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氯乙酰胺类除草剂微生物降解研究进展*

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摘 要 氯乙酰胺类除草剂主要分子结构由苯环和氯乙酰胺基团组成，在世界范围内的产量和使用量仅次于草甘膦和磺酰脲类除草剂. 其大量使用导致在环境中残留量不断增加，已经对生态环境和人类健康造成了严重威胁. 该类除草剂在环境中的消解主要是通过微生物代谢实现的，本文从微生物降解菌株资源、降解途径、关键基因和酶等几个方面综述了国内外微生物降解该类除草剂的最新研究进展. 细菌主要采用厌氧谷胱甘肽–共轭脱氯和好氧脱烷基–苯环开环代谢途径降解该类除草剂；真菌降解该类除草剂的途径多样化且比较复杂，尚需进一步研究. 从细菌中克隆出*N*-脱烷基酶、酰胺酶和羟化酶基因，但是对于上述酶学特性研究不够深入. 同时指出，目前有关微生物降解该类除草剂的研究主要集中于甲草胺、乙草胺和丁草胺，对于异丙草胺和异丙甲草胺微生物降解的代谢途径和分子机制方面的研究仍较少，在今后的工作中要加强异丙草胺和异丙甲草胺微生物降解方面的研究. 此外，微生物降解该类除草剂的新型基因为抗除草剂作物新品种的构建提供了良好的基因资源.（图6 表1 参69）

关键词 氯乙酰胺类除草剂；微生物降解；生物修复
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Research progress in microbial degradation of chloroacetanilide herbicides*

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Abstract Chloroacetanilide herbicides are a class of highly efficient preemergence herbicides that are widely used across the world. Presently, the production and use of the chloroacetanilide herbicides is less than glyphosate and sulfonylurea herbicides. Due to their widespread use, long persistence, and high-water solubility, some of these herbicides have frequently been detected in the environment and have shown toxic effects on humans. Microbial metabolism is the most important factor in the degradation of chloroacetanilide herbicides in the environment. In this paper, we summarized the latest research progress on the microbial strain isolation, identification, biodegradation pathway, and molecular mechanisms (such as key genes and enzymes) of these herbicides. The anaerobic-bacteria degradation of the pathway is glutathione-acetanilide conjugates which can be further converted to sulfonated metabolites. The aerobic-bacteria degradation of the pathway is mainly initiated by *N*/*C*-dealkylation events, followed by aromatic ring hydroxylation and cleavage processes. The fungal degradation pathways of chloroacetanilide herbicides are more complex, and therefore further research is needed. Though, the genes of *N*-dealkylase, amidase and hydroxylase have been cloned, the characteristics of these enzymes have not been thoroughly studied. Currently, the research has focused on biodegradation of alachlor, acetochlor and butachlor; however, relatively little is known about the metabolic pathways and molecular mechanisms for biodegradation of propisochlor and metolachlor. Therefore, we should strengthen the research on biodegradation of propisochlor and metolachlor. In particular, research is needed on the chiral selectivity of the microorganisms and enzymes to the isomers of metolachlor. In addition, new genes for microbial degradation of this herbicide may provide genetic resources for transgenic crops.

Keywords chloroacetanilide herbicide; microbial degradation; bioremediation

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氯乙酰胺类除草剂是酰胺类除草剂一个重要分支，主要分子结构由苯环和氯乙酰胺基团组成，是一种选择性触杀型芽前土壤处理剂，其结构通式如图1所示.

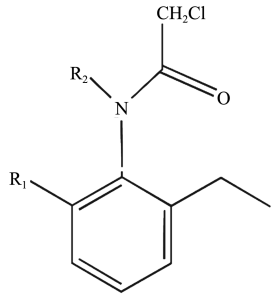

图1 氯乙酰胺类除草剂的核心结构.
Fig. 1 The core structures of chloroacetanilide herbicides.

目前市场上所售卖的品种大约53个[1]，据统计市场上占主导地位的品种为甲草胺（Alachlor）、乙草胺（Acetochlor）、丙草胺（Pretilachlor）、异丙草胺（Propisochlor）、丁草胺（Butachlor）和异丙甲草胺（Metochlor）等6种，上述6种氯乙酰胺类除草剂分子结构上主要区别在苯环及氯乙酰胺基团上取代基，如图2所示.

该类除草剂主要用于防除大豆、玉米、花生和棉花等作物中一年生禾本科杂草及部分小粒种子的阔叶杂草[1]，施入表层土壤中的除草剂主要通过幼芽或根系进入杂草中进而抑制杂草细胞分裂，导致杂草死亡[2, 3]. 因为该类除草剂的优点，截至2018年该类除草剂年产量与使用面积仅次于草甘膦和磺酰脲类，居世界第三位，其中以甲草胺、乙草胺、丁草胺和异丙甲草胺的销售量最大[4, 5]. 该类化学结构稳定，半衰期长，广泛而大量的使用使得其在环境中残留量逐渐积累，因此严重威胁着生态环境安全；目前一些研究发现该类除草剂对生物机体神经内分泌系统、免疫及生殖统有潜在的影响[6]. 2008年美国环保局将甲草胺和乙草胺定为B-2致癌物质，将丁草胺和异丙甲草胺定为L2和C类致癌物，并且把饮水中甲草胺的最大残留限量定为 < 2 μg/L[7].

研究表明该类除草剂在环境中的消解主要通过微生物代谢完成[8-10]，截止目前，已经筛选到大量氯乙酰胺类除草剂降解菌株，阐明了该类除草剂微生物代谢的部分途径，克隆出若干个降解关键基因. 本文主要介绍了近年来国内外对氯乙酰胺类除草剂的微生物降解方面取得的进展，为该类除草剂的微生物降解和生物修复的研究提供参考.

1 降解氯乙酰胺类除草剂的微生物

由于该类除草的高选择性和高活性，自上市以后得到了广泛使用，因此很早就有学者研究该类除草剂的微生物降解，目前已报道的氯乙酰胺类除草剂降解菌株按照年份汇总于表1.

从表中可以看出能够降解该类除草剂的微生物菌株种类很多既有细菌和真菌，也有放线菌. 其中分离出的细菌最多，真菌次之，放线菌最少. 细菌主要包括芽孢杆菌属、节杆菌、假单胞杆菌属、红球菌属、鞘氨醇单胞、副球菌属、申氏杆菌属和伯克氏菌属等大约18属；真菌主要包括丝核菌属、毛壳菌属、毛霉属、镰刀菌属、头芽胞菌属、曲霉属、青霉属、链霉菌属和酵母菌等大约12属；放线菌只有4株. 从菌株的分离生境方面统计，发现这些报道的菌株大多数分离于污染的土壤或农药厂活性污泥.

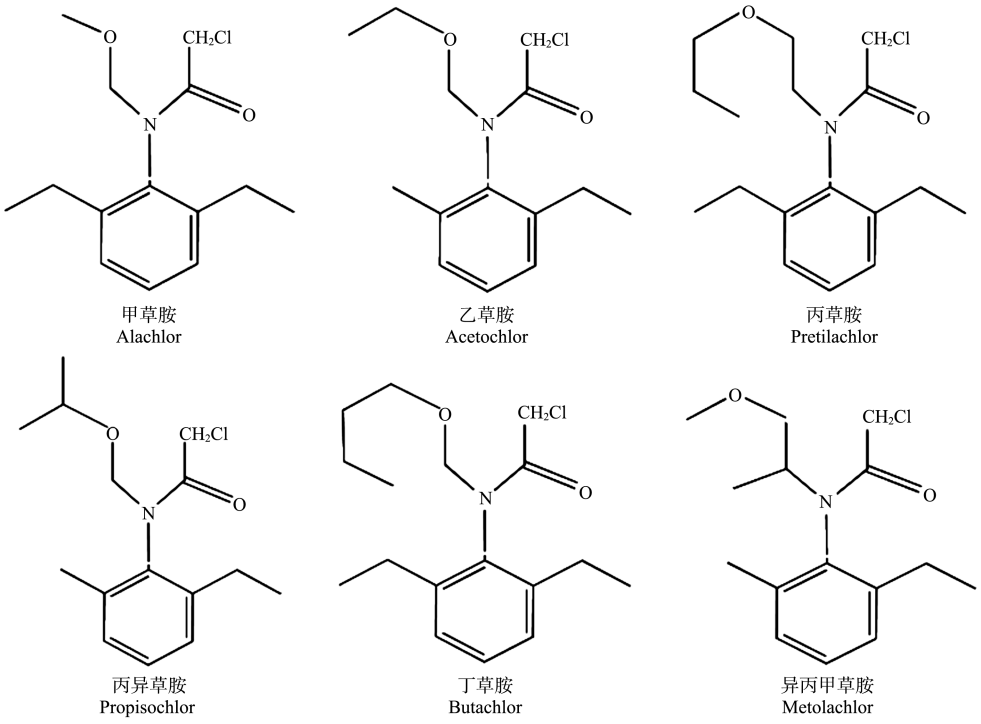

图2 常用氯乙酰胺类除草剂分子结构式.
Fig. 2 The chemical structures of some widely used Chloroacetanilide herbicides.

表1 已经报道的氯乙酰胺除草剂降解菌

Table 1 The reported chloroacetamide herbicides degrading-microorganisms

	年份 Year	降解菌株 Strain	降解底物 Substrate	分离地区 Isolation
细菌 Bacteria	1987	*Bacillus circulans* *Bacillus megate* [11]	异丙甲草胺 Metochlor	美国 USA
	2000	*Arthrobacter* sp. WY306 [12]	丁草胺 Butachlor	中国 China
	2006	*Pseudomonas oleovorans* LCa2 [13]	甲草胺、乙草胺和丁草胺 Alachlor, Acetochlor and Butachlor	中国 China
	2008	*Pseudomonas putida* ER1 and ER2 [14]	丁草胺 Butachlor	中国 China
	2008	*Bacillus megaterium* *B. circulans* *Bacillus cereus* RM2 *Bacillus thuringiensis* [15]	甲草胺、乙草胺、丁草胺和异丙甲草胺 Alachlor, Acetochlor, Butachlor and Metochlor	中国 China
	2009	*Acinetobacter* sp. LYC-1 [16]	丁草胺 Butachlor	中国 China
	2009	*Pseudomonas stutzeri* BD-1 [17]	丁草胺 Butachlor	中国 China
	2010	*Stenotrophomonas acidaminiphila* JS-1 [18]	丁草胺 Butachlor	沙特阿拉伯 Saudi Arabia
	2011	*Ensifer adhaerens* A-3 [19]	丁草胺 Butachlor	中国 China
	2013	*Rhodococcus* sp. T3-1 [20]	甲草胺、乙草胺和丁草胺 Alachlor, Acetochlor and Butachlor	中国 China
	2011	*Shinella* sp. Y-4 [21]	乙草胺 Acetochlor	中国 China
	2011	*Paracoccus* sp. Y3B-1 [22]	甲草胺、乙草胺和丁草胺 Alachlor, Acetochlor and Butachlor	中国 China
	2011	*Shinella maltophilia* CSUFTM78[23]	乙草胺 Acetochlor	中国 China
	2011	*Paracoccus* sp. FLY-8 [24]	甲草胺、乙草胺、丁草胺和异丙甲草胺 Alachlor, Acetochlor, Butachlor and Metochlor	中国 China
	2012	*Catellibacterium caeni* DCA-1 [25]	丁草胺 Butachlor	中国 China
	2012	*Rhodococcus* sp. B1 [26]	甲草胺、乙草胺和丁草胺 Alachlor, Acetochlor and Butachlor	中国 China
	2013	*Sphingomonas* sp. DC-6 [27]	甲草胺、乙草胺和丁草胺 Alachlor, Acetochlor and Butachlor	中国 China
	2013	*Mycobacterium* sp. J7A [28]	丁草胺 Butachlor	韩国 Korea
	2013	*Stenotrophomonas* sp. M-3 [29]	乙草胺 Acetochlor	中国 China
	2013	*Sphingobium* sp. DC-2 [30]	甲草胺、乙草胺和丁草胺 Alachlor, Acetochlor and Butachlor	中国 China
	2013	*Achromobacter* sp. D-12 [31]	乙草胺 Acetochlor	中国 China
	2013	*Pseudoxanthomonas* sp. Y4-6 [32]	异丙甲草胺 Metochlor	中国 China
	2013	*Delftia* sp. C-5 [33]	丁草胺 Butachlor	中国 China
	2015	*Bacillus* sp. BTC-3 [34]	丁草胺 Butachlor	中国 China
	2015	*Burkholderia* sp. WN-3 [35]	乙草胺 Acetochlor	中国 China
	2015	*Pseudomonas aeruginosa* JD115 [36]	乙草胺 Acetochlor	中国 China
	2015	*Bacillus subtilis* L3 [37]	乙草胺 Acetochlor	中国 China
	2016	*Klebsiella variicola* B2 [38]	乙草胺 Acetochlor	中国 China
	2016	*Pseudomonas* sp. A-1 [39]	乙草胺 Acetochlor	中国 China
	2016	*Bacillus* sp. hys-1 [40]	丁草胺 Butachlor	中国 China
	2018	*Serratia ureilytica* AS1 [41]	丁草胺 Butachlor	印度 India
真菌 Fungus	1975	*Rhizoctonia solani* [42]	甲草胺 Alachlor	美国 USA
	1975	*Chaetomium globosun* [43]	甲草胺 Alachlor	
	1978	*Mucor sufui* NTU-358 [44]	丁草胺 Butachlor	日本 Japan
	1986	*Acremonium recifei* *Aspergillus flavus* *Fusarium solani* *Penicillium Variabile* *Trichoderma* sp. [45]	异丙甲草胺 Metochlor	美国 USA
	1987	*Fusarium* sp. *Mucor racemosus*[46]	异丙甲草胺 Metochlor	美国 USA
	1991	*Fusarium solani* *Fusarium oxysporum* [47]	丁草胺 Butachlor	印度 India
	1991	*Phanerochaete chrysosporium* *Rhizoctonia praticola* *Syncephalastrum racemosum* [48]	异丙甲草胺 Metochlor	美国 USA
	1995	*Penicillium chrysosporium* [49]	异丙甲草胺 Metochlor	美国 USA
	1997	*Cunninghamella elegans* ATCC 36112 [51]	甲草胺和异丙甲草胺 Alachlorr and Metochlor	美国 USA
	2004	*Streptomyces* sp. LS166, LS17 and LS182 [52]	甲草胺 Alachlor	巴西 Brazil
	2010	*Candida xestobii* [53]	甲草胺和异丙甲草胺 Alachlorr and Metochlor	美国 USA
	2013	*Paecilomyces marquandii* [54]	甲草胺 Alachlor	波兰 Poland
放线菌 Actinomyces	1985	*Actinomycetes* sp. [55]	异丙甲草胺 Metochlor	美国 USA
	1987	*Actinomycete* sp. [56]	异丙甲草胺 Metochlor	美国 USA
	1991	*Streptomyces* sp.	异丙甲草胺 Metochlor	美国 USA
	1996	*Streptomyces* sp. PS1	甲草胺 Alachlor	美国 USA

1.1 放线菌

1985年, Krause等首次报道了一株能够降解异丙甲草胺的放线菌, 该菌株在含有0.04%酵母膏培养基中可以将50 mg/L异丙甲草胺在14 d内全部降解[55]; 随后1987年Saxena等发现*Actinomycete* sp. 27 d对初始浓度为100 mg/L的异丙甲草胺降解率60% [56], 90年代又有两株链霉菌从环境中分离出来, 但是对于降解效率没有研究这也是截止目前从环境中分离获得的4株放线菌.

1.2 真 菌

真菌作为微生物的一个庞大类群, 功能多样, 大量菌株具有降解异生质的能力. 从1975年第一株甲草胺降解真菌*Rhizoctonia solani* [42]报道以来, 截止目前大约12余属真菌能够降解氯乙酰胺类除草剂, 主要为毛霉属、镰刀菌属、曲霉属、青霉属、链霉菌属和酵母菌属等. 虽然真菌对该类除草剂有很强的降解能力, 但是由于真菌代谢产物多, 因此有关真菌降解该类除草剂的研究主要侧重于菌株的筛选和降解效率的测定, 对于真菌降解该类除草剂的代谢途径、参与其中的降解酶与基因方面的研究还处于起步阶段. 单一菌株降解能力和效率最高的是*Penicillium marquandii*, 该菌株7 d内对500 mg/L的甲草胺降解率为96.3% [54].

1.3 细 菌

在研究微生物降解氯乙酰胺类除草剂最初的一段时间, 报道的具有降解功能的微生物多数为真菌, 直到20世纪初陆续有该类除草剂降解细菌从环境中被分离和鉴定, 截止目前有芽孢杆菌属、节杆菌属、假单胞菌属、红球菌属、鞘氨醇单胞属、鞘酯菌属、副球菌属、申氏杆菌属和伯克氏菌属等18属32株细菌被分离和鉴定. 大多数菌株采用共代谢的方式降解氯乙酰胺类除草剂, 有些需菌株联合起来才能够矿化该类除草剂.

联合降解该类除草剂的细菌种类和数量比较多, 例如*Mycobacterium* sp. J7A 和*Sphingobium* sp. J7B 组合成菌系可以矿化丁草胺[28], *Rhodococcus* sp. T3-1、*Delftia* sp. T3-6和 *Sphingbium* sp. MEA3-1组成菌系可以矿化甲草胺、乙草胺和丁草胺[20], *Stenotrophomonas acidaminiphila* JS-1、*Stenotrophomonas marcescens* 66C-1、*Pseudomonas shunanensis* 66C-2、*Pseudomonas putida* 66C-3和*Pseudomonas nitroreducens* 66C-4菌株等比例混合构成的降解菌系HXDW可以利用乙草胺为唯一碳源、但不能利用丁草胺和异丙甲草胺等其他氯乙酰胺类除草剂为唯一碳源; 有些虽独自可以降解, 但是只能将该类除草剂转化为某种中间产物, 例如*Rhodococcus* sp. B1 [26]、*Sphingobium* sp. DC-2 [30]和*Bacillus* sp. hys-1 [40]; 有些虽独自也可以降解, 但是只能以某种氯乙酰胺类除草剂为唯一碳源或氮源生长例如*Shinella* sp. Y-4 [21]、*Delftia* sp. C-5 [33]、*Bacillus* sp. BTC-3 [34]、*Acinetobacter* sp. LYC-1 [35]、*Klebsiella variicola* B-2 [38]和*Pseudomonas aeruginosa* JD115 [36].

单一矿化该类除草剂的细菌资源只有*Paracoccus* sp. FLY-8 [24]和*Sphingomonas* sp. DC-6 [27]. 这两株微生物于2011年和2013年被Zhang和Chen等从农药厂活性污泥获得, 研究发现菌株*Paracoccus* sp. FLY-8能够矿化甲草胺、乙草胺、丙草胺、丁草胺、异丙草胺和异丙甲草胺; 菌株*Sphingomonas* sp. DC-6能够矿化甲草胺、乙草胺和丁草胺, 但对异丙甲草胺、丙草胺和异丙草胺却没有降解效果, 在初始浓度为100 mg/L时, 48 h 对丁草胺、乙草胺和甲草胺的降解率分别为76.7%、93.6%和98.6%.

2 降解氯乙酰胺类除草剂的主要途径

截止目前研究者筛选到种类多样的氯乙酰胺类除草剂降解菌株, 不仅研究了其降解特性, 同时对该类除草剂的降解途径进行了深入分析, 但是由于产物的复杂性、不稳定性及菌体产生的次级代谢产物的影响, 导致了中间产物的鉴定困难. 研究表明在各种微生物酶的催化下该类除草剂发生脱氯、羟基化、*N*-脱烷基和*C*-脱烷基等生理生化过程, 目前已报道的微生物降解该类除草剂的主要为细菌代谢途径和真菌代谢途径, 其中细菌代谢途径又分为两条: (1)厌氧谷胱甘肽–共轭脱氯途径; (2)好氧脱烷基及苯环开环途径.

2.1 厌氧谷胱甘肽–共轭脱氯途径

早在1998年Stamper 等在研究氯乙酰胺类除草剂降解时发现, 微生物体内的谷胱甘肽-*S*-转移酶能够亲核攻击甲草胺、异丙甲草胺和毒草胺的2-氯基团并形成谷胱甘肽-乙酰胺中间态, 其进一步形成无除草活性的乙烷磺酸ESA (Ehtnaesulofnic acid) [57], 如图3所示.

2.2 好氧脱烷基及苯环开环途径

该降解途径最初在丁草胺降解菌*Paracoccus* sp. FLY-8中发现, 首先丁草胺在*C*-脱烷基酶作用转成甲草胺, 然后在*N*-脱烷基酶作用下转化成2,6-二乙基-2-氯乙酰苯胺(CDEPA), 进一步在酰胺酶的作用下转化成2,6-二乙基苯胺(DEA), 进入苯胺和邻苯二酚代谢途径, 继而开环直至完全降解[24], 如图4所示.

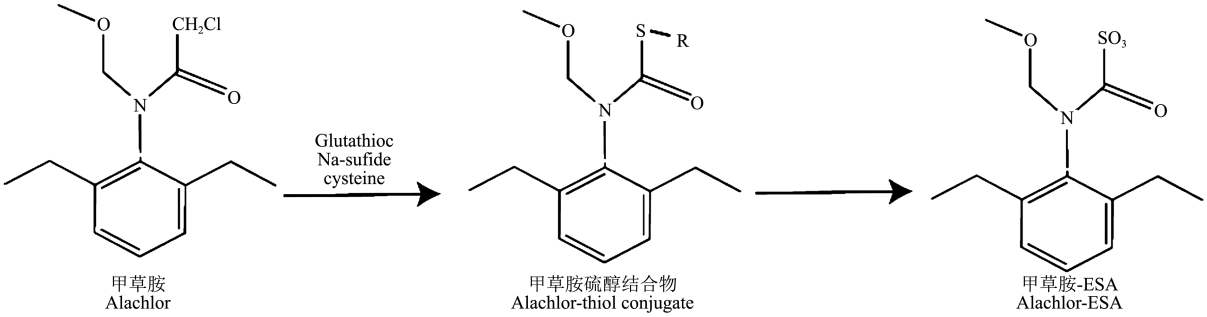

图3 谷胱甘肽介导甲草胺的降解[57].
Fig. 3 Thiol-alachlor conjugation and subsequent degradation to alachlor-ESA [57].

目前该类代谢途径也在某些单菌或混合降解菌系中发现乙草胺降解菌*Sphingobium* sp. DC-6 [27], 丁草胺混合降解菌系*Mycobacterium* sp. J7A 和 *Sphingobium* sp. J7B [28], 乙草胺混合降解菌系*Rhodococcus* sp. T3-1、*Delftia* sp. T3-6和*Sphingobium* sp. MEA3-1 [20, 58-60], 该途径在菌株*Sphingobium* sp. DC-6 [61-62]研究最为详细(图5).

图4和图5可以看出, 甲草胺、乙草胺或丁草胺首先在*N*-脱烷基酶作用转化成2,6-二乙基-2-氯乙酰苯胺(CDEPA)

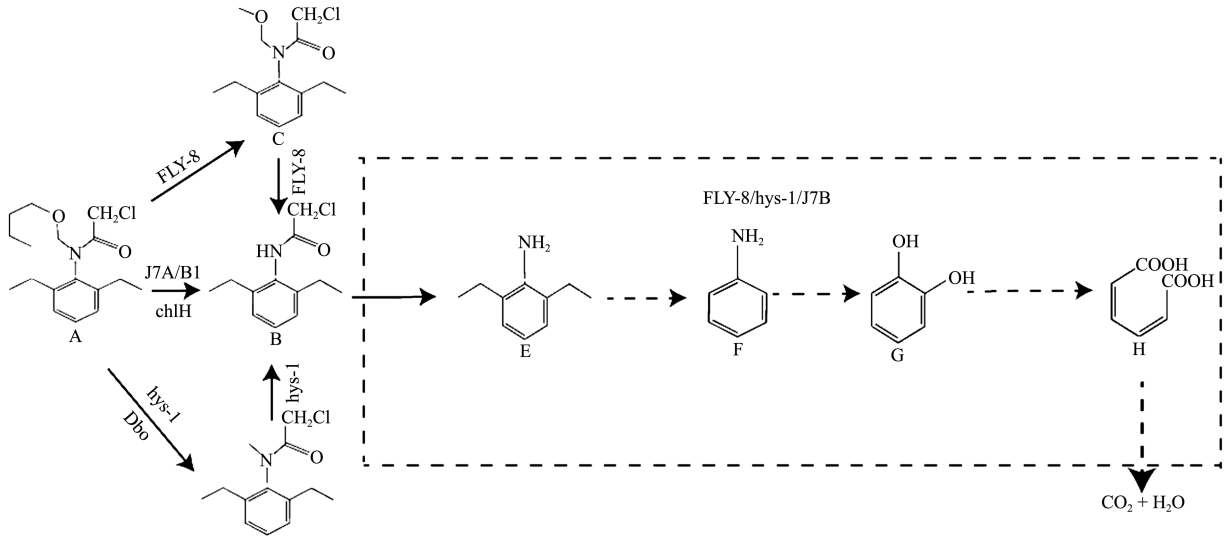

图4 菌株J7A、J7B、FLY-8、hys-1和B1降解丁草胺的途径. A: 丁草胺; B: 2,6-二乙基-2-氯乙酰苯胺; C: 甲草胺; D: 2,6-二乙基-2-氯-N-甲基-乙酰苯胺; E: 2,6-二乙基苯胺; F: 苯胺; G: 邻苯二酚; H: 粘康酸. 降解酶: ChlH, 丁草胺*N*-脱烷基水解酶; Dbo, 丁草胺*N*-脱烷基酯酶.

Fig. 4 The proposed metabolic pathways of butachlor by strain J7A, J7B, FLY-8, hys-1and B1. A: Butachlor; B: 2-chloro-*N*-(2,6-diethylephenyl)-*N*-methylacatanide; C: Alachlor; D: 2-chloro-*N*-(2,6-diethylphenyl)-*N*-acetamide; E: 2,6-diethylaniline; F: Aniline; G: Catechol; H: Muconic acid. Enzymes: ChlH, The hydrolase responsible for the *N*-dealkylation of butachlor from strain B1; Dbo, the esterase responsible for debutoxylation of butachlor from strain hys-1.

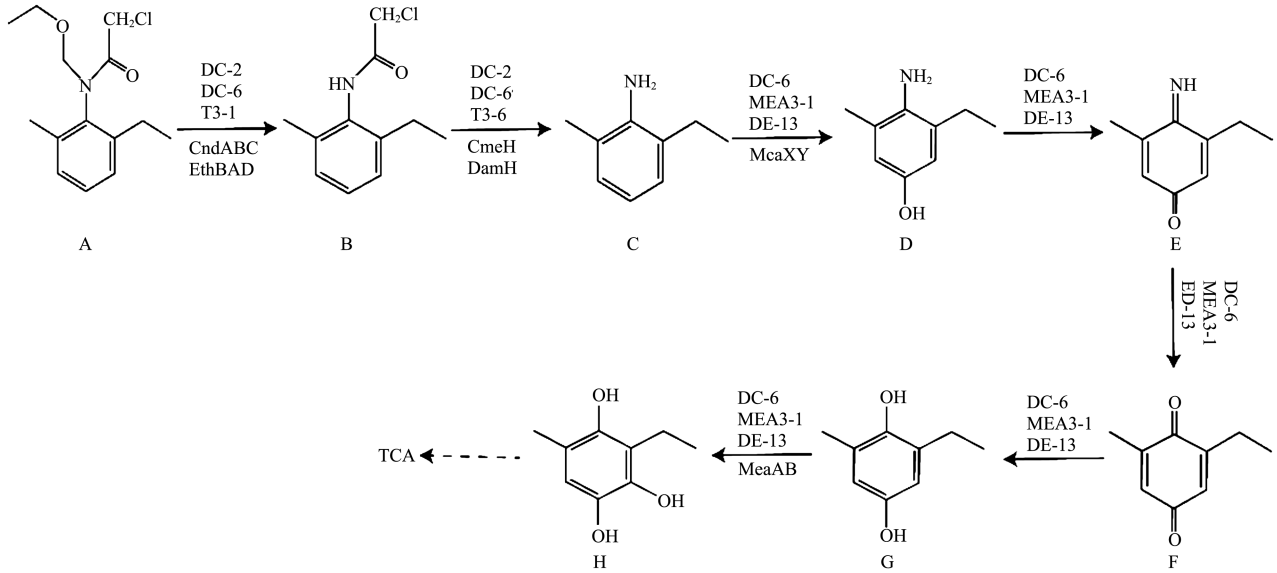

图5 菌株DC-2、DC-6、T3-1、T3-6、MEA3-1和DE-13降解乙草胺或其中间产物的代谢途径. A: 乙草胺; B: 2-甲基-6-乙基2-氯乙酰苯胺; C: 2-甲基-6-乙基苯胺; D: 4-羟基-2-甲基-6-乙基苯胺; E: 2-甲基-6-乙基对苯醌胺; F: 2-甲基-6-乙基对苯二醌; G: 2-甲基-6-乙基对苯二酚; H: 2-甲基-3-羟基-6-乙基对苯二酚. 酶: CndABC, 菌株DC-2或DC-6中的乙草胺*N*-脱烷基酶; EthBAD, 菌株T3-1中的乙草胺*N*-脱烷基酶; CmeH, 菌株DC-2中2-甲基-6-乙基2-氯乙酰苯胺水解酶; DamH, 菌株T3-6中2-甲基-6-乙基2-氯乙酰苯胺水解酶; MeaXY, 菌株DE-13中2-甲基-6-乙基苯胺单加氧酶; MeaAB, 菌株MEA3-1中2-甲基-6-乙基对苯二酚单加氧酶.

Fig. 5 The proposed metabolic pathways of acetochlor or metabolites by strain DC-2, DC-6, T3-1, T3-6, MEA3-1 and DE-13. A: Acetochlor; B: 2-chloro-*N*-(2-methyl-6-ethyl)acetamide; C: 2-methyl-6-ethyl-aniline; D: 4-hydroxy-2-methyl-6-ethylaniline; E: 2-methyl-6-ethyl-benzoquinone imine; F: 2-methyl-6-ethyl-benzoquinone; G: 2-methyl-6-ethyl-hydroquinone; H: 3-hydroxy-2-methyl-6-ethyl-hydroquinone. Enzymes: CndABC, three-component Rieske non-Heme iron oxygenase system responsible for the *N*-dealkylation of acetochlor from DC-2 or DC-6; EthBAD, cytochrome P450 oxygenase system responsible for the *N*-dealkylation of acetochlor from strain T3-1; CmeH, 2-chloro-*N*-(2-methyl-6-ethyl)acetamide hydrolase from strain DC-2; DamH, 2-chloro-*N*-(2-methyl-6-ethyl) acetamide hydrolase from strain T3-6; MeaXY, 2-methyl-6-ethyl-aniline monooxygenase system from strain DE-13; MeaAB, 2-methyl-6-ethyl-hydroquinone monooxygenase system from MEA3-1.

或2-甲基-6-乙基2-氯乙酰苯胺（CMEPA），接着在酰胺酶作用下转化成2,6-二乙基苯胺（DEA）或2-甲基-6-乙基苯胺（MEA），然后转化成4-羟基-2,6-二乙基苯胺（4-OH-DEA）或4-羟基-2-甲基-6-乙基苯胺（4-OH-MEA），进一步转化成2,6-二乙基对苯二酚（DEHQ）或2-甲基-6-乙基对苯二酚（MEHQ），之后转化成3-羟基-2,6-二乙基对苯二酚（3-OH-DEHQ）或2-甲基-3-羟基6-乙基对苯二酚（3-OH-MEHQ），然后苯环打开进入TCA循环直至完全降解.

2.3 真菌代谢途径

真菌虽然降解氯乙酰胺类除草剂效率比细菌高，但是其有复杂的初级或次级代谢产物，导致真菌代谢途径鉴定困难，研究表明真菌代谢途径较细菌复杂. 1991年Liu等研究发现*P. chrysosprium*、*R. praticola*和*S. racemosum* 的纯培养均能降解异丙甲草胺，使异丙甲草胺发生脱氯、脱烷基和烷基侧链的羟基化[48]. 如图6所示，在真菌*F. solani*的作用下丁草胺通过脱氯、羟基化、脱烷基化、环化等酶促反应产生15种中间代谢产物[47]，最终转化成2-甲基-6-乙基苯胺.

3 与氯乙酰胺类除草剂降解有关的基因和酶

随着该类降解微生物资源数目不断增加，也间接促进了研究者对该类除草剂降解的分子机制深入探索，有关该类除

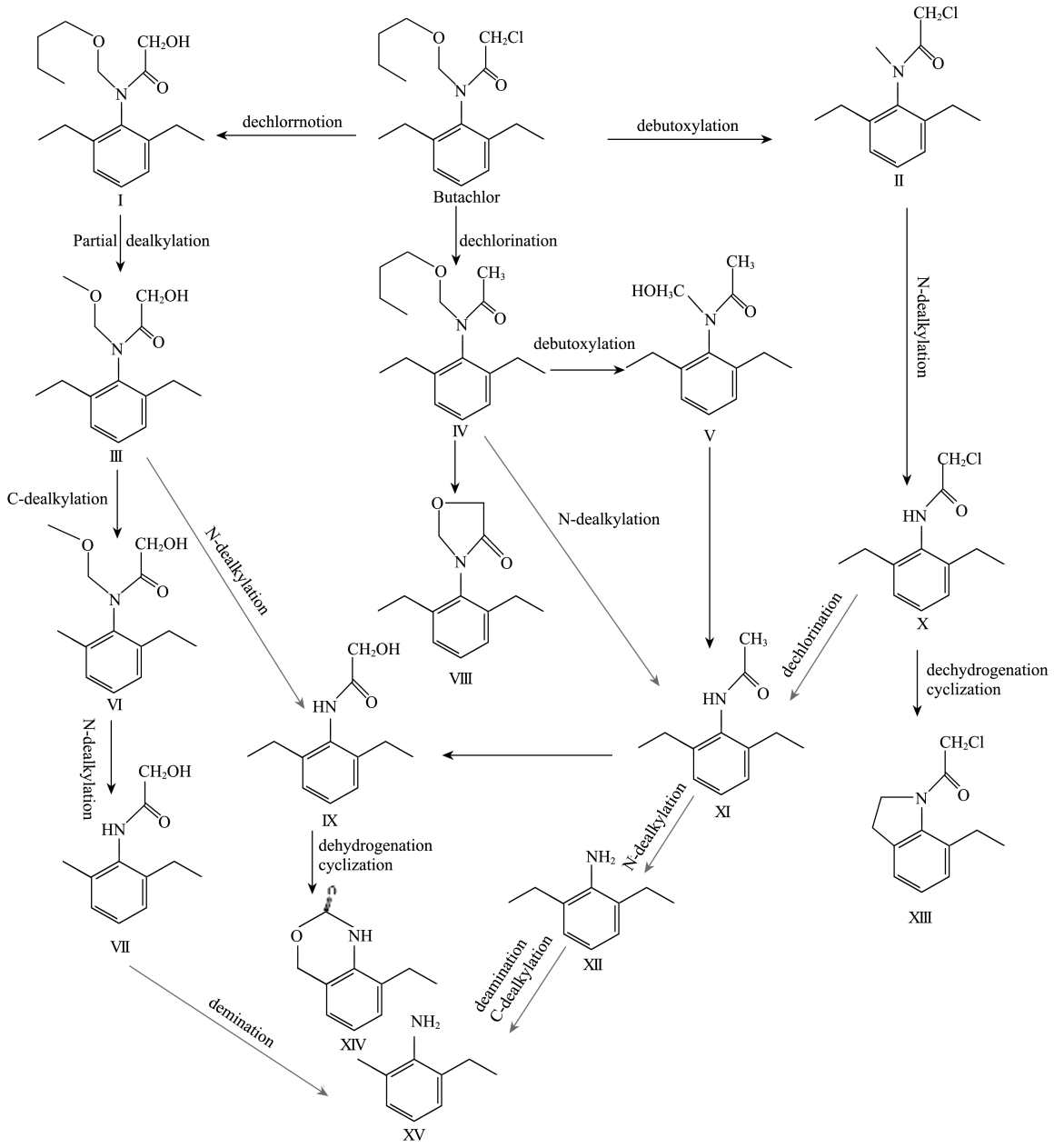

图6 *Fusarium solani*降解丁草胺代谢途径[47].

Fig. 6 The proposed metabolic pathways of Butachlor by strain *Fusarium solani* [47].

草剂降解相关基因和酶等方面的研究也获得较大进展. 2013年Liu等通过蛋白层析技术首次从菌株*Rhodococcus* sp. B1中分离纯化出水解酶ChlH, ChlH是一个大小约为45 × 10^3的单体蛋白, 能够直接催化甲草胺、乙草胺和丁草胺*N*-脱烷基生成CDEPA或CMEPA, 研究发现其催化效率随着*N*-烷氧链长度增加催化效率下降[26]. 2013和2014年, Li等[30]和Wang等[58]分别从菌株*Sphingobium* sp. DC-2和*Delftia* sp. T3-6克隆到编码CmeH和DamH的酰胺酶基因, CmeH和DamH属于水解酶, 并且只能作用于CDEPA或CMEPA为底物中的酰胺键, 将二者分别转化成DEA或MEA, 但是不能催化转化底物甲草胺、乙草胺和丁草胺. 2014年Chen等从*Sphingomonas* sp. DC-6中克隆催化甲草胺、乙草胺和丁草胺*N*-脱烷基酶CndABC基因, 氨基酸序列分析CndA为Type Iv*α*型Riseke非血红素铁氧化酶家族[61]. 同年Wang等从菌株*Rhodococcus* sp. T3-1克隆催化甲草胺、乙草胺和丁草胺*N*-脱烷基酶的*eth*BAD, EthB为细胞色素P450单加氧酶[59]. 2016年Gao等从菌株*Bacillus* sp. hys-1克隆出编码*C*-脱烷基酶基因*dbo*, Dbo是一个大小约为28 × 10^3的单体蛋白, 氨基酸序列分析该酶为羧酸酯酶, Dbo催化甲草胺、乙草胺和丁草胺转化成2-氯-*N*-2,6-二乙基-*N*-甲基乙酰胺或2-氯-*N*-2-甲基-6-二乙基-*N*-甲基乙酰胺[40]. 2015年Dong等通过比较基因组学和2D双向电泳技术从菌株*Sphingobium* sp. MEA3-1中克隆出MEHQ或DEHQ单加氧酶基因*mea*A, MeaA属于黄素单加氧酶家族, 氧化酶MeaA和还原酶MeaB组成酶系将MEHQ或DEHQ转化成3-羟基-2,6-二乙基对苯二酚(3-OH-DEHQ)或2-甲基-3羟基-6-乙基对苯二酚(3-OH-MEHQ)[60]. 2017年Cheng等同样通过比较基因组学的方法从菌株*Sphingobium baderi* DE-13中克隆出DEA或MEA羟化酶基因 *mea*XY, MeaXY为双组分核黄素单加氧酶, 在NADH和FMN作为辅因子存在下MeaXY将DEA或MEA转化成4-OH-DEA或4-OH-MEA[62].

4 总结与展望

自从氯乙酰胺类除草剂研发投入市场上以来, 该类除草剂及其代谢产物在环境残留、降解和迁移一直备受关注. 环境中存在大量的细菌、真菌和放线菌, 这些微生物在该类除草剂的降解过程起着重要的角色, 研究发现生物修复是一种低廉、高效及环保友好型一项技术. 迄今为止, 对于氯乙酰胺类除草剂中甲草胺、乙草胺和丁草胺微生物降解已经取得很大进展, 上述3种除草剂的降解菌不仅菌种资源丰富, 而且降解效果高效; 但是对于氯乙酰胺类除草剂中的异丙草胺和异丙甲草胺微生物降解进展缓慢, 特别是对不同手型构型的异丙甲草胺的微生物降解研究近乎空白, 在今后的工作中要加强异丙草胺和异丙甲草胺降解细菌菌株的筛选. 在细菌的氯乙酰胺基团上取代基的脱烷基及苯环开环途径中3-OH-DEHQ或3-OH-MEHQ如何开环并最终进入TCA循环, 目前还尚未完全阐明, 此方面研究亟待加强. 虽然经过研究人员的努力, 从细菌中克隆到了若干个负责氯乙酰胺类除草剂降解的基因, 其中较有代表性的关键基因有*cnd*ABC、*eth*BAD、*cme*H、*dam*H、*mea*XY和*mea*BA, 由于某些基因编码的酶为氧化还原酶类, 目前对于体外CndABC、EthBAD、MeaXY和MeaBA催化特性还有待进一步研究阐述.

随着转基因工程技术的发展以及抗除草剂基因的获得, 截止2017全球共有328个抗除草剂转化事件被相关国家授权, 主要为棉花、大豆、油菜和玉米抗除草剂新品种的构建[63]. 微生物来源的草甘膦(*goxv247*, 来源于*O. anthropistrain* LBAA的草甘膦氧化酶基因)[64]、草铵膦(*bar*, 来源于*S. hygroscopicus*膦丝菌素*N*-乙酰转移酶基因; *pat*, 来源于*S. viridochromogenes*膦丝菌素-乙酰转移酶基因)[65]、2,4-二氯苯氧乙酸(*add-1*, 来源于鞘氨醇杆菌*S. herbicidovorans*的芳氧基链烷酸酯双加氧酶基因)[66]、麦草畏(*dmo*, 来源于*S. maltophilia* DI-6单加氧酶)[67]、磺酰脲类(*S4-HrA*, 来源于烟草*Nicotiana tabacum* cv. *Xanthi*的突变乙酰乳酸合酶基因)[68]和溴苯腈(*bxn*, 来源于*K. pneumoniae* subsp. *ozaenae*的腈水解酶基因)[69]降解基因已经成功转到相应作物中并带来巨大的经济利益. 因此氯乙酰胺类除草剂降解基因*cnd*ABC、*eth*BAD、*cme*H、*dam*H、*mea*XY和*mea*BA有望在转基因作物领域大显身手.

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