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Synthesis and anti-biofilm activities of dihydro-pyrrol-2-one derivatives on *Pseudomonas aeruginosa*

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ABSTRACT

Biofilm formation is an important reason for bacterial resistance to antimicrobials. Many compounds with dihydro-pyrrol-2-one (DPO) have antibacterial effects. It is prospective to base on DPO skeleton to design new compounds for biofilm inhibition. DPO was designed by a novel method of tandem cyclization between ethyl glyoxalate and amines, the series of DPO derivatives were synthesized by change of the amines. Their activities were evaluated by the inhibition of biofilm in *Pseudomonas aeruginosa*. The interaction of DPO derivatives with mannitol dehydrogenase (MDH) or extracellular DNA (eDNA) in the biofilm was simulated by molecular docking to reveal possible mechanism. 19 new DPO derivatives were synthesized and identified, 15 of them had antibacterial activities, but only 5 of them had more than 50% inhibition on biofilm of *P. aeruginosa* at 50 μg/mL. The MDH activity and eDNA content in biofilm decreased significantly after treatment of the DPO derivatives in concentration dependence. The simulation reveals that strong interaction exists between the five DPO derivatives and MDH or eDNA, which are involved in anti-biofilm mechanism. The synthetic method of DPO derivatives is practical to provide effective anti-biofilm agents for *P. aeruginosa*, and they take effect through inhibition on MDH and eDNA of biofilm.

Dihydro-pyrrol-2-one (DPO), a key building block for constructing a variety of bioactive ingredients or intermediates, widely exists in pheromone, alkaloids, steroids, heme, chlorophyll and other compounds, and it has very strong antibacterial and other pharmacological activities.[1] The inherent ring-conjugate system and chirality of DPO contribute to its high region-selectivity and stereo-selectivity in chemical reaction,[2] which are conducive to the functionalization of specific sites in the molecules.[3] Therefore, DPO synthesis plays an important role in rational drug design and drug screening.

Many methods exist today for the synthesis of DPO derivatives, including the multistep method,[4] the two component method,[5–7] the three component method[8,9] and the multi-component method.[10,11] The first method involves a cumbersome and inefficient process of separation and purification, the second and the third methods need the complex substrates, which are difficult to synthesize, and the last one requires many kinds of raw materials. We designed a new tandem reaction with two simple components (aldehydes and amines) to synthesize DPO derivatives.

Pseudomonas aeruginosa is the major pathogenic bacterium for pulmonary nosocomial infection, and it easily acquire drug resistance, leading to the deficiency of therapeutic effects.[12] Biofilm formation is an important reason for bacterial resistance and diseases recurrence.[13] It is meaningful to find out effective anti-biofilm agents for therapy of *P. aeruginosa* induced infections. DPO has small molecular structure, may play a role in anti-biofilm with strong permeability. It is prospective to design and screen out anti-biofilm agents with DPO skeleton based on new synthetic methods.

The synthetic route of DPO derivatives was designed as shown in Figure 1. They were synthesized by ethyl glyoxalate and amines with anhydrous sodium sulfate as water absorbent, Pd $(TFA)_2$ as catalyst and toluene as solvent. Each substrate was repeated five times to ensure the synthesis yield.

Conditions for the palladium-catalyzed cascade cyclization of ethyl glyoxalate with *p*-anisidine were optimized in our previous research,[14] DPO structure was formed in tandem reaction. Different DPO derivatives were synthesized by using different amines. 19 kinds of DPO derivatives were obtained when 20 amines reacted with ethyl glyoxalate. The structures and yields of DPO derivatives are listed in Table 1.

The synthesis of DPO was previously achieved by the multi-step reaction like other five member nitrogen heterocyclic rings.[10,11] but the entire synthesis process is extremely cumbersome and complicated because the by-product must be purified in each step.

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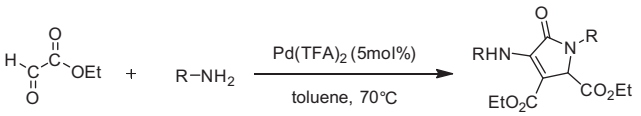


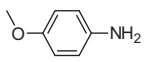
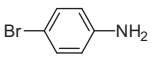
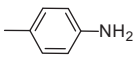
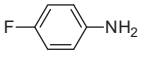
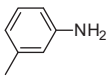
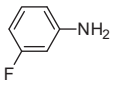
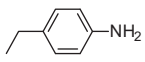
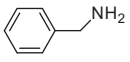
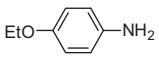
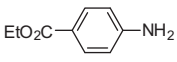
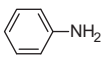
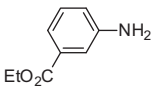
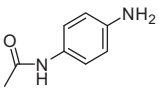
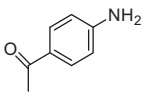
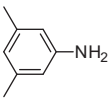
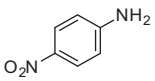
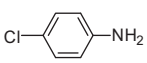
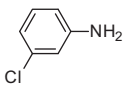
Figure 1. The synthetic route of dihydro-pyrrol-2-one.

We designed a new synthetic strategy in tandem catalytic system to achieve the diversity and complexity of products by adjusting the variety and portion of the substrates. Tandem reaction makes raw materials to form complex structure without isolation of the intermediates. It greatly reduces expenditure of solvent, energy and time, and is a promising method in the field of organic synthesis.

Based on low-cost raw materials, ethyl glyoxalate and amines are chosen to build DPO structure. The mechanism is that ethyl glyoxalate interacts with equivalent amine to form acyl imines by dehydration. At the same time, palladium combines with ethyl glyoxalate to be a chelate by oxidative addition. Finally, acyl imines and the chelate continuously react to get DPO through cross coupling or cyclization.[15,16] The reaction mechanism is illustrated in Figure 2. Arylamines have good adaptability with either electron-withdrawing groups or electron-donating groups such as methyl, alkyl, alkoxy, halogen, ester, acetyl aniline and ketone, and can react with ethyl glyoxalate to reach high yield. However, arylamines with *meta*-substituted group have slight decrease in yield than with *para*-substituted group, it may be attributed to steric hindrance on amino nitrogen atom. *p*-Nitro aniline cannot generate the desired product, it may be due to the strong electron withdrawing effect of *p*-nitro inhibiting the formation of imine.[17]

DPO derivatives exhibit different biological activities if there is change of substituent in γ-lactam ring, especially the 3-amino substituted compounds because its enamine structure can be further functionalized.[18] For example, α,β-unsaturated lactam structure of DPO has Michael addition reaction with stable carbon anions, nitrogen nucleophiles and Gilman reagents, which are owing to the presence of a double bond in the ring prone to epoxidation and hydroxylation.[19] In addition, DPO dihydropyrrolo ring can be oxidized to be pyrrole, and reduced to be pyrrolidone, which

Table 1
Yields of DPO derivatives in ethyl glyoxalate reaction with amines

Sample no.	Amine	Yield (%)	Sample no.	Amine	Yield (%)
1		67.5 ± 3.4	11		73.7 ± 2.6
2		61.0 ± 2.4	12		71.5 ± 3.1
3		36.3 ± 2.0	13		36.3 ± 3.6
4		70.5 ± 3.6	14		40.8 ± 2.5
5		63.2 ± 3.3	15		57.7 ± 4.5
6		57.5 ± 4.6	16		69.7 ± 3.7
7		38.2 ± 2.9	17		26.0 ± 3.8
8		34.8 ± 2.3	18		0.0 ± 0.0
9		78.2 ± 2.8	19		55.3 ± 4.9
10		64.2 ± 3.5	20		34.8 ± 3.9

Yield was expressed as mean ± standard deviation ($n = 5$).

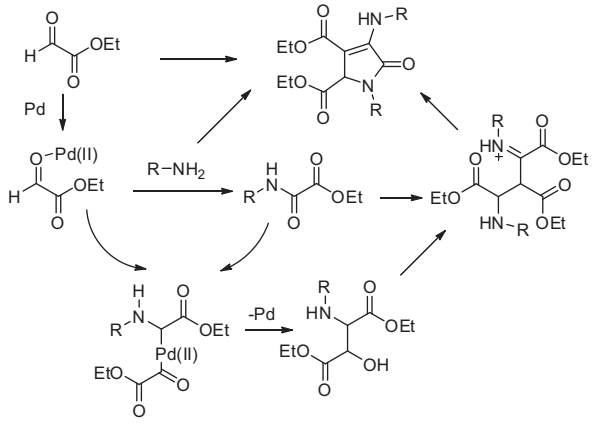


Figure 2. Reaction mechanism of ethyl glyoxalate and amines.

has various pharmacological activities, such as anti-inflammation, anti-tumor, anti-HIV and preventing senile dementia.[20]

Our research disclosed the effects of DPO derivatives on bacterial growth and biofilm formation. Bacterial biofilm were measured according to the reference.[21] 50% inhibitory concentrations (IC_{50}) of the compounds on bacterial growth and biofilm formation were determined by dilution until the inhibition rate reached 50%.

Of 19 DPO derivatives synthesized by our method, fifteen had a certain inhibitory effect on *P. aeruginosa* at 50 μg/mL concentration, eight of them had more than 50% growth inhibition rate, and the compound nos. **1** and **10** had no significant difference from erythromycin on the inhibition of bacterial growth. The results are shown in Table 2. Five compounds (**1**, **4**, **5**, **10** and **11**) had more than 50% inhibition on biofilm of *P. aeruginosa*, and were more effective than erythromycin, which is one of popular drugs currently for inhibition of *P. aeruginosa* and was used as the control in the experiment, but had less effect due to bacterial resistance. Other anti-biofilm agents such as carbenicilli and roxithromyci have IC_{50} from 64 to 256 μg/mL on growth of *P. aeruginosa*.[22] Compound no. **10** has the lowest IC_{50} on both bacterial growth (27.8 μg/mL) and biofilm formation (28.8 μg/mL) among DPO derivatives. It is different from other pyrrole–imidazole alkaloids such as fluconazol, ketoconazole and clotrimazole, those only show effects on fungi with 64 μg/mL of IC_{50} but no inhibitory effect on *P. aeruginosa*.[23] It suggests that compound no. **10** [*diethyl 1-(3-chlorophenyl)-4-((3-chlorophenyl)amino)-5-oxo-2,5-dihydro-1H-pyrrole-2,3-dicarboxylate*] is a good candidate of antibiofilm agents.

Table 2
Inhibition of DPO derivatives on bacterial growth and biofilm formation of *Pseudomonas aeruginosa*

Compound no.	Growth inhibition rate (%)	IC_{50} (μg/mL)	Biofilm inhibition rate (%)	IC_{50} (μg/mL)
1	80.8 ± 5.9	34.2 ± 4.0	61.4 ± 4.8**	40.2 ± 4.1
2	34.0 ± 4.0**	/	13.6 ± 4.6	/
3	55.5 ± 6.6**	48.4 ± 5.1**	29.3 ± 3.3	/
4	65.8 ± 2.5**	42.2 ± 3.0**	53.2 ± 2.7**	47.4 ± 4.0
5	63.1 ± 3.6**	46.6 ± 5.6**	53.8 ± 3.9**	48.4 ± 3.8
6	50.4 ± 6.0**	50.0 ± 2.5**	35.5 ± 4.5	/
8	32.5 ± 5.5**	/	28.9 ± 3.2	/
9	68.6 ± 3.8**	41.6 ± 3.2**	20.7 ± 2.2	/
10	83.0 ± 4.8	27.8 ± 4.0	70.2 ± 5.2**	28.8 ± 4.5
11	76.8 ± 5.8**	38.4 ± 3.0	65.4 ± 3.1**	39.0 ± 4.4
13	20.3 ± 6.4**	/	27.5 ± 5.8	/
15	44.7 ± 5.8**	/	37.8 ± 2.4	/
16	15.4 ± 6.6**	/	42.0 ± 2.2	/
19	26.6 ± 6.0**	/	11.8 ± 3.1	/
20	40.9 ± 6.0**	/	41.3 ± 5.4	/
Erythromycin	67.6 ± 5.9	33.2 ± 5.6	45.7 ± 4.1	/

Data were presented as mean ± standard deviation ($n = 5$).
** $p < 0.01$, compared with erythromycin.

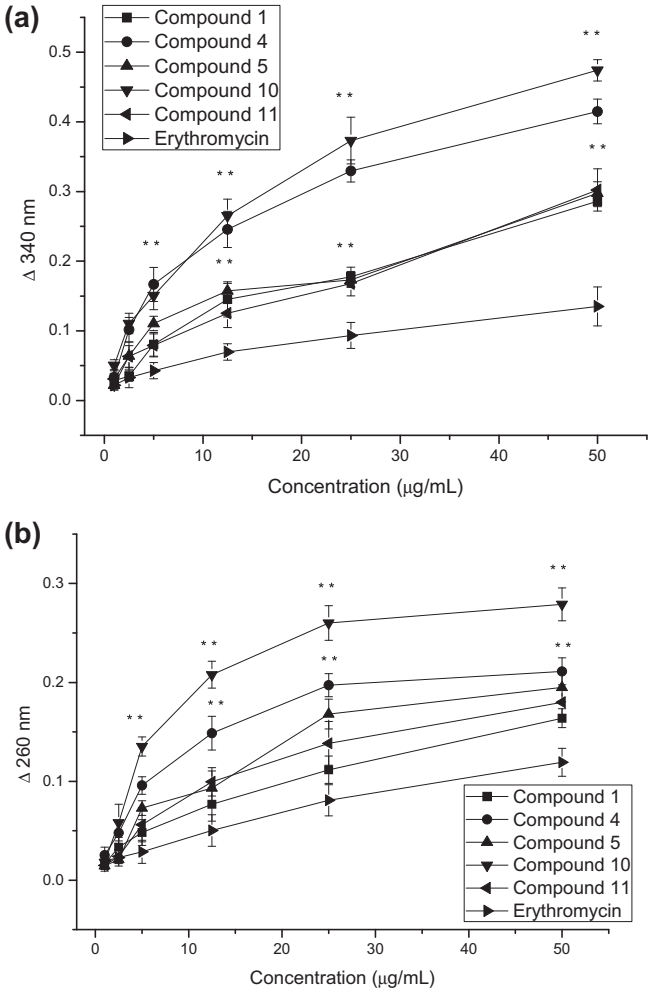


Figure 3. Activity of mannitol dehydrogenase (a) and content of extracellular DNA (b) in bacterial biofilm affected by the DPO derivatives ($\bar{x} \pm s$, $n = 5$). They were measured, respectively, by a decrease in the absorbance of reactive mixture at 340 nm and biofilm lysate at 260 nm compared to untreated controls. **, $p < 0.01$, compared with erythromycin.

The expression of specific genes is involved in biofilm formation and responsible for bacterial drug resistance.[24] The main difference between biofilm bacteria and planktonic bacteria is that biofilm bacteria are tightly packed and wrapped in its own secreted extracellular polysaccharide matrix called extracellular polymeric substances (EPS). The main components of EPS is alginate.[25] Mannitol dehydrogenase (MDH) is a key enzyme in alginate synthesis process of *P. aeruginosa*.[26] In addition, large amount of extracellular DNA (eDNA) is found in the biofilm,[27] it not only affects the formation of biofilms, but also increases the resistance of biofilms by chelating cation.[28] It is proved that eDNA enzymes can clear early and immature biofilm in vitro,[29] and biofilm formation can be regulated by MDH and eDNA.[30]

In order to discuss the mechanism of DPO derivatives inhibiting bacterial biofilm, MDH or eDNA in biofilm were measured, respectively, by a decrease in the absorbance of reactive mixture at 340 nm and biofilm lysate at 260 nm compared to untreated controls according to the references.[31,32] ΔA_{340} and ΔA_{260} increased significantly ($p < 0.01$) after the treatments of the DPO derivatives at concentration dependence as shown in Figure 3. It suggests that they play a role in the inhibition of MDH and eDNA in the biofilms.

Table 3
Binding energy of DPO derivatives with mannitol dehydrogenase and eDNA

DPO derivatives	Mannitol dehydrogenase		eDNA	
	CDOCKER energy (kcal/mol)	CDOCKER interaction energy (kcal/mol)	CDOCKER energy (kcal/mol)	CDOCKER interaction energy (kcal/mol)
1	−22.21 ± 2.40	41.88 ± 3.36	−40.67 ± 0.64	20.09 ± 1.04
4	−23.84 ± 1.96	43.18 ± 2.81	−45.03 ± 1.10	18.82 ± 1.37
5	−19.75 ± 1.96	44.57 ± 3.81	−37.43 ± 1.37	21.94 ± 1.29
10	−28.18 ± 0.94	38.60 ± 1.12	−46.39 ± 0.55	18.99 ± 1.54
11	−15.27 ± 0.71	45.47 ± 1.93	−37.62 ± 1.12	20.87 ± 0.68

Data were presented as mean ± standard deviation (n = 10 poses).

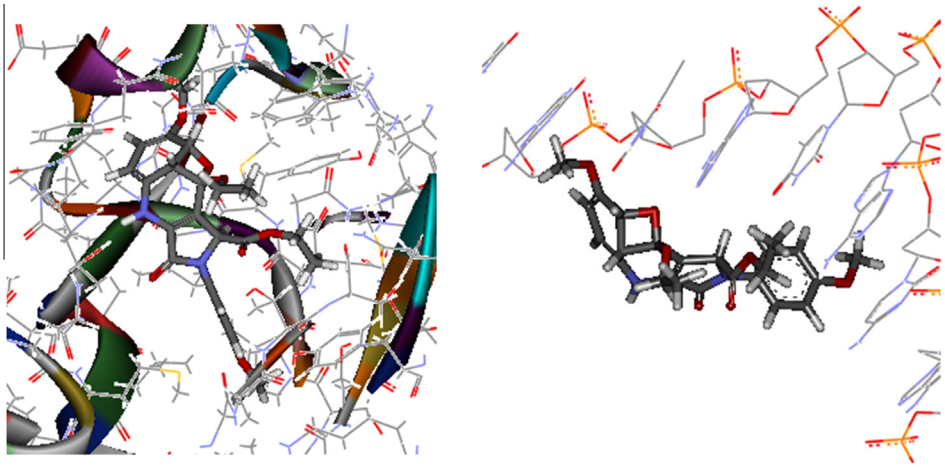


Figure 4. Diagram of DPO derivative binding with mannitol dehydrogenase (a) and eDNA (b).

Simulation of molecular docking provides a simple way to research the interaction between DPO derivatives and MDH or eDNA. Based on the lock-key principle and complementary structural hypothesis, molecular docking simulates mutual interaction between ligand and receptor.[33] If receptor and ligand can interact, they must approach each other, and then combines in a particular conformation of the binding site, finally reach stable complex by adjusting conformation. It is helpful to drug design through adding or removing some electron withdrawing or donating groups to obtain the binding substance in the lowest energy according to principle of energy complementation. Correct affinity prediction is conducive to drug design and screening. The semi-flexible docking applied in this research is suitable for docking of macromolecule receptor and small molecule ligand. The main factors affecting the binding stability of ligand and receptor are hydrophobic force and bonding force. Free energy value is an important parameter for evaluation of docking affinity, binding activity and stability of receptor and ligand, and it can be used to judge the interaction of ligand and receptor. The simulation shows that only five (**1**, **4**, **5**, **10** and **11**) among the 19 DPO derivatives can bind to MDH and eDNA. Each molecule successfully docks in 10 poses. The binding energy is listed in Table 3. They all have positive CDOCKER interactive energy and negative CDOCKER energy, suggesting that they can spontaneously bind to MDH and eDNA, and interact with them. Compound No.10 has the highest CDOCKER energy on both MDH and eDNA suggesting that the strong molecular interaction contributes to its anti-biofilm activity.

DPO derivatives (**1**, **4**, **5**, **10** and **11**) have characteristic structure of amine connected, respectively, with benzyloxy, phenethyl, phenyl ethoxy, *m*-chlorophenyl and *p*-bromophenyl group. Some groups such as benzyl, *p*-chlorophenyl, *p*-fluorophenyl, and 4-fluorobenzene in DPO derivatives have little differences, but cannot act with MDH and eDNA. It suggests that the substituent groups, the size of atoms and the charge all affect the three-dimensional structure of the final product, and then influence its binding force. Mimic diagram of compound 10 binding to MDH and DNA was shown in Figure 4, it illustrates that they can well bind with each other by hydrogen bonds.

The docking simulation shows a good correlation between interaction and anti-biofilm effect. It suggests that molecular docking can be used to predict the drug's effect on biofilm inhibition. The interaction between DPO derivatives and MDH and eDNA can be used to speculate the biofilm inhibition.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.12.010.

References and notes

1. Khan, A. T.; Ghosh, A.; Musawwer Khan, M. *Tetrahedron Lett.* **2012**, *53*, 2622.
2. Merino, P.; Castillo, E.; Franco, S. *Tetrahedron: Asymmetry* **1998**, *9*, 1759.
3. Palacios, F.; Vicario, J.; Aparicio, D. *Eur. J. Org. Chem.* **2006**, *2006*, 2843.
4. Peifer, C.; Selig, R.; Kinkel, K. *J. Med. Chem.* **2008**, *51*, 3814.
5. Yavari, I.; Mokhtarporyani-Sanandaj, A.; Moradi, L. *Tetrahedron* **2008**, *64*, 5221.
6. Demir, A. S.; Emrullahoglu, M.; Ardahan, G. *Tetrahedron* **2007**, *63*, 461.
7. Jourdan, F.; Kaiser, J. T.; Lowe, D. J. J. *Synth. Commun.* **2005**, *35*, 2453.

8. Yavari, I.; Moradi, L.; Nasiri, F. J. *Monatsh. Chem.* **2005**, *136*, 1757.
9. Alves, J. C. F. *J. Braz. Chem. Soc.* **2007**, *18*, 855.
10. Sun, J.; Wu, Q.; Xia, E. Y. *Eur. J. Org. Chem.* **2011**, *2011*, 2981.
11. Tekkam, S.; Alam, M. A.; Jonnalagadda, S. C. *Chem. Commun.* **2011**, 3219.
12. Quartin, A. A.; Scerpella, E. G.; Puttagunta, S.; Kett, D. H. *BMC Infect. Dis.* **2013**, *13*, 561.
13. Blackledge, M. S.; Worthington, R. J.; Melander, C. *Curr. Opin. Pharmacol.* **2013**, *13*, 699.
14. Luo, Y. T.; Lu, X. X.; Ye, Y.; Guo, Y.; Jiang, H. F.; Zeng, W. *Org. Lett.* **2012**, *14*, 5640.
15. Ma, G. N.; Zhang, T.; Shi, M. *Org. Lett.* **2009**, *11*, 875.
16. Qian, B.; Guo, S.; Shao, J. *J. Am. Chem. Soc.* **2010**, *132*, 3650.
17. Borrione, E.; Prato, M.; Scorrano, G. *J. Heterocycl. Chem.* **1988**, *25*, 1831.
18. El-Fatah, M. E. A.; Soliman, S. A.; Farahat, S. M. *Indian J. Heterocycl. Chem.* **2005**, *14*, 225.
19. Bashiardes, G.; Safir, I.; Barbot, F. *Tetrahedron Lett.* **2003**, *44*, 8417.
20. Kawasuji, T.; Fuji, M.; Yoshinaga, T.; Sato, A.; Fujiwara, T.; Kiyama, R. *Bioorg. Med. Chem.* **2007**, *15*, 5487.
21. Robert, W. H.; Justin, J. R. *J. Am. Chem. Soc.* **2007**, *129*, 6966.
22. Li, Y.; Xie, Y. Y.; Chen, R. X.; Xu, H. Z.; Zhang, G. J.; Li, J. Z.; Li, X. M. *Biomed. Environ. Sci.* **2009**, *22*, 170.
23. Zhang, S. Q.; Lin, H.; Miao, Q.; Liu, W.; Cao, Y. B.; Jiang, Y. Y. *Chin. J. Mycol.* **2012**, *7*, 4.
24. Jensen, P. Ø.; Bjarnsholt, T.; Phipps, R.; Rasmussen, T. B.; Calum, H.; Christoffersen, L.; Moser, C.; Williams, P.; Pressler, T.; Givskov, M.; Høiby, N. *J. Microbiol.* **2007**, *53*, 1329.
25. Orgad, O.; Oren, Y.; Walker, S. L.; Herzberg, M. *Biofouling* **2011**, *27*, 787.
26. Banerjee, P. C.; Vanags, R. I.; Chakrabarty, A. M. *J. Bacteriol.* **1983**, *155*, 238.
27. Nur, A.; Hirota, K.; Yumoto, H. *J. Appl. Microbiol.* **2013**, *115*, 260.
28. Mulcahy, H.; Charron, M. L.; Lewenza, S. *PLoS Pathog.* **2008**, *4*, 1213.
29. Ou, Y. Z.; Zhu, Y. L.; Chen, J. M. *Fudan Univ. J. Med. Sci.* **2006**, *33*, 569.
30. Donlan, R. M.; Costerton, J. W. *Clin. Microbiol. Rev.* **2002**, *15*, 167.
31. Ortiz, M. E.; Fornaguera, M. J.; Raya, R. R.; Mozzi, F. *Appl. Microbiol. Biotechnol.* **2012**, *95*, 991.
32. Wu, J.; Xi, C. *Appl. Environ. Microbiol.* **2009**, *75*, 5390.
33. Duan, A. X.; Chen, J.; Liu, H. D. J. *Anal. Sci.* **2009**, *25*, 473.