专论与综述

二硝基苯胺类除草剂微生物降解研究进展

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摘　要：二硝基苯胺类除草剂是一类广谱、高效且广泛使用的除草剂，微生物的降解代谢作用是其在环境中消解的最主要因素。分离筛选除草剂的高效降解菌株、分析其降解途径并阐明其微生物降解机制，可为除草剂残留污染的微生物降解修复提供理论依据和优良的降解菌株、降解基因和酶资源。本文简述了二硝基苯胺类除草剂的微生物降解菌株、降解代谢途径和降解基因/酶的研究进展，为进一步研究该类除草剂的微生物降解及其污染生物修复提供理论依据和资源。

关键词：二硝基苯胺类除草剂，微生物降解，降解资源，生物修复

Research progress on microbial degradation of dinitroaniline herbicides

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Abstract: Dinitroaniline herbicides are a class of highly efficient pre-emergence herbicides, with broad spectrum and wide application. Microbial catabolism plays the most important role in the dissipation of the dinitroanilines in the environment. Isolating highly efficient herbicides-degrading microorganisms, studying the metabolic pathways, and elucidating the degradation mechanisms will provide the theoretical basis and degrading resources for the study on the transformation mechanism and ecological security of the herbicides in the environment, and for the microbial remediation of the herbicides residual contamination in the environment. This paper summarizes the research progress on microbial degradation of dinitroaniline herbicides, including degrading bacteria, metabolic pathways, and the genes/enzymes involved in the degrading progress. The aim of this review is to provide theoretical basis and resources for the bioremediation of dinitroaniline herbicides residual contamination in environment.

Keywords: Dinitroaniline herbicides, Microbial degradation, Degradation resources, Bioremediation

我国是农业生产和农产品出口贸易大国，农业的可持续发展关乎国民经济的健康、持续发展。在保证粮食增产的前提下，除草剂的使用量也随着大幅度增高。二硝基苯胺类除草剂主要包括氟乐灵、二甲戊灵、仲丁灵、安磺灵(图 1)等，是一类广谱、高效的选择性芽前土壤处理剂，具有高效低毒、除草谱广和持效期长等优点，在化学除草应用中占很大比重[1]。该类除草剂被广泛应用于棉花、花生、大豆、小麦、水稻、玉米等多种作物农田中，防除一年生禾本科杂草和部分一年生阔叶杂草[2-3]，其除草机理是通过抑制细胞的有丝分裂与分化，破坏细胞的正常分裂，从而造成杂草的死亡[4]。二硝基苯胺类除草剂的纯品多为橘黄色晶体，结构通式为2,6-二硝基苯胺，在我国使用的代表产品是二甲戊灵、氟乐灵和仲丁灵。

二硝基苯胺类除草剂化学性质稳定，残留期长[5-8]，易挥发，易进入环境中，已成为一类环境污染物[9-12]，对生态环境和人类健康造成严重威胁。二硝基苯胺类除草剂对水生生物和无脊椎动物高毒[5,13-19]，因含二硝基苯胺结构可形成致癌物质——亚硝胺，对人体健康也有潜在危害[20-21]。此外，氟乐灵对某些后茬作物存在危害[17]，造成内分泌紊乱[17]；对哺乳动物细胞有基因毒性，其致癌性被认定为 C 类，即可疑的人类致癌剂[19,22-25]。美国国家环境保护局 (US Environmental Protection Agency，USEPA)已将二甲戊灵和氟乐灵归类为持久性生物积聚有毒物质[12]。欧洲一些国家已对氟乐灵采取禁用或限用的措施[22,26-29]，但由于性价比较高，它们在中国仍是首选的除草剂。因此，二硝基苯胺类除草剂在生态环境中迁移、转化、降解以及生态安全性评估已引起了广泛关注。

二硝基苯胺类除草剂在环境中的消解方式主要有挥发、光解和微生物降解[30-32]，大量研究表明微生物的降解代谢在其中起主要作用[33-36]。本文简述目前国内外关于二硝基苯胺类除草剂微生物降解菌株、降解代谢途径以及降解基因和酶方面的研究进展，旨在为二硝基苯胺类除草剂残留的微生物降解修复提供理论依据和优良的降解菌株、基因及酶资源。

1 二硝基苯胺类除草剂微生物降解菌株

大量研究表明微生物的降解代谢作用是环境中二硝基苯胺除草剂消解的主要因素，目前国内外已筛选到很多能降解该类除草剂的纯品系菌株，这些降解菌株涉及真菌、细菌和放线菌，种属分布非常广泛，具有丰富的多样性(表 1)。目前分离筛选到的二硝基苯胺除草剂微生物降解菌株绝大多数是降解二甲戊灵和氟乐灵的菌株，而已报道的仲丁灵和安磺灵的纯品系降解菌株非常少；具有降解活性的细菌主要来源于芽孢杆菌属(*Bacillus* sp.)和假单胞菌属(*Pseudomonas* sp.)，具有降解活性的真菌主要是曲霉属(*Aspergillus* sp.)菌株和尖孢镰刀菌(*Fusarium oxysporum*)，具有降解活性的放线菌目前只有二甲戊灵降解菌株 *Actinomycetes* sp. 42。
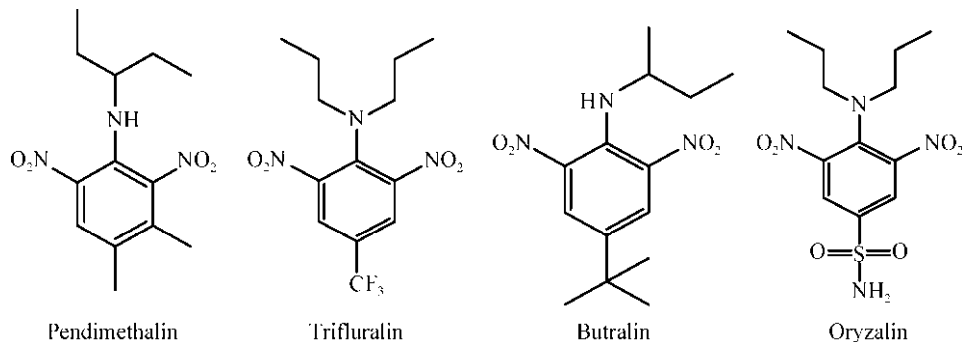

图 1 二硝基苯胺类除草剂主要品种的化学结构式
Figure 1 Chemical structures of the major products of dinitroaniline herbicides

表 1 已报道的二硝基苯胺类除草剂降解菌株
Table 1 The reported dinitroaniline herbicides-degrading microorganisms

降解菌株 Degrading strains		降解底物 Degrading substrates	来源 References
细菌 Bacteria	*Pseudomonas* sp.	Trifluralin	[37-38]
	Bacteroides ruminicola subsp. *brevis* GA-33	Trifluralin	[39]
	Azotobacter vinelandii	Pendimethalin	[40]
	Alcaligenes sp. T-a *Moraxella* sp. T-b	Trifluralin	[41]
	Azotobacter chroococcum	Pendimethalin	[42]
	Bacillus megaterium	Pendimethalin	[43]
	Pseudomonas sp.		
	Rhizoctinia sp.		
	Rhizoctinia bataticola		
	Pyricularia oryzae Cav		
	Herbaspirillum sp. *Bacillus* sp. *Klebsiella* sp.	Trifluralin	[44]
	Bacillus sp. HB-7	Pendimethalin	[45]
	Bacillus circulans	Pendimethalin	[46]
	Pseudomonas aeruginosa	Pendimethalin	[47]
	Bacillus mycoides		
	Bacillus cereus		
	Bacillus megaterium E22	Pendimethalin	[48]
	Pseudomonas putida E15	Pendimethalin	[49]
	Bacillus lehensis XJU	Pendimethalin	[50]
	Shewanella marisflavi EP1	Pendimethalin	[51]
	Leucobacter sp. FJ-01	Trifluralin	[52]
	Bacillus subtilis Y3	Pendimethalin, Trifluralin, Butralin, Oryzalin	[53-54]
	Pseudomonas resinovorans E20	Pendimethalin	[55]
	Bacillus simplex *Bacillus muralis* *Micrococcus luteus* *Micrococcus yunnanensis* *Clostridium tetani*	Trifluralin	[34]
	Bacillus safensis FO-36b	Pendimethalin	[56]
	Bacullus subilis subsp. *inaquosorum* KCTC13429		
	Bacillus cereus ATCC14579		
	Bacillus sp. D8	Trifluralin	[57]
	Serratia sp. JY-2	Pendimethalin	[58-59]
	Pseudomonas sp. JY-5		
	Bacillus cereus	Pendimethalin	[60]
	Asaccharospora irregularis		
	Paracoccum sp. P13	Pendimethalin	[61]
放线菌 Actinomycetes	*Actinomycetes* sp. 42	Pendimethalin	[43]

(待续)

(续表 1)

真菌 Fungi	*Aspergillus niger*	Trifluralin	[62]
	Lachnospira multiparus D-32	Trifluralin	[39]
	Paecilomyces sp.	Trifluralin, Butralin	[63-64]
	Candida sp.	Trifluralin	[65]
	Aspergillus carneus	Trifluralin	[66]
	Fusarium oxysporum		
	Trichoderma viride		
	Aspergillus flavus	Pendimethalin	[67]
	Aspergillus terreus		
	Fusarium solani		
	Fusarium oxysporum		
	Penicillium citrinum		
	Penicillium simplicissimum		
	Fusarium oxysporum	Pendimethalin	[68]
	Paecilomyces variotii		
	Rhizoctonia bataticola		
	Aspergillus niger 2012	Pendimethalin	[43]
	Fusarium oxysporum		
	Paecilomyces variotii		
	Aspergillus terreus YF-209	Pendimethalin	[69]
	Monilochaetes sp. YF-212		
	Aspergillus furnigatus YF-216		
	Fusarium oxysporum	Pendimethalin	[70]
	Aspergillus oryzae		
	Lentinula edodes		
	Penicillium brevicompactum		
	Lecanicillium saksenae		
	Phanerochaete chrysosporium	Pendimethalin	[71]
	Aspergillus tubingensis Qsun-6	Pendimethalin	[72]
	Penicillium thrichoderma	Trifluralin	[34]
	Penicillium simplicissimum		
	Penicillium talaromyces		
	Metacordyceps chlamydosporia		
	Stachybotrys chartarum		
	Alternia alternata		
	Lentinula edodes EL1	Pendimethalin	[35]
	Clavispora lusitaniae YC2	Pendimethalin	[73]

在表 1 的二硝基苯胺除草剂降解细菌菌株中，朱鲁生等以二甲戊灵为唯一碳源选择性富集筛选二甲戊灵降解菌株，并在转接培养的同时不断提高培养基中的二甲戊灵浓度加强筛选效果，最终得到一株二甲戊灵降解菌株 HB-7，鉴定为芽孢杆菌属 (*Bacillus* sp.)；不同的培养条件会影响菌株的生长和降解效率，在最适 pH 7.0 和 30 °C 的条件下，再外源添加少量葡萄糖(0.1%)作为碳源可以提高菌株降解二甲戊灵的效率，24 h 内能降解 90.0%以上的 100 mg/L 二甲戊灵[45]。Ni 等从活性污泥中通过选择富集的方法分离筛选到一株二甲戊灵降解菌株——枯草芽孢杆菌(*Bacillus subtilis*) Y3，并通过定时取样的方法对该菌降解二甲戊灵的效果进行监测，该菌能在 60 h 内降解 100 mg/L 二甲戊灵，也能在相同时间内降解 100 mg/L 的仲丁灵和安磺灵以及 60 mg/L 氟乐灵[53-54]。陈贝贝等以氟乐灵为唯一碳

源从连作 15 年以上的棉田土壤中选择分离到一株氟乐灵降解菌株 D8，鉴定为芽孢杆菌(*Bacillus* sp.)，并通过单因素实验对菌株 D8 降解氟乐灵的降解特性进行研究，菌株 D8 在氟乐灵初始浓度为 50 mg/L、接种量 4%、外加氮源(酵母浸粉) 0.1%、pH 5.0、培养温度 37 °C 的条件下，3 d 对氟乐灵的降解率达到 59.9%以上[57]。胡佳月等采用以蛋白胨为氮源、二甲戊灵和少量葡萄糖为碳源的富集培养基，从长期施用二甲戊灵的棉田土壤中富集筛选能够降解二甲戊灵的菌株，并通过不断提高培养基中的二甲戊灵浓度来转接培养进一步选择筛选，最终得到 2 株二甲戊灵降解菌株——嗜线虫沙雷氏菌(*Serratia* sp.) JY-2 和假单胞菌(*Pseudomonas* sp.) JY-5，对这 2 株菌的降解特性进行了研究，结果表明这 2 株菌在最佳条件(即外加碳源量 0.5%、菌株接种量 10%、pH 7.0、培养温度 30 °C、初始二甲戊灵浓度 200 mg/L)下作用 3 d 对二甲戊灵的降解率能分别达到 83.3%和 82.8%[58-59]。Archana 等用以二甲戊灵为唯一碳源的选择培养基从受农药污染的土壤中富集筛选到的 2 株二甲戊灵降解细菌，通过菌株形态、生理生化特征和 16S rRNA 基因序列将这 2 株菌鉴定为蜡样芽孢杆菌(*Bacillus cereus*)和不规则梭菌(*Asaccharospora irregularis*)，这 2 株细菌能在二甲戊灵初始浓度为 500 mg/L 的条件下生长，每隔 7 d 对这 2 株菌降解二甲戊灵的情况进行测定，结果表明这 2 株细菌在 28 d 内分别能降解 408 mg/L 和 422 mg/L 二甲戊灵[60]。Ni 等通过富集培养的方法从果园土壤中分离到的一株二甲戊灵降解菌株——副球菌属(*Pacacoccus* sp.) P13，它能以二甲戊灵为唯一碳源和能源生长，2 d 能降解 100 mg/L 二甲戊灵，5 d 能降解 200 mg/L 二甲戊灵[61]。

在已公开报道的二硝基苯胺类除草剂降解真菌菌株中，Zayed 等测定了埃及土壤中 3 个主要真菌类群肉色曲霉(*Aspergillus carneus*)、尖孢镰刀菌(*Fusarium oxysporum*)和绿色木霉(*Trichoderma virida*)降解转化氟乐灵的能力，并对氟乐灵的转化产物进行了鉴定，这 3 株菌 10 d 内对初始浓度

200 mg/L 氟乐灵的转化率均高于 90.0%，其中绿色木霉(*T. virida*)对氟乐灵的转化率最高，高达 97.0%[66]。林爱军等通过以二甲戊灵为唯一碳源的无机盐培养基对污泥中的微生物进行富集培养，并不断提高二甲戊灵的浓度进行转接培养，最终分离筛选到多株二甲戊灵降解真菌菌株，选取了其中降解效率较高且对二甲戊灵耐受性较强的 3 株真菌——土生曲霉(*Aspergillus terreus*) YF-209、长梗串孢霉属(*Monilochaetes* sp.) YF-212 和烟色曲霉(*Aspergillus furnigatus*) YF-216 进行了降解特性的测定，结果表明不同的培养条件对微生物降解二甲戊灵的效果有较大影响，但这 3 株真菌在外加蔗糖浓度为 0.5%−1.0%、二甲戊灵初始浓度为 100 mg/L、pH 6.0−8.0、温度 20−30 °C 的条件下，菌株的生长量和对二甲戊灵的降解量都能达到最大，这 3 株真菌 YF-209、YF-212 和 YF-216 在 5 d 内对 100 mg/L 二甲戊灵的降解率分别为 66.5%、78.4%和 71.3%[69]。宫宏琨等以加入适量二甲戊灵的察氏培养基为富集培养基，以富集培养基中加入 100 mg/L 二甲戊灵开始富集筛选二甲戊灵降解菌株，并在转接培养过程中逐步提高富集培养基中二甲戊灵的浓度，最终达到 5 000 mg/L，稀释涂布获取单菌落，并将各个不同的单菌落转接到以二甲戊灵为唯一碳源的选择培养基中进一步纯化，同时在转接培养时也不断提高二甲戊灵的浓度，最终结合菌株降解实验得到一株二甲戊灵降解真菌 Qsun-6，被鉴定为塔宾曲霉(*Aspergillus tubingensis*)，该菌株在 5 d 内对 100 mg/L 二甲戊灵的降解率能达到 75.2%，对菌株 Qsun-6 降解二甲戊灵的特性进行研究，发现在外源添加适量速效碳源(如 1%蔗糖)、二甲戊灵浓度为 100 mg/L、pH 6.0 和 30 °C 的条件下，菌株的生长量和对二甲戊灵的降解率都能达到最大[72]。Han 等以二甲戊灵为碳源的 Czapek 培养基为富集培养基，从连续 15 年施用二甲戊灵的棉田土中分离筛选二甲戊灵降解菌株，同样在富集的过程中不断提高培养基中的二甲戊灵浓度，最终得到一株二甲戊灵降解菌株 YC2，被鉴定为葡萄牙棒孢酵母(*Clavispora*

lusitaniae)，该酵母在 5 d 内能降解 60.0%以上的 200 mg/L 二甲戊灵，8 d 的降解率能达到 74.0%[73]。

2 二硝基苯胺类除草剂的微生物降解途径

目前国内外分离到许多株二硝基苯胺类除草剂降解菌株，研究了菌株的降解特性，通过液相色谱、气相色谱、质谱和核磁共振等技术鉴定了降解过程中产生的代谢产物，并根据鉴定到的代谢产物结构分析了微生物降解该类除草剂的代谢途径。研究表明各种微生物菌株降解二甲戊灵、氟乐灵、仲丁灵和安磺灵的方式主要包括硝基还原、氧化 N 端脱烷基、环化生成苯并咪唑、芳甲

基氧化等[39,41-43,46-47,50,53-54,62,65-68]，各个微生物菌株利用二硝基苯胺类除草剂的方式不尽相同，这也造成了二甲戊灵、氟乐灵等除草剂的代谢产物多种多样。纵观现有公开发表的研究成果，二硝基苯胺除草剂的微生物降解途径研究往往停滞于降解过程的前 3 步(即降解的上游代谢途径)，而完整的矿化途径尚无研究报道。

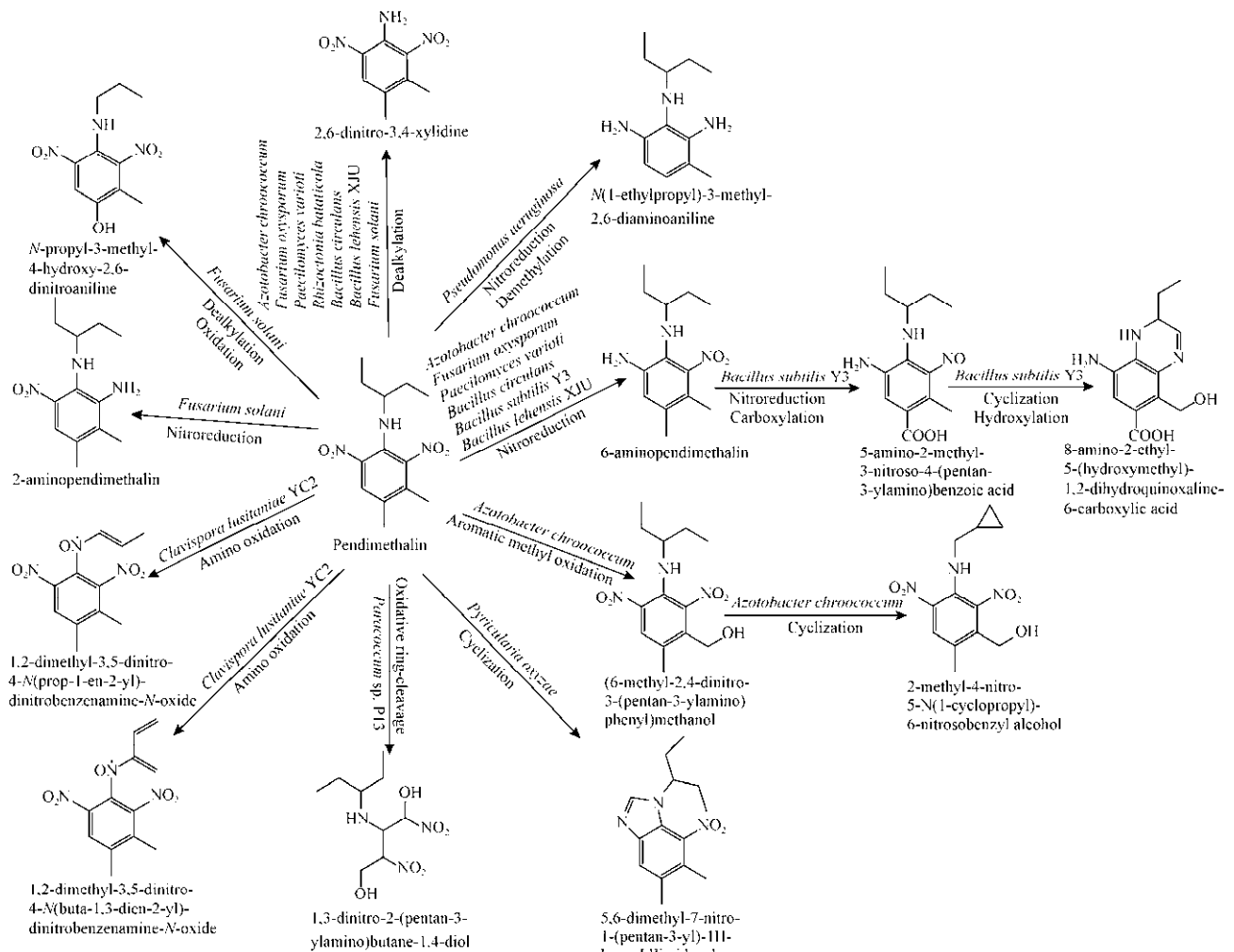已公开报道的微生物降解二甲戊灵、氟乐灵、仲丁灵和安磺灵的部分代谢途径分别见图 2−5。其中，*B. subtilis* Y3 能以硝基还原的方式起始降解二甲戊灵生成 6-氨基二甲戊灵[*N*-(1-乙基丙基)-2-硝

图 2 推测的二甲戊灵微生物降解途径
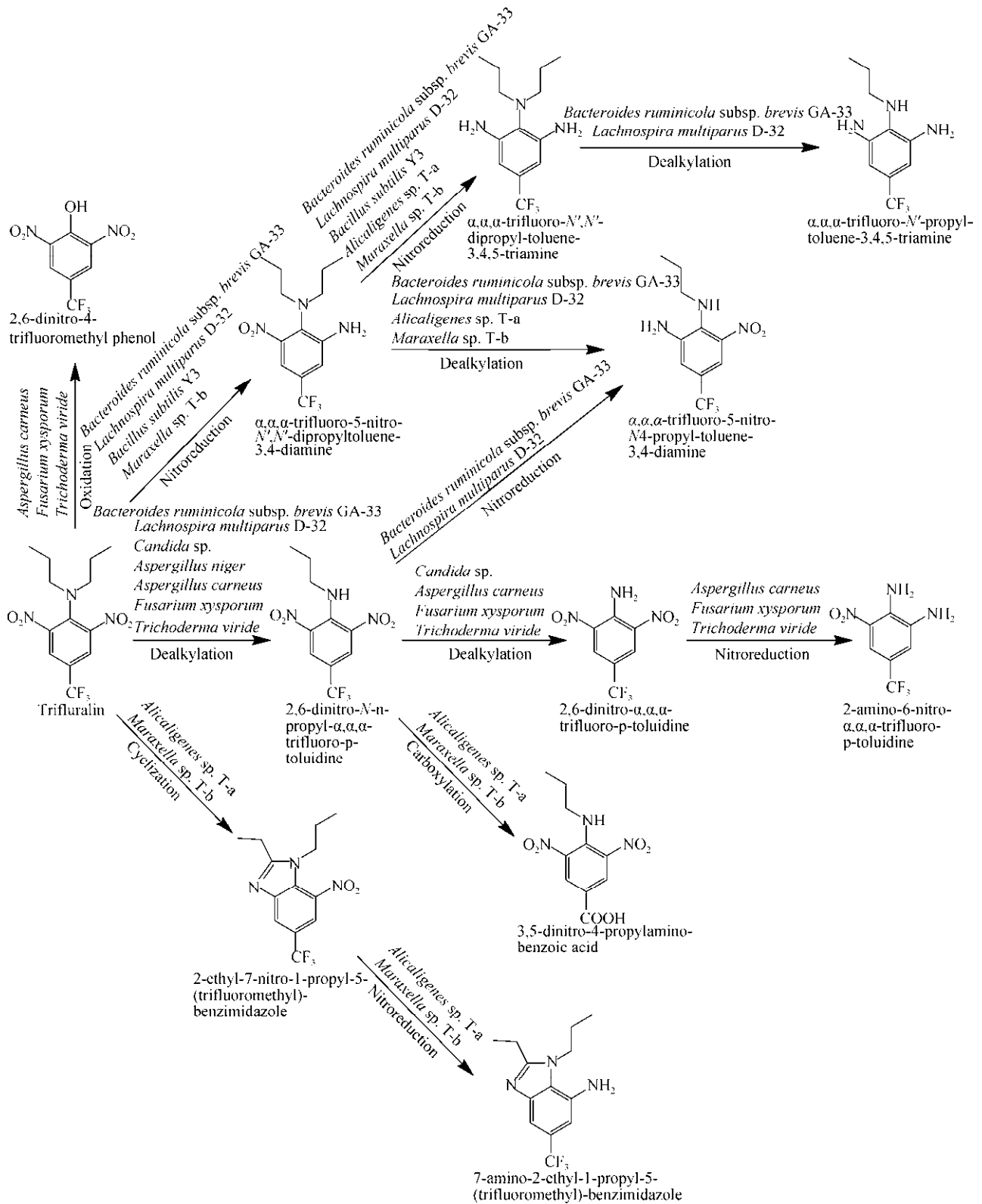**Figure 2 Proposed metabolic pathways of pendimethalin by pure isolates**

图 3   推测的氟乐灵微生物降解途径

Figure 3   Proposed metabolic pathways of trifluralin by pure isolates
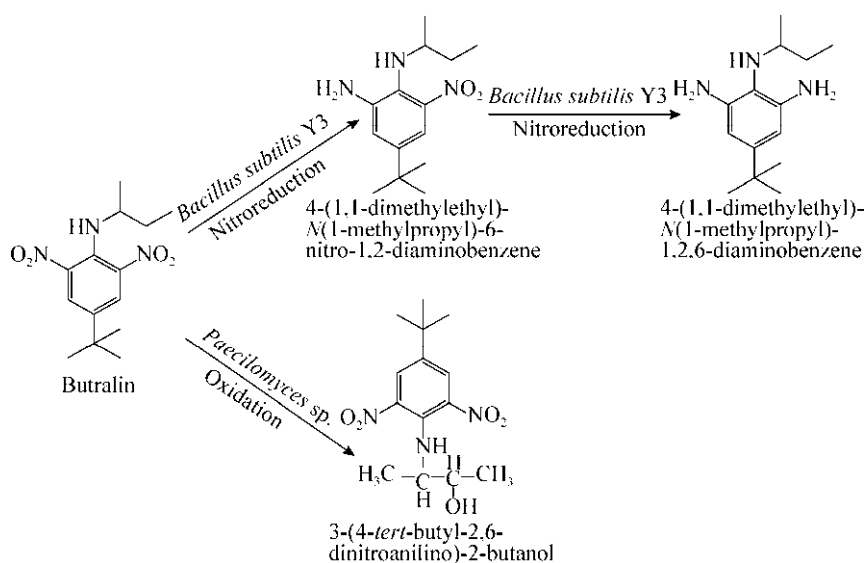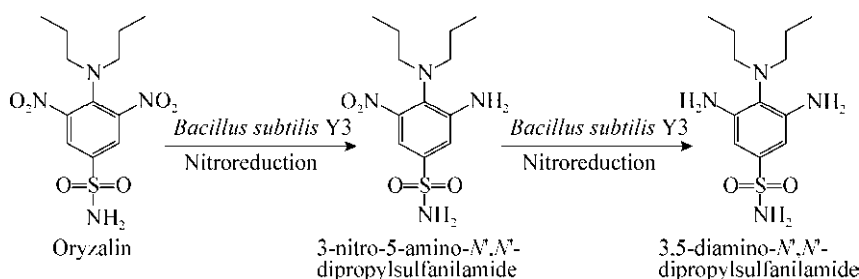
图 4　推测的仲丁灵微生物降解途径
Figure 4　Proposed metabolic pathways of butralin by pure isolates

图 5　推测的 *B. subtilis* Y3 降解安磺灵的部分代谢途径
Figure 5　Proposed partial metabolic pathway of oryzalin by *B. subtilis* Y3

基-6-氨基-3,4-二甲苯胺]，进而又通过硝基还原作用和羧化作用生成 5-氨基-2-甲基-3-亚硝基-4-(戊烷基-3-氨基)-苯甲酸，随后发生环化和羟基化作用得到 8-氨基-2-乙基-5-(羟甲基)-1,2-二氢喹喔啉-6-羧酸[53]。菌株 Y3 还能连续催化还原氟乐灵、仲丁灵和安磺灵的 2 个对称的硝基基团[54]。此外，褐球固氮菌(*Azotobacter chroococcum*)、尖孢镰刀菌(*F. oxysporum*)、环状芽孢杆菌(*B. circulans*)和芽孢杆菌(*Bacillus lehensis*) XJU 均能分别以 *N* 端氧化脱烷基和硝基还原的方式开始降解二甲戊灵[42-43,46,50]。在氟乐灵的微生物降解中，栖瘤胃拟杆菌短亚种(*Bacteroides ruminicola* subsp. *brevis*) GA-33 和多对毛螺菌(*Lachnospira multiparus*) D-32 也能分别通过 *N* 端氧化脱烷基和硝基还原两种方式开始降解氟乐

灵[39]。此外，在产碱杆菌属菌株(*Alcaligenes* sp.) T-a 和莫拉菌属菌株(*Moraxella* sp.) T-b 降解氟乐灵的过程中，不仅检测到了硝基还原产物和脱烷基产物，也检测到了环化产物苯并咪唑，说明这 2 株菌可以通过多种途径代谢氟乐灵[41]。

3　二硝基苯胺类除草剂的降解基因和酶

　　二硝基苯胺类除草剂的微生物降解菌株资源在不断地丰富，这也促进了微生物降解该类除草剂分子机制的研究，但目前该类除草剂的降解基因和酶方面的研究报道还是很少。2004 年，朱鲁生等采用碱裂解法从二甲戊灵降解菌株 *Bacillus* sp. HB-7 中提取到一个大小约为 20 kb 的质粒，该质粒可以产生能够降解二甲戊灵的酶系[45]；同年，Bellinaso 等通过 PCR 和 DNA 探针杂交技术在 4 株

氟乐灵降解菌株(*Klebsiella oxytoca*、*Herbaspirillum seropedicae* 和 2 株 *Bacillus megaterium*)中均克隆到了 *nodB* (编码萘 1,2-双加氧酶)相似基因，说明在这 4 株菌中都可能有一个双加氧酶参与氟乐灵的代谢，但是还需要进一步的测序和蛋白质组学等实验证明[74]。2015 年，More 等通过蛋白纯化技术从环状芽孢杆菌(*B. circulans*)中纯化到了二甲戊灵硝基还原酶，该酶单体分子量为 27 kD，需要 NADPH 作为辅因子，能催化还原二甲戊灵、氟乐灵、地乐胺等二硝基苯胺除草剂，也能还原对硝基苯胺、

2,4,6-三硝基甲苯(TNT)、2,4-二硝基甲苯和 2-硝基苯甲酸等硝基芳烃化合物，但对二甲戊灵和氟乐灵具有更高的催化活性[75]。2016 年，Ni 等通过降解酶蛋白纯化、肽指纹图谱分析和基因组学分析等方法从菌株 *B. subtilis* Y3 中克隆到负责二甲戊灵第一步降解的硝基还原酶基因 *pnr*，并通过降解酶催化产物纯化和核磁共振技术鉴定到 PNR 还原二甲戊灵的产物为 6-氨基二甲戊灵，PNR 还能连续催化还原氟乐灵、仲丁灵和安磺灵的 2 个对称的硝基基团(图 6)[54]。
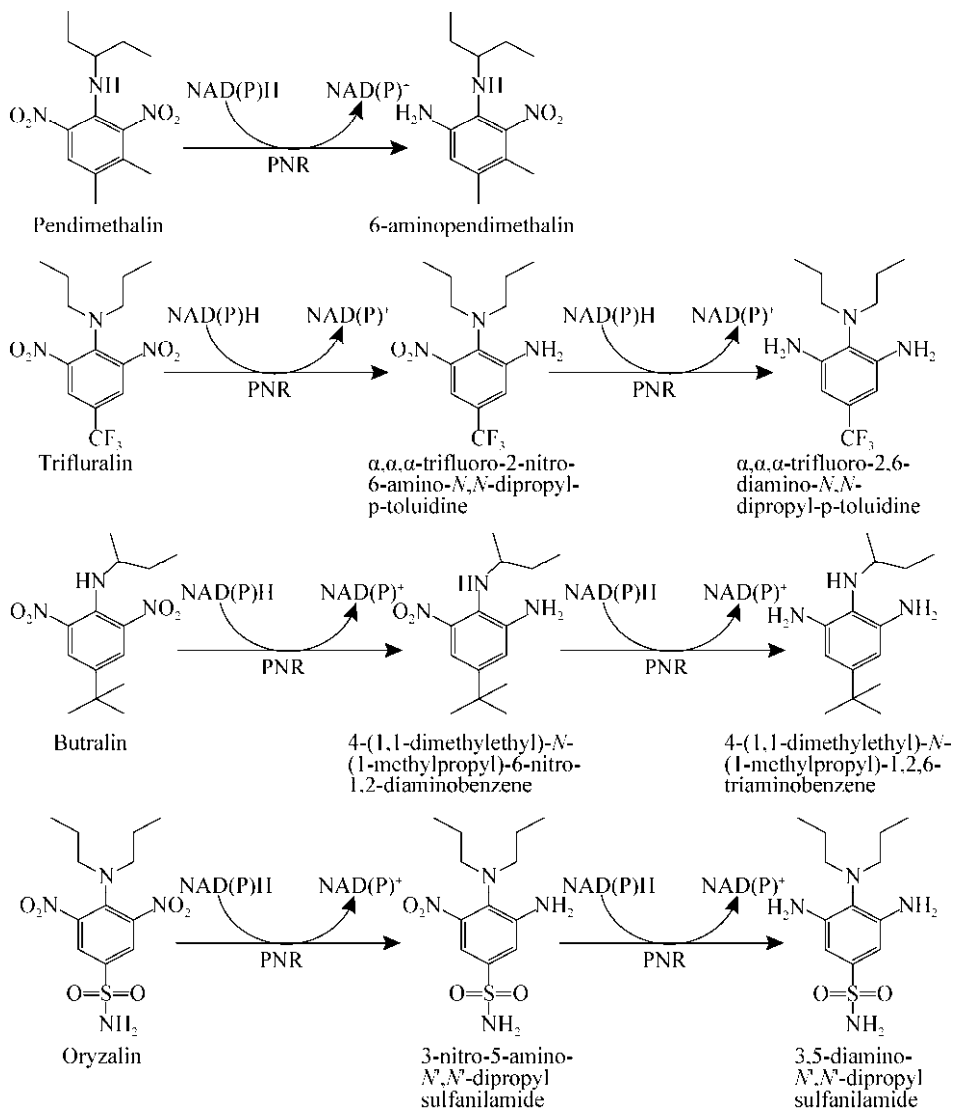

图 6　硝基还原酶 PNR 催化还原二甲戊灵、氟乐灵、仲丁灵和安磺灵[54]
Figure 6　Nitroreduction of pendimethalin, trifluralin, butralin, and oryzalin by PNR[54]

4　展望

　　二硝基苯胺类除草剂是一类广谱高效且广泛使用的选择性除草剂，其在环境中的残留对生态环境和人类健康都具有潜在危害，微生物因其数量、种类和代谢类型的多样性以及适应性强等特性而在二硝基苯胺类除草剂的降解中占据至关重要的位置。目前国内外已经积累了许多二硝基苯胺类除草剂的降解资源，但是当前对该类除草剂的微生物降解研究还比较浅。例如：(1) 菌株的降解特性研究较少；(2) 高效的降解菌株资源较少；(3) 现有的除草剂降解途径绝大多数是基于中间代谢产物的鉴定分析推测而来的，而且降解途径的研究往往止步于降解转化的前 3 步，该类除草剂在环境的矿化并没有研究清楚；(4) 虽然脱烷基和硝基还原是该类除草剂微生物降解的主要方式，但是研究发现还存在很多其他的降解方式(图 2−3)，对于这些降解过程中得到的降解产物是不是具有生态毒性还需要进一步验证；(5) 降解基因和酶的研究进展缓慢，缺乏对菌株降解机制的深入和全面研究等，从而限制了对该类除草剂残留污染修复的降解菌剂、降解基因和酶资源的开发和利用。

　　除草剂降解/脱毒酶基因在抗除草剂转基因工程领域具有潜在的应用价值，而我国目前还缺乏具有自主知识产权、性能优良的除草剂降解/脱毒酶基因。抗除草剂转基因工程可以极有效地控制农田杂草，其中最为成功的是抗草甘膦转基因作物，已经大量种植。但长期使用草甘膦带来严重的杂草抗性问题，急需发掘其他除草剂降解/脱毒酶基因，为研发新的抗除草剂转基因作物提供基因资源。二硝基苯胺类除草剂是一类广泛使用、杀草谱较广的除草剂，可作为抗除草剂转基因工程的靶标除草剂，因此它的降解/脱毒酶基因在构建抗除草剂作物中具有良好的应用前景，而要构建具备完善降解体系的抗二硝基苯胺类除草剂转基因作物，就迫切需要进一步探索除草剂的矿化过程，全面深入解析其降解机制。

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