagler, K., Krawczyk, A.O., De Jong, A., Madela, K., Hoffmann, T., Laue, M., Kuipers, O.P., Bremer, E., Moeller, R., 2016. Identification of differentially expressed genes during *bacillus subtilis* spore outgrowth in high-salinity environments using RNA sequencing. Front. Microbiol. 7, 1564.

Nakatsu, C.H., Barabote, R., Thompson, S., Bruce, D., Detter, C., Brettin, T., Han, C., Beasley, F., Chen, W., Konopka, A., Xie, G., 2013. Complete genome sequence of *Arthrobacter* sp. strain FB24. Stand. Genomic Sci. 9, 106–116.

Saum, S.H., Muller, V., 2007. Salinity-dependent switching of osmolyte strategies in a moderately halophilic bacterium: glutamate induces proline biosynthesis in *halobacillus halophilus*. J. Bacteriol. 189, 6968–6975.

Shapir, N., Mandelbaum, R.T., Gottlieb, H., 1998. Atrazine degradation in saline wastewater by *Pseudomonas* sp. strain ADP. J. Ind. Microbiol. Biotechnol. 20, 153–159.

Shir, M.A., Dehghani, M., Samaei, M.R., 2016. Isolation of atrazine degrading bacteria in semi-salinity medium. J. Health Sci. Surveill. Syst. 4, 121–128.

Singh, B., Singh, K., 2016. Microbial degradation of herbicides. Crit. Rev. Microbiol. 42, 245–261.

Singh, P., Suri, C.R., Cameotra, S.S., 2004. Isolation of a member of *Acinetobacter* species involved in atrazine degradation. Biochem. Biophys. Res. Commun. 317, 697–702.

Vanraes, P., Willems, G., Nikiforov, A., Surmont, P., Lynen, F., Vandamme, J., Van Durme, J., Verheust, Y.P., Van Hulle, S.W., Dumoulin, A., Leys, C., 2015. Removal of atrazine in water by combination of activated carbon and dielectric barrier discharge. J. Hazard. Mater. 299, 647–655.

Wang, Q., Xie, S., 2012. Isolation and characterization of a high-efficiency soil atrazine-degrading *Arthrobacter* sp. strain. Int. Biodeterior. Biodegrad. 71, 61–65.

Wang, J., Zhu, L., Wang, Q., Wang, J., Xie, H., 2014. Isolation and characterization of atrazine mineralizing *Bacillus subtilis* strain HB-6. PLoS One 9, e107270.

Zhang, Y., Jiang, Z., Cao, B., Hu, M., Wang, Z., Dong, X., 2011. Metabolic ability and gene characteristics of *Arthrobacter* sp. strain DNS10: the sole atrazine-degrading strain in a consortium isolated from black soil. Int. Biodeterior. Biodegrad. 65, 1140–1144.

Zhao, X., Wang, L., Ma, F., Bai, S., Yang, J., Qi, S., 2016. Pseudomonas sp. ZXY-1, a new isolated and highly efficient atrazine-degrading bacterium, and optimization of biodegradation using response surface methodology. J. Environ. Sci., http://dx.doi.org/10.1016/j.jes.2016.06.010.