

Degradation of Atrazine and Phenol by a Bacterial Consortium and Its Use in Bioremediation of Atrazine-Contaminated Soil

Jincheng Yao, Ying Li*, Huizhuan Wang, Defu Chen, Shumin Niu, Baoli Cai

College of Life Sciences, Nankai University, Tianjin, China

Email: caibaoli@nankai.edu.cn

Abstract: Herbicide atrazine is an endocrine disrupting chemical. It has been used widely over the last 50 years. This has resulted in the contamination of surface water, groundwater and soil and threatened to human health. For the bioremediation of atrazine-contaminated environments, we isolated a bacterial consortium that can degrade atrazine and phenol from a co-culture of *Pseudomonas* sp. ADP and mixed atrazine-degrading bacteria. The bacterial consortium consisted of two members of *Arthrobacter* sp. AD25 from the mixed bacteria and *Pseudomonas* sp. ADP-V which is a mutant strain of ADP. AD25 contains *trzN*-, *atzB* and *atzC* genes and can degrade atrazine to cyanuric acid. ADP-V contains *atzDEF* genes and can degrade cyanuric acid to CO₂ and NH₃. The biodegradation experiments indicated that after the minimal medium containing 500 mg/L atrazine as nitrogen source and 3000 mg/L sucrose as carbon was inoculated with the consortium and incubated at 30°C with shaking for 72 hours, atrazine was removed by 96%. After the minimal medium containing 500 mg/L atrazine as nitrogen source and 800 mg/L phenol as carbon was inoculated with the consortium and incubated at 30°C with shaking for 6 d, atrazine and phenol were removed by 80% and 98%, respectively. A bioremediation trial of atrazine-contaminated soil with phosphate biostimulation has indicated that the consortium can degrade as high as 99% of atrazine contained in soil (100 mg/kg) after incubating for 15 d at 30°C. These results proved that the bacterial consortium as a good candidate for use in bioremediation of atrazine and phenol-contaminated wastewater and soil.

Keywords: bacterial consortium; atrazine; phenol; biodegradation; bioremediation

一个细菌聚生体的阿特拉津和苯酚降解特性及其在阿特拉津污染土壤生物修复中的应用

姚金城, 李 颖*, 王绘砖, 陈德富, 牛淑敏, 蔡宝立

南开大学生命科学学院, 天津, 中国, 300071

Email: caibaoli@nankai.edu.cn

摘 要: 除草剂阿特拉津已被确认是一种内分泌干扰剂。这种除草剂已在世界范围内使用 50 多年, 造成对表面水、地下水和土壤的污染, 因此对人类健康构成威胁。为了对阿特拉津污染环境进行生物修复, 我们从一个降解阿特拉津的混合菌群和 *Pseudomonas* sp. ADP 的共培养物中分离到一个高效降解阿特拉津和苯酚的细菌聚生体 (consortium)。聚生体由两个菌株组成: 一个是来自混合菌群的 *Arthrobacter* sp. AD25, 它含有 *trzN*、*atzB* 和 *atzC* 基因, 能将阿特拉津降解成氰尿酸; 另一个是 *Pseudomonas* sp. ADP-V, 它是 ADP 的突变菌株, 含有 *atzDEF* 基因, 能将氰尿酸降解成 CO₂ 和 NH₃。在以阿特拉津 (500 mg/L) 为氮源、蔗糖 (3000 mg/L) 为碳源的液体培养基中接种细菌聚生体, 30°C 震荡培养 72 h 以后, 阿特拉津降解率为 96%。在以阿特拉津 (500 mg/L) 为氮源、苯酚 (800 mg/L) 为碳源的液体培养基中接种细菌聚生体, 30°C 震荡培养 6 d 以后, 阿特拉津降解率为 80%, 苯酚降解率为 98%。在阿特拉津含量为 100 mg/kg 并补充适量磷酸盐的土壤中接种细菌聚生体, 于 30°C 培养 15 d 以后, 阿特拉津去除率为 99%。上述结果表明, 该细菌聚生体在阿特拉津、苯酚污染土壤和水的生物修复中有很好的应用潜力。

关键词: 细菌聚生体; 阿特拉津; 苯酚; 生物降解; 生物修复

1 引言

阿特拉津 (Atrazine) 是一种三嗪类除草剂, 主要用于玉米、高粱和甘蔗地的杂草防除。由于阿特拉津已经在世界范围内使用50多年, 所以导致对环境的污染, 在污染严重的地区, 土壤、地表水和地下水中阿特拉津的浓度分别达到1100 $\mu\text{g/g}$ 、16 $\mu\text{g/L}$ 和1500 $\mu\text{g/L}$ ^[1]。阿特拉津已经被确认是一种内分泌干扰剂, 它能干扰激素的调节功能, 诱导肿瘤发生, 所以对其污染的环境进行生物修复引起广泛重视^[2,3]。自1985年以来, 研究人员用富集培养法从不同的污染位点分离和鉴定了大量来自不同属的阿特拉津降解细菌, 例如 *Acinetobacter*、*Agrobacterium*、*Alcaligenes*、*Aminobacter*、*Arthrobacter*、*Chelatobacter*、*Nocardia*、*Nocardioides*、*Polaromonas*、*Pseudaminobacter*、*Pseudomonas*、*Ralstonia*、*Rhizobium*、*Rhodococcus*、*Sinorhizobium* 和 *Stenotrophomonas* 等^[4-7]。其中研究最深入的是 *Pseudomonas* sp. ADP 和 *Arthrobacter aureus* TC1, 前者含有 *atzABCDEF* 6个降解基因, 能将阿特拉津彻底降解成 CO_2 和 NH_3 ^[8], 后者含有 *trzN-atzBC* 3个降解基因, 能将阿特拉津降解成氰尿酸^[9]。此外, 还发现由几种或多种细菌组成的聚生体 (consortium) 和群落 (community) 具有更好的阿特拉津降解能力^[10,11]。近年来, 用阿特拉津降解细菌修复废水和污染土壤取得良好效果^[12-14]。

我们将阿特拉津降解速度快但降解不完全的混合菌群 (将阿特拉津降解成氰尿酸) 和降解完全但降解速度慢的 *Pseudomonas* sp. ADP 进行长期驯化培养, 从中筛选到一个阿特拉津降解速度快和降解完全的细菌聚生体, 而且该聚生体还能降解苯酚。聚生体由两个菌株组成, 一个是来自混合菌群的 *Arthrobacter* sp. AD25, 它含有 *trzN*、*atzB* 和 *atzC* 基因, 能将阿特拉津降解成氰尿酸; 另一个是 *Pseudomonas* sp. ADP-V, 它是 ADP 的突变菌株, 含有 *atzDEF* 基因, 能将氰尿酸降解成 CO_2 和 NH_3 ^[15]。本文报道该聚生体的阿特拉津和苯酚降解特性以及用于阿特拉津污染土壤的生物修复效果。

资助信息: 中国国家自然科学基金项目 (40971183)
现在工作单位: 北京市农业部畜禽产品质量安全检验检测中心

2 材料和方法

2.1 菌株、培养基和生长条件

Pseudomonas sp. ADP 和 *Arthrobacter aureus* TC1 为 LP Wackett 教授惠赠, 降解阿特拉津的混合细菌和聚生体为本实验室培育和分离。阿特拉津无机盐培养基 (g/L): 0.9 g KH_2PO_4 , 6.5 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mL 微量元素溶液^[16], 3 g 蔗糖, 3 g 柠檬酸钠, 高压灭菌前加入阿特拉津甲醇悬液 (50 mg/mL) 至终浓度为 500 mg/L。阿特拉津-苯酚无机盐培养基: 用终浓度为 800 mg/L 的苯酚代替蔗糖, 其它成分与阿特拉津无机盐培养基相同。苯酚无机盐培养基: 用终浓度为 400 mg/L 的 $(\text{NH}_4)_2\text{SO}_4$ 0.4 代替阿特拉津, 其它成分与阿特拉津-苯酚无机盐培养基相同。上述 3 个菌株均在转速为 150 r/min 的摇床中于 30℃ 培养。

2.2 污染土壤的生物修复

离心收集来自 20 mL 菌液的细胞, 先后用 0.9% 的 NaCl 溶液和无菌水洗细胞, 并悬浮于含有 3 g/L 蔗糖和 0.3 g/L 磷酸盐 (0.2 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.1 g KH_2PO_4) 的溶液中, 使细胞浓度为 10^8 个/mL。在 9 cm 直径的无菌培养皿中加入 10 g 灭菌土和 1 mg 阿特拉津 (50 mg/mL 甲醇溶液), 65℃ 加热 30 min 使甲醇挥发, 然后加入 1 mL 菌悬液, 30℃ 保温 15d。每天定量喷洒少量无菌水, 使土壤保持湿润。分别在实验的 0、1、2、3、5、10 和 15 d 从每个试验组中取出 3 个培养皿, 储存于 -20℃^[7]。

2.3 阿特拉津测定

用二氯甲烷萃取含有阿特拉津的水样或土样, 再用 0.22 μm 的滤膜过滤, 然后用美国 CoMatro-6000 HPLC 检测。色谱柱 250 mm $\times$ 4.6 mm $\times$ 5 μm 。检测条件: 乙腈和水为流动相, 乙腈所占比例随时间变化: 0-6 min 10%-25%, 6-21 min 25%-65%, 21-23 min 65%-100%, 25 min 100%; 流速 1.0 mL/min, 检测器温度 35℃, 检测波长 220 nm^[6]。

2.4 氰尿酸测定

用 HPLC 测定氰尿酸含量^[17]。将细菌培养液 10000r/min 离心 15 min, 上清液用 0.22 μm 的滤膜过滤, 取 10 μL 样品经 Waters 600E 高效液相色谱仪的自

动进样器进样,通过 996 Photodiode Array 检测器检测特征峰的保留时间和峰面积,然后计算样品中氰尿酸的含量。色谱操作条件:色谱柱为 150 mm × 3.9 mm Symmetry RP18 (5 μm),流动相甲醇:水 (5 mmol/L KH₂PO₄+H₃PO₄缓冲液, pH3) 为 10:90,柱温为室温,检测波长 200 nm,流速 0.7 mL/min。

2.5 苯酚测定

用标准的 4-氨基安替比林法测定细菌培养液中的苯酚浓度^[18]。

3 结果与讨论

3.1 细菌聚生体的阿特拉津降解特性

根据降解基因组成分析,聚生体中的 *Arthrobacter* sp. AD25 含有 *trzN*、*atzB* 和 *atzC* 基因,能将阿特拉津降解成氰尿酸, *Pseudomonas* sp. ADP-V 含有 *atzDEF* 基因,能将 AD25 的降解产物氰尿酸进一步降解成 CO₂ 和 NH₃^[15],所以最终结果是阿特拉津被完全矿化。降解实验表明,聚生体的阿特拉津降解速度和降解率明显好于 ADP,在含有 500 mg/L 阿特拉津的培养基中培养 24 h 以后,能将阿特拉津去除 85%,相同条件下 ADP 的阿特拉津去除率只有 25%。培养 72 h 以后,聚生体的阿特拉津降解率为 96%,ADP 的阿特拉津降解率仅为 86% (图 1)。图 2 的结果表明,聚生体对阿特拉津降解的彻底性接近于 ADP。聚生体在含有 500 mg/L 阿特拉津的培养基中培养 72 h 后氰尿酸的浓度为 55 μg/mL,明显低于混合菌群培养液的氰尿酸浓度 (282 μg·mL⁻¹),但高于 ADP 培养液的氰尿酸浓度 (数值为零),这表明聚生体不仅能将阿特拉津降解成氰尿酸,而且能使氰尿酸进一步降解,但培养液中有少量氰尿酸积累。出现这种情况的原因是 AD25 降解阿特拉津产生氰尿酸的速度快于 ADP-V 的氰尿酸降解速度。

3.2 细菌聚生体的苯酚降解特性

虽然 *Pseudomonas* sp. ADP 菌株能够降解阿特拉津和苯酚,以阿特拉津为氮源、苯酚为碳源生长^[19],但聚生体的苯酚降解能力以及同时降解阿特拉津和苯酚的能力均好于 ADP。在以苯酚为碳源、硫酸铵为氮源生长时,聚生体对苯酚的降解要比 ADP 菌株快,2 d 就可以将 800 mg/L 的苯酚降解 99% 以上,而 ADP 在相同条件下只能使苯酚降解 60%,4 d 后才能

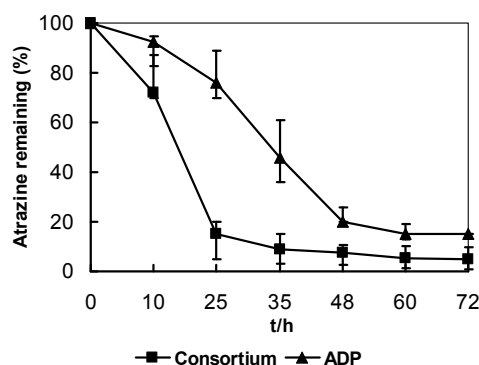


Figure 1. Atrazine degradation curves of bacterial consortium and *Pseudomonas* sp. ADP

图 1. 细菌聚生体和 ADP 菌株的阿特拉津降解曲线

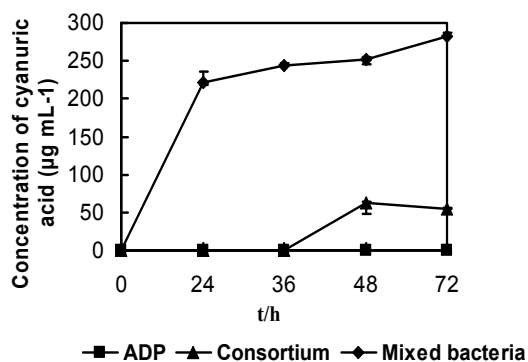


Figure 2. Cyanuric acid accumulation of mixed bacteria, consortium and ADP in atrazine minimal medium

图 2. 混合细菌、聚生体和 ADP 在阿特拉津培养基中的氰尿酸积累

完全降解 (图 3)。在以阿特拉津为氮源、苯酚为碳源的培养基中,聚生体对阿特拉津的降解效果也好于 ADP,培养 6 d 以后,聚生体可将阿特拉津降解 80%,而 ADP 菌株只能将阿特拉津降解 60%。两者对苯酚的降解差别不大 (图 4)。

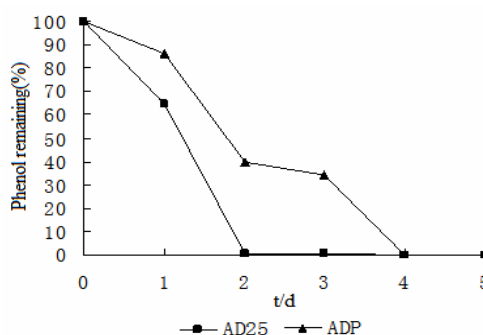
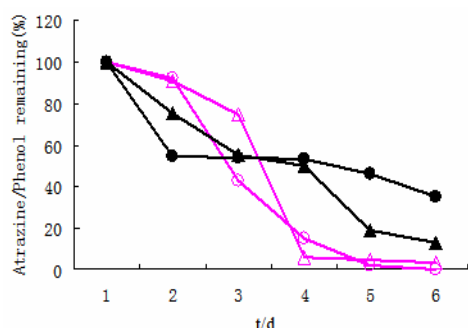


Figure 3. Degradation of phenol by bacterial consortium and ADP

图 3. 细菌聚生体和 ADP 对苯酚的降解



▲ : consortium-atrazine; △: consortium-phenol;
●: ADP-atrazine; ○: ADP-phenol

Figure 4. Degradation of atrazine and phenol by bacterial consortium and ADP

图 4. 细菌聚生体和 ADP 对阿特拉津及苯酚的降解

3.3 阿特拉津污染土壤的生物修复

Silva 等^[14]证明,在用 ADP 菌株进行污染土壤的生物修复时,加入适量的碳源和磷酸盐能明显提高阿特拉津的降解率。本实验为了提高细菌对土壤中阿特拉津的降解效果,将细菌细胞悬浮于蔗糖 (3 g/L) 和磷酸盐 (0.3 g/L) 溶液中,然后加到土壤中。30℃保温 15 d 以后,接种聚生体的土壤中阿特拉津浓度由 100 mg/kg 降低到 1.1 mg/kg,去除率为 99%。在未接种降解菌的土壤中 (CK),阿特拉津浓度由 100 mg/kg 降到 82 mg/kg,降解率为 18%,这是由化学降解作用引起的 (图 5)。上述结果表明,细菌聚生体在阿特拉津污染土壤的生物修复方面有很好的应用潜力。

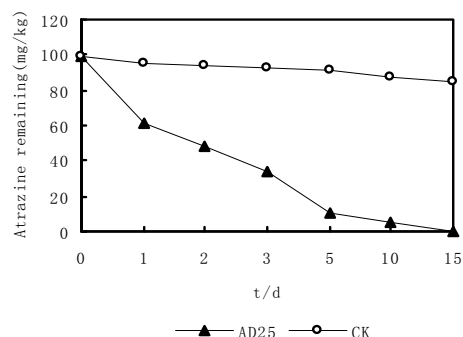


Figure 5. Degradation of atrazine in contaminated soil by bacterial consortium

图 5. 细菌聚生体对土壤中的阿特拉津的降解

4 结论

(1)由 *Arthrobacter* sp. AD25 和 *Pseudomonas* sp. ADP-V 组成的细菌聚生体能同时高效降解除草剂阿

特拉津和苯酚。

(2) 细菌聚生体在 30℃条件下 15 d 内将土壤中 100 mg/kg 的阿特拉津去除 99%, 表明它在阿特拉津污染土壤的生物修复中有很好的应用潜力。

References (参考文献)

- [1] Struthers JK, Jayachandran K, Moorman TB. Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. *Appl Environ Microbiol*, 1998, 64: 3368-3375.
- [2] Singh P, Suri CR, Cameotra SS. Isolation of a member of *Acinetobacter* species involved in atrazine degradation. *Biochem Biophys Res Commun*, 2004, 317: 697-702.
- [3] Gammon DW, Aldous CN, Carr Jr WC, Sanborn JR, Pfeifer K. A risk assessment of atrazine use in California: human health and ecological aspects. *Pest Management Science*, 2005, 61: 331-355.
- [4] Wackett LP, Sadowsky MJ, Martinez B, Shapir N. Biodegradation of atrazine and related *s*-triazine compounds: from enzymes to field studies. *Appl Microbiol Biotechnol*, 2002, 58: 39-45.
- [5] Cai BL, Han YN, Liu B, Ren Y, Jiang S. Isolation and characterization of an atrazine-degrading bacterium from industrial wastewater in China. *Lett Appl Microbiol*, 2003, 36: 272-276.
- [6] Devers M, Azhari NE, Kolic NU, Martin-Laurent F. Detection and organization of atrazine-degrading genetic potential of seventeen bacterial isolates belonging to divergent taxa indicate a recent common origin of their catabolic functions. *FEMS Microbiol Lett*, 2007, 273: 78-86.
- [7] Li QY, Li Y, Zhu XK, Cai BL. Isolation and characterization of atrazine-degrading *Arthrobacter* sp. AD26 and use of this strain in bioremediation of contaminated soil. *J Environ Sci*, 2008, 20(10): 1226-1230.
- [8] Martinez B, Tomkins J, Wackett LP, Wing R, Sadowsky MJ. Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. *J Bacteriol*, 2001, 183: 5684-5697.
- [9] Sajjaphan K, Shapir N, Wackett LP, Palmer M, Blackmon B, Tomkins J, Sadowsky MJ. *Arthrobacter aurescens* TC1 atrazine catabolism genes *atrZ*, *atrB*, and *atrC* are linked on a 160-kilobase region and are functional in *Escherichia coli*. *Appl Environ Microbiol*, 2004, 70: 4402-4407.
- [10] Yang C, Li Y, Zhang K, Wang X, Ma C, Tang H, Xu P. Atrazine degradation by a simple consortium of *Klebsiella* sp. A1 and *Comamonas* sp. A2 in nitrogen enriched medium. *Biodegradation*, 2010, 21: 97-105.
- [11] Kolic NU, Hrsak D, Kolara AB, Petric I, Stipicevic S, Soulas G, Martin-Laurent F. Combined metabolic activity within an atrazine-mineralizing community enriched from agrochemical factory soil. *Int Biodeter Biodegr*, 2007, 60: 299-307.
- [12] Shapir N, Mandelbaum RT, Gottlieb H. Atrazine degradation in saline wastewater by *Pseudomonas* sp. strain ADP. *J Ind Microbiol Biotechnol*, 1998, 20: 53-159.
- [13] Newcombe DA, Crowley DE. Bioremediation of atrazine-contaminated soil by repeated applications of atrazine-degrading bacteria. *Appl Microbiol Biotechnol*, 1999, 51: 877-882.
- [14] Silva E, Fialho AM, Sa-Correia, Burns RG, Shaw LJ. Combined bioaugmentation and biostimulation to cleanup soil contaminated with high concentrations of atrazine. *Environ Sci Technol*, 2004, 38: 632-6379.
- [15] Li Y, Li QY, Zhu XK, Cai BL. Isolation and identification of herbicide atrazine-degrading bacterial consortium. *Acta Scientiarum Naturalium Universitatis Nankaiensis*, 2007, 40(6): 56-61. (Ch)

李颖, 李清艳, 朱希坤, 蔡宝立. 降解除草剂阿特拉津的细

- 菌聚生体的分离和鉴定. 南开大学学报(自然科学版), 2007, 40(6):56-61.
- [16] Scholtz R, Wackett LP, Egli C, Cook AM, Leisinger T. Dichloromethane dehalogenase with improved catalytic activity isolated from a fast-growing dichloromethane-utilizing bacterium. *J Bacteriol*, 1988, 170: 5698-5704.
- [17] Cantu R, Evans O, Kawahara FK, Wymer LJ, Dufour AP. HPLC determination of cyanuric acid in swimming pool waters using phenyl and confirmatory porous graphitic carbon columns. *Anal. Chem*, 2001, 73: 3358-3364.
- [18] Arutchelvan V, Kanakasabai V, Nagarajan S, Muralikrishnan V. Isolation and identification of novel high strength phenol degrading bacterial strains from phenol-formaldehyde resin manufacturing industrial wastewater. *J Hazard Mater*, 2005, B127: 238-243.
- [19] Neumann G, Teras R, Monson L, Kivisaar M, Schauer F, Heipieper HJ. Simultaneous degradation of atrazine and phenol by *Pseudomonas* sp. strain ADP: effects of toxicity and adaptation. *Appl Environ Microbiol*, 2004, 70: 1907-1912.