doi: 10.13241/j.cnki.pmb.2015.09.040

土壤中抗性基因的产生,扩散传播以及消减的研究进展 *

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摘要:近年来,土壤中残留的大量抗生素不可避免的导致耐药微生物和抗性基因的增加和扩散,引起一系列土壤污染和生态风险。作为一类新兴污染物,抗性基因的污染水平已经远远超出我们的预想,因此对土壤中抗性基因的分布水平、扩散传播及消减技术的研究刻不容缓。本文对国内外土壤中抗生素和抗性基因残留水平进行了总结分析,探讨了土壤中抗性基因的产生、扩散的内在动力和机制。同时,分析了土壤中抗性基因分布和扩散的影响因素,如:抗生素残留水平,土壤理化性质和环境条件等。在此基础上,探讨了土壤抗性基因阻隔和消减技术,包括传统降解方法:高温,光照催化、微波 -H_2O_2- 微生物联合处理技术等,并提出新型消解技术:取代活性基团、靶位修饰以及改变外排泵的通透性等。讨论未来在控制抗性基因生态风险,降低其在土壤中的丰度,有效阻截技术的发展趋势。

关键词:抗生素;抗性基因;土壤;生态风险;消减技术

中图分类号:X53;R978.1 **文献标识码**:A **文章编号**:1673-6273(2015)09-1752-08

Emergence, Spread and Elimination of Antibiotic Resistance Genes in Soil- A review*

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ABSTRACT: In recent years, various antibiotic residuals in soils inevitably result in an increase and spread of resistance microorganism and antibiotic resistance genes (ARGs), and lead to a series of soil ecological risks. As an emerging pollutant, the residual level of ARGs in soils is actually much higher than we thought previously, thus the relevant studies are urgent to understand the distribution level and spread of ARGs, and proceed for elimination technologies. In this paper, we firstly summarized the residual level of antibiotics and ARGs in soils at home and abroad, and discussed the intrinsic driving force and mechanism of emergence and spread of ARGs, and potential threat to ecological environment. In addition, we analyzed the factors that influencing the distribution and spread of ARGs in soils, such as the residual concentration of antibiotics, soil physical and chemical properties, environmental conditions, and so on. Then we further discussed the elimination techniques of ARGs, including high temperature, photocatalysis, microwave-H_2O_2-microbial integrated-treatment technology, and proposed new methods, such as replaced active groups, changed the target position and the permeability of efflux pump. Finally, this study highlighted the trends in controlling the residual abundance and ecological risks of ARGs in soils.

Key words: Antibiotics; Antibiotic resistance genes; Soil; Ecological risk; Elimination technology

Chinese Library Classification(CLC): X53; R978.1 Document code: A

Article ID: 1673-6273(2015)09-1752-08

前言

抗生素类药物被广泛用于治疗传染性疾病和作物增产剂[1,2]。例如,在美国,每年抗生素的产量为 22.7 万吨,其中 17.8-70%都用于畜禽养殖[3]。在上世纪 90 年代,欧盟有 70%的抗生素类药物用于疾病预防,其余的抗生素主要作为食品添加剂用于促进畜禽动物的生产[4]。在中国,每年用于畜禽养殖的兽用抗生素就超过 8 万吨以上,而其中以四环素的使用量最大[5]。人或动物体内摄入的抗生素无法完全吸收和代谢,随排泄物进入环境中。研究表明,约 25%-75%的抗生素以母体化合物的形式随粪尿排出体外。调查表明,粪便中四环素类抗生素的残留浓度范围为 0.1-46.0 mg/kg[6]。土壤中的抗生素的残留也有广泛报道,例如施有机肥土壤中金霉素残留浓度高达 0.01-1.08 mg/kg[7,8]。含有抗生素类药物的排泄物以有机肥料的途径施用

* 基金项目:国家自然科学基金项目(41371256);江苏省高校自然科学研究项目(13KJB610001)
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(收稿日期:2014-10-23 接受日期:2014-11-08)

于农田和耕地土壤后，抗生素就可能被植物根系吸收并在植物体内积累富集，进而抑制植物生长[9]，同时对人体也存在一定的健康风险。

这篇综述旨在总结目前土壤中抗性基因的残留水平及其生态风险，主要涉及到土壤微生物群落平衡和生态功能以及对人体健康的影响。进一步阐述相关的消减方法，为土壤抗性基因的阻隔及消减提供一定的理论依据和参考。

1 抗性基因在土壤和粪便中的分布

1.1 粪便中抗性基因的残留水平

抗生素对原著微生物和菌落产生选择压力，致使粪便中抗性基因的出现和高浓度残留。并且这种选择压力决定抗性细菌的种类和水平[10]，选择压力的强弱水平与抗生素残留浓度密切相关，Ji 等人[11]通过检测中国畜禽养殖场粪便中抗生素浓度与抗性基因丰度，发现 sul2 基因的丰度与相应的抗生素含量呈正相关（r=0.847），而 tet(O)和 tet(W)基因丰度与相应抗生素浓度的响应水平较低。Schwaiger 等[12]通过检测调查德国某农场的粪便样品（n=179）中抗性基因残留情况，发现有 147 个粪便样品残留强力霉素抗性基因，而其中 86%的样品携带 tet (M)基因，64%携带 tet(L)基因，7%携带 tet(S)，5%携带 tet(O)基因。很明显，抗性基因在粪便中的检出率较高且种类繁多，俨然已成为抗性基因的另一个天然的基因库。然而，粪便中的抗性基因不可能持久性存在，必然会通过一系列传输途径进入土壤等其他环境介质中，造成致病基因和抗性基因在更广泛的环境中分布。目前，粪便中各类抗性基因的残留丰度已明显高于土壤抗性基因的丰度水平，例如，在欧洲的畜禽粪便样品中，四环素类和红霉素类抗性基因的丰度明显高于土壤中，丹麦，意大利，西班牙以及英国的抗性基因丰度范围分别为：9.0-9.7、8.3-8.4、8.1-8.4 以及 6.3-9.4(log copies/g)[13,14]。通过人类的农业活动，粪便中高丰度的抗性微生物和抗性基因必然会经过一系列传播扩散过程进入土壤[15,16]水体，底泥等其他环境介质中，造成抗性基因更广范围的输送和富集。

粪便的堆积和施肥等农业活动将携带高浓度抗性基因的菌株和抗性微生物群落带入土壤生态中，并导致抗性基因在土壤中的传播和暴露，一项研究也印证了这种传播过程。经报道，四环素类抗性基因（tetB, tetC, tetO 以及 tetW）在美国落基山国家公园的土壤中未被检出，而这些抗性基因却在附近施有机肥的农田中被普遍发现[17]。因此，考虑到粪便中抗性基因的高丰度和传播的多途径，其生态风险不应被忽视。

1.2 土壤中抗生素的残留水平及抗性基因的分布

由于畜禽动物体内的抗生素很难完全吸收，大部分的抗生素以尿液或粪便的形式排入土壤中，造成土壤抗生素残留浓度增加。据报道，在畜禽养殖场的有机肥检测结果中，磺胺甲基嘧啶为 0.4 mg/kg[18]，洁霉素为 0.24 mg/kg[18]，环丙沙星(0.37-0.40 mg/kg)[6,19]，恩诺沙星(0.06-1.35 mg/kg)[5]，以及土霉素(0.50-2.68 mg/kg)[7,20]均有被检测出。目前，土壤中抗生素的输入已经远远超出其生态自净水平，不可避免的导致抗生素在土壤中的积累与富集，并且可能导致土壤抗性基因的产生、扩散和传播。

土壤抗性基因的生态风险已经远远超过抗生素类有机物本身。目前，土壤中各类抗性基因已经被广泛鉴别和检测出。例如，在土壤中，仅四环素类抗性基因就有 40 余种，其中 tet(A)，tet(O)，tet(W)以及 tet(Z)基因等被频繁检出[21-23]。而磺胺类抗性基因(sul)，四环素类抗性基因(tet)，红霉素抗性基因(erm)等其他抗生素类基因也被多次报道[24,25]。经过一系列抗生素的诱导和土壤传播介质的运行过程，抗性基因已经广泛的分布在全球土壤环境中，很明显，土壤已经成为各类抗性基因最大的基因库[26]。考虑到四环素类和红霉素类在全球范围内的高使用频率和水平，其对应的抗性基因 tet 和 erm 基因在区域土壤中的残留丰度被表现在图 1。在一些国家的耕地中，比如挪威，法国和中国，土壤中的抗性基因丰度超过了 7.0，甚至超过了 8.5(log copies/g)。而在丹麦和英国，其土壤中抗性基因的残留丰度和水平就相对低些，各类抗性基因残留丰度范围在 4.3-7.2 之间。由于不同地区各类抗生素的使用量和传播环境条件不同，土壤中不同抗性基因残留水平与丰度也不尽相同。在荷兰农田中，β-内酰胺酶类，红霉素类以及四环素类抗性基因的残留丰度分别在 2.8-4.7,4.1-4.9 和 4.4-5.3，而三类抗性基因中分别以 blaTEM，erm(F)和 tet(Q)的丰度最高[14]。在意大利，erm，tet 基因丰度分别在 5.9-7.3 和 6.5-8.0，其土壤中 tet(W)和 erm(V)的丰度最高。而在中国耕田中，tet 基因被频繁的检测出，其中以 tet(C)丰度最高，为 8.9，而 tet(M)的丰度最低，低于 2.0[27]。因此，土壤中高浓度抗生素残留对造成多种类和高丰度抗性基因的出现创造了可行条件。另外，抗性菌株和耐药微生物的传播机制，条件和环境也为抗性基因的更广泛分布扩散和富集提供了可能，加剧了抗性基因污染土壤的修复难度。目前，兽用抗生素的使用量剧增且已经超过医疗抗生素，在未来几年，土壤中抗性基因残留丰度的持续增加将成为可预见的趋势，因此土壤生态纳污能力正面临严峻的考验。土壤中抗性基因的消解能力主要与抗生素残留种类和浓度，抗性基因传播途径以及抗性基因的扩增传播速率有直接的关系。因此，严格控制土壤抗生素的残留量是避免抗性细菌和抗性基因出现的最有效的途径和方法。

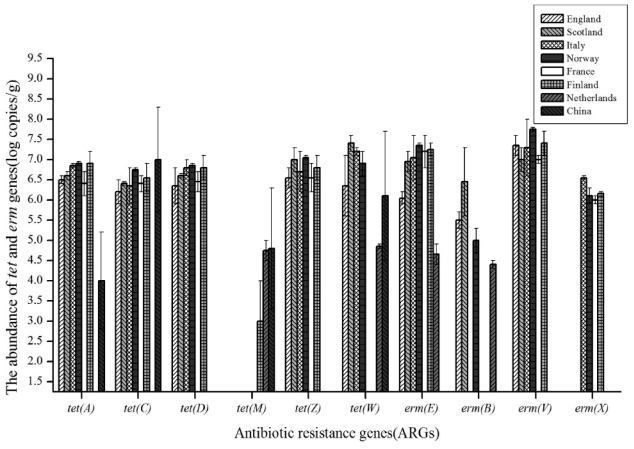

图 1 农田土壤中抗性基因 tet 和 erm 的残留丰度[14,25,27-30]

Fig. 1 The abundance of tet and erm genes in farmland soils

土壤抗性基因出现后，紧接着将会在土壤微生物群落间扩散转移。经过对比发现，含抗生素残留的土壤对土壤微生物和群落结构有显著地抗性诱导，并且在这种诱导能力的促使下，各类抗性细菌和抗性基因逐步出现。Heuerh 和 Smalla 发现[31]，添加猪粪的土壤，抗性细菌和耐药微生物的数量有显著的增加。同样，在泰乐菌素污染的土壤中，微生物群落也出现短暂的耐药性[32]。随着各种传播载体和媒介的作用，进一步加剧了耐药微生物和抗性基因的转移，并且转移后的抗性微生物对土壤

原著微生物群落同样具有一定的诱导风险。以上这种抗性基因的出现 - 传播 - 再出现的循环风险将进一步证实扩散传播对抗性基因的生态风险能力。与此相反,也有研究者认为抗性细菌和耐药微生物的出现与抗性基因的传播转移并没有必然的关联。Sudeshna 等[33]证实,对比施有机肥的土壤与对照土壤,其抗性微生物的数量和水平并没有发生明显的变化,并未出现抗性微生物的出现 - 传播 - 再出现的递增模式。Davelos 也认为,在人类未发现抗生素之前,许多土壤中抗性细菌就已经存在,提出抗性细菌与抗生素残留浓度和传播水平没有一定的相关性[33-35]。

2 土壤抗性基因的生态风险

土壤中高浓度抗性基因会引起一系列潜在的生态风险,比如,侵入动物及人体细胞组织[36,37],渗透植物根系并富集于植物体内[38],感染或诱导原著微生物群落并破坏菌落结构和多样性。目前,在食用动植物体内已被检出耐药菌株和抗性基因。如 Aarestrup 等[39]分别从人、鸡、猪体内分离出屎肠球菌和粪肠球菌中发现,3 种不同来源的球菌有相似的耐药谱和耐药基因,如氯霉素耐药菌均含 catpIP501 耐药基因,耐庆大霉素屎肠球菌均含 aac6-aph2 耐药基因,四环素耐药菌含 tet(M)耐药基因,表明耐药细菌可能会在人和动物间传播。另据报道,一些致病细菌(*R. solanacearum* 和 *Acinetobacter sp.*)能通过根系吸收进入烟草体内[40]。通过观察抗性基因在转基因植物和土壤微生物之间的传递过程,发现抗性基因也存在于转基因植物中,bla_{TEM}116 基因在转基因玉米(Bt176)中被频繁检出[41]。土壤中抗性基因残留对作物生长和生态影响不容忽视。

除了对食用动物和植物的生态影响之外,土壤耐药细菌和抗性基因对土壤原著微生物的数量和功能产生不利的影响,使得耐药和致病细菌更广泛的分布于土壤中,并可能致使微生物多样性的减少以及土著微生物生物功能的缺失,极大程度上破坏原生态平衡。Chee-Sanford 等[42]认为,抗性细菌和耐药微生物加剧了土壤原著微生物的抗性转变。因此,土壤中大量微生物群落在高浓度抗生素的选择压力下,可能转变成抗性菌株和耐药细菌,直接导致群落数量减少和土壤生物活性功能的减弱。抗生素化学特性以及对土壤微生物活性影响见表 1。

表 1 土壤中抗生素的化学特性以及残留水平

Table 1 Properties of antibiotic and the residual level in soils

Antibiotics	Half-time (d)	Medial lethal concentration(EC_{50}) (mg/L)	Soil adsorption coefficients (Koc)	Residuals in soils (mg/kg)	References
Tetracycline	24.2-48	2.2	40000	0.02-0.9	[2,6,20,43]
Oxytetracycline	18-79	4.18	27792-93317	0.027-2.68	[9,44-48]
Ciprofloxacin	18.3-43.9	0.25	1127-61000	0.37-0.4	[6,19,20,45,49]
Norfloxacin	91.2	30.78	7800-15800	0.015-0.15	[5,13,50,51]
Enrofloxacin	10.2-180	0.173	16510-99980	0.02-0.06	[6-7,45,52]
Sulfadiazine	10-67	-	80-170	0.009-0.04	[5-6,53,54]
Trimethoprim	22-41	112	1680-3390	0.003-0.1	[6,13,55,56]
Sulfamethoxazole	12-18	13.7	37-125	0.091-0.48	[52,57,58]
Tylosin	3.3-8.1	0.95	128-7988	0.003-0.05	[59-61]
Penicillin	15-55	84.6	1.67	0.085	[2,13,61,62]

抗生素类药物一旦进入土壤生态环境后,对土壤微生物群落会有很大影响。一方面抗生素能直接抑制或杀死土壤中某些微生物生长繁殖,尤其是对细菌的抑制效果最为明显[63]。同时,残留的抗生素能够影响微生物群落分布和活性功能[64]。另一方面,土壤中高浓度抗生素的残留诱导了抗性菌株的出现和繁殖。Knapp 等[14]发现,在荷兰,某些抗性基因的丰度已经超过 70 年代抗性基因丰度的 15 倍,其原因是有机肥的大量使用导致土壤抗性基因的增加和传播。Sengelov[65]也认为,粪便中高浓度抗生素诱导了农田中四环素类抗性基因的扩散。虽然一些抗性基因可能会随着时间逐渐恢复到先前残留水平,但对于大环内酯类和链霉素,其抗性基因丰度在 8 个月内只有微弱的波动和降低。因此,土壤抗性基因丰度的增加与抗生素的残留水平有直接的原因,在抗生素的选择压力下,土壤中抗性细菌在数量、多样性以及抗性强度上都有明显的提高。这些菌株通过质粒或染色体等媒介在致病性微生物和耐药微生物之间传播转变,使得大量微生物群落不仅携带一定的耐药性甚至是致病性基因,而且很难阻隔其在土壤中的传播途径和方向,这可能是抗性基因最大的生态风险[66]。到目前为止,16 种四环素类耐药基因,3 种磺胺类耐药基因,10 种 β- 内酰胺类耐药基因均在沉积物和土壤等环境介质中被发现[20]。

抗性基因无论是对植物、动物或是微生物的生态风险都将直接或间接影响到人体健康。如果抗性菌株和耐药微生物进入人体免疫系统,对人体健康和组织功能的影响是无法预测的。抗生素抗性基因能够以食物链的方式给人类健康带来潜在的威胁。在许多国家的肉类、牛奶等食品中,检测到的肠球菌携带大量耐药性基因[67,68]。这些抗性基因一旦侵入人体细胞就可能感染正常细胞,从而降低人体免疫力。另外除了肉类传播之外,饮水也是抗性基因进入人体的重要方式。据报道,水体及水生生物体内的各类抗性基因污染已经十分的普遍[69]。饮用水能检测到不同程度的四环素类和磺胺类抗性基因被一再检测出[70,71]。同时,在饮用水源中分离的 Enterobacteriaceae 菌株中有 4 种抗性基因被同时检测出[72]。因此,抗性基因能通过多种途径进入土壤生物和人体系统,这种潜在的生态风险和毒性应该引起足够的重视。

3 土壤中抗性基因的传播扩散过程及影响因素

土壤微生物是抗性基因组的主要携带和传播者,其对抗生素类药剂的抗性一般表现为内在抗性和获得性抗性两种。内在抗性是指某些细菌本身对一些特定抗生素不敏感。而获得性抗性是由于抗生素药物对细菌或真菌遗传环境造成的选择压力而致使其系列基因的改变。土壤微生物可通过多种途径获取抗性。通过以基因突变的方式表达潜在的耐药基因,也可通过不同细菌群落间抗性基因水平传递获得抗性[73]。而抗性基因的水平移动是一个多步骤过程,携带抗性基因的游离分子在特殊环境下能使受体细胞与土壤的细菌细胞之间发生基因的水平扩散转移,从而将抗性基因从游离 DNA 分子中完全的转移到细菌体内,使该细菌获取抗性,而细胞中的遗传物质通过载体(如质粒)、直接(如结合)或者间接(如转化)的形式进一步转移[44]。在这些移动遗传载体如质粒、转座子、整合子等的参与条件下,使得抗性基因能够在同种甚至不同菌株间发生水平扩散,这也极大的促使抗性基因在群落菌株间的传播扩散[74]。比如,转座子就被猜测是抗性基因 TEM β-lactamase (bla_{TEM}) 在 Haemophilus influenzae 和 Neisseria gonorrhoeae 之间传播的媒介[75,76]。此外,携带抗性基因的细菌其自身的代谢也是抗性基因转移的另一途径和方式,这是由于携带抗性载体的细菌死亡后,其细胞内部的裂解,使得体内的 DNA 分子释放到土壤且持久性暴露在生态系统中,并在特定条件和载体的协助下,出现抗性基因水平转移的生态风险[77-79]。

除了借助遗传因子传播转移以外,外部环境因素也是土壤抗性基因传播的重要途径。(1)物理外力,这是一个天然的传播工具致使土壤中耐药细菌和抗性基因广泛的分布在全球土壤环境中,比如:风,河流等自然外力。在美国北科罗拉多州,四环素抗性基因(tetW 和 tetO)在不同的水体(环礁湖和沟渠)被同时检测出[80]。(2)土壤生物,土壤动物为抗性基因的传播提供一种便利和生物机制[81]。一项研究显示,在土壤生物量较丰富的墨西哥,其土壤动物体内检测到携带抗性基因的大肠杆菌数量要明显高于相对贫瘠的澳大利亚[82]。(3)在一定程度上,人类的农业生产和种植灌溉也促进了抗性基因在更广范围的传播迁移。据报道,在英格兰农田中,几乎每个田鼠体内都发现了耐 β- 内酰胺细菌,动物抗性细菌的出现与农用抗生素的使用有直接的联系[73]。总之,抗生素耐药菌株和抗性基因借助以上多种途径已经在全球土壤中传播。即使在偏僻的玻利维亚甚至是无人活动的阿拉斯加也有耐药细菌和 β 内酰胺酶基因被发现[83,84]。所以,土壤抗性基因高效的传播速率和复杂的转移过程应该引起更多的关注。

高浓度抗生素残留及其选择压力是抗性基因转移传播的主导因素,而除此之外,土壤的理化性质等环境条件也影响着土壤抗性基因的转播扩散。土壤 pH,重金属,有机质以及环境温度等条件能影响抗性基因的传播过程。例如,Berg 等[85]发现,经硫酸铜处理的土壤,抗性微生物的数量显著提高。土壤中抗性基因丰度与重金属含量的显著相关性[86]。此外土壤 pH 值也能影响或抑制抗性基因的水平传播,例如:大肠杆菌抗性基因在中性环境中抑制效果最不明显[87]。同时,土壤光照条件能够影响土壤中生物有效性碳的水平,直接影响土壤异养菌与抗性细菌的竞争优势[44]。

4 土壤中抗性基因的传播阻隔和消减方法

4.1 土壤中抗性基因的传播阻隔

抗性基因借助特定的传播机制和水平扩散使得携带抗性的微生物在土壤微生物群落间更广范围的扩散转移,这种转移方向的不确定性和转移过程高效性已成为最严重的生态风险之一,因此阻隔抗性基因的传播方式将成为污染控制的重要途径。目前,土壤中抗性基因的阻隔方法主要从土壤理化性质等外部条件考虑,如:土壤杀菌剂,盐类化合物,湿度,有机质等。其目的在于通过降低微生物群落数量和活性,破坏抗性基因的传播载体和条件,以到达阻隔土壤抗性基因的传播环境。通过调节土壤理化条件可以控制抗性基因在土壤中的转移扩散能力[88,89]。并且考虑到土壤中重金属含量与抗性基因丰度显著的相关性和促进性[90,91],降低土壤重金属含量也将成为修复抗性基因污染的重要途径。另外,外部环境(温度和光照)也能通过提高异养菌数量和活性的方式削弱抗性微生物的竞争优势,以降低抗性基因在土壤群落间的传播水平。

4.2 土壤抗性基因的消减

土壤抗性基因的消解最简单且最直接的方式应从土壤抗生素的降解着手,因为抗性基因的累积是由于高浓度抗生素富集并在相关质粒载体的诱导条件下产生的,而高温、光照以及提高湿度处理都是降解抗生素和抗性基因最常见的方法。

高温是一种能够降低抗生素和抗性基因浓度的重要方法,高温环境能够防止或抑制抗性微生物繁殖生长,并破坏抗性微生物的分子结构。Pei 等[92] 在不同处理温度下抗性基因的降解效果,其中四环素抗性基因 tet(W)、tet(O)以及磺胺类抗性基因 sul1、sul2 在 20℃ 处理后的丰度明显低于 4℃ 环境下的水平。因此生物降解反应器温度越高,其抗性基因的去除效果更加显著。因此,提高温度对土壤抗性基因的抑制和消减有一定的作用,但其最佳温度范围仍有待研究分析。

相比高温环境中,在光照条件下,抗生素抗性微生物和抗性基因能够被加速消减且消减效果较为明显。在无光环境中,土壤微生物初级产物能力较低,耐药细菌与宿主细胞不发生光催化降解。Engemann 等[93]研究表明光照条件下四环素类抗性基因 tet(O)、tet(W)、tet(M)和 tet(Q),的一级消减系数(kd)在 -0.75 至 -0.84d^{-1} 之间,而避光条件下,抗性基因的一级消减系数(kd)仅为 -0.49d^{-1}。另据报道,添加感光剂能对携带抗性基因的 S. aureus 菌株有很好的去除效果[94,95]。在无光的环境介质中,如底泥或废水池中,抗性基因和耐药微生物去除的时间更长,且抗性水平更高。因此,在一定程度上,光照能显著地抑制或降解土壤抗性细菌和抗性基因的残留。

除此之外,土壤含水率也是影响抗生素和耐药微生物去除的关键因素,80%含水率的土壤在消减速率和半衰期方面明显优于 20%,可能与微生物活性有关[96]。同时,也可能与含水率改变带来的土壤间隙度,离子电位和吸附系数的改变有关[97]。改变土壤湿度其本质在于创造最佳的微生物降解条件,但是对于某些抗生素如磺胺类,微生物引起的降解效果并不理想,其原因是抗生素药剂的抑菌或杀菌作用。

相关的研究表明,利用微波 -H_2O_2- 微生物联合降解技术,不仅避免了抗生素的抑菌效果和低降解率,同时在微波 -H_2O_2 降解过程中的产物也被证实对微生物并无毒性和抑制性,并且

最终产物对土壤环境无二次污染。赵方等[98]研究发现，当微波功率为 900W，辐照时间超过 13min，H_2O_2 的添加量 0.25ml 时，磺胺二甲基嘧啶(SM_2)的降解率高达 97%。在微波条件的辅助下，强氧化剂 H_2O_2 能有效破坏分子键和分子结构，使抗生素失去活性和抑菌性，为微生物的进一步降解提供理想条件。抗生素分子结构的改变或者活性基团的取代，将能够有效阻截抗性基因的出现和传播[99,100]，也为土壤抗性基因的新型消解技术提供理论支持。

另外，通过修饰抗生素的作用靶位使抗生素无法与其结合，在很大程度上避免了抗性微生物和抗性基因的产生。通过 miaA 修改 tRNA 紧邻反密码子 37 位的腺苷酸，能有效的降低抗性基因(tetM)的耐药程度；修饰 rpsL30S 亚基的 S12 蛋白对四环素抗性基因(tetO)的耐药性有明显的降低；通过一系列突变和转录，改变 23S rRNA 的 V 区的肽基转移酶区的靶位后，便可使大环内酯类(MALs)和林可酰胺类(LINs)抗生素失活，而此两类抗生素具有交互重叠的联结位点[101]。因此，利用修饰抗生素主要靶位，使其失活和失抑，从而有效阻止抗生素与微生物的结合，避免抗性基因的富集。另外，改变特异的抗生素外排泵的通透性将抗生素排出细胞外，以此降低细胞内抗生素的浓度，降低微生物抗性和抗性基因诱导风险[101]。研究表明，acrA 和 acrB 基因组成的操作子能与特定外膜孔 TolC 发生协同作用，将大量的抗生素搬运到细胞外[101]。关于抗生素靶位的修饰、基团取代以及外排泵通透性的改变的研究并不成熟，目前对于相关的消减技术仅仅停留在理论研究，这些生物技术运用到大规模土壤抗生素污染修复工作还有很大难度，因此涉及到实践工程运行还未有相应的报道。

5 结论与展望

当前，关于土壤抗性基因的消除和抗性基因的传播阻隔技术研究较少，一些涉及到抗性基因消减的方法主要从降解土壤抗生素残留浓度方面考虑，对于土壤抗性基因和抗性微生物的消减方法仍处于理论性研究，而用于规模化土壤抗性基因污染修复困难很大，并且单种抗性基因和抗性微生物消减技术仍然存在降解周期长、降解率不稳定、工程运行难等缺点，需要进一步探讨新型消减技术(微生物消减、植物吸收消减)，或者通过将传统的抗生素降解方法(高温、光催化、生物吸附等)和新型的抗性基因消减方法(修饰作用靶位、改变外排泵通透性以及取代关键基团)进行优势联合，研究确定最佳联合消减技术和抗性菌群的去除方法。但目前，土壤中耐药微生物和抗性基因污染已经广泛存在，其生态风险缺乏严格的管控，而涉及到土壤抗性基因的去除方法仅仅停留在抗生素的降解。对于相应的抗性基因和抗性细菌的消减技术，如：关键基因基团的替代、细胞膜上形成多糖类的阻隔等，仅仅存在实验性研究且缺乏完善的消减运行过程，这将大大增加土壤抗性基因污染的生态风险。因此，在未来关于抗性基因消减技术的创新将是降低土壤抗性基因污染风险的前沿方向，并且整合多种修复技术和阻隔抗性微生物的传播途径将对控制土壤抗性微生物和抗性基因的产生和扩散传播起至关重要的作用。

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