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Bt 毒素表达应用及其残留风险与免疫检测研究进展

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摘要: Bt 毒素是苏云金芽孢杆菌产生的一类生物大分子蛋白质,对多种常见的农林害虫甚至卫生媒介蚊虫都有特异性毒杀活性,是具备重大经济价值和生态环境效益的绿色抗虫材料。然而,随着 Bt 毒素制剂和转基因抗虫作物长期应用,致使靶标害虫抗药性进化加快,并对非靶标生物的交互毒性等潜在风险加大,因此对其残留监测成了农业食品和环境安全风险评估的重要内容。本研究梳理了 Bt 毒素传统的依托微生物表达体系的制剂和植物表达体系的转基因抗虫作物应用及其对靶标害虫抗药性和非靶标生物交互毒性潜在风险的研究现状,概述了针对 Bt 毒素残留分析的免疫检测研究进展;并结合本研究团队近年来依托热门的噬菌体展示抗体库技术,在 Bt 毒素特异性基因工程抗体创制以及 Bt 毒素抗虫模拟物靶向设计等方面的最新研究成果,探讨了基于 Bt 毒素的新型安全杀虫蛋白质创新研发与应用策略及其毒素蛋白质残留检测技术创新等未来潜在发展动向和可行捷径,为进一步围绕 Bt 毒素的相关研究提供有价值的文献资料和新的思路。

关键词: Bt 毒素; 杀虫蛋白质; 蛋白质表达; 转基因作物; 农药残留; 免疫检测
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Research progress on the expression and application of Bt toxin and its residue risk and immunoassay

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Abstract: Bt toxin is a kind of biological macromolecular protein produced by *Bacillus thuringiensis*, which has specific toxic activity for many common agricultural and forestry pests and even health mosquito vectors, and is a green insect-resistant material with great economic value and ecological and environmental benefits. However, with the long-term use of Bt toxin preparations and Bt-transgenic crops, the potential risks of their exposure, such as driving the evolution of resistance to target pests and cross-toxicity to non-target organisms, have attracted much attention. Therefore, monitoring their residues has become an important part of agriculture, food and environmental safety risk assessment. This paper reviewed the current status of research on the application of Bt toxin traditional preparations based on microbial expression system and transgenic insect-resistant crops based on plant expression system, as well as the potential risk of resistance to target pests and cross-toxicity of non-target organisms, and summarized the research progress of immunoassay for Bt toxin residues monitoring. Combined with the latest research results of our research team in the creation of Bt toxin-specific genetic engineering antibodies and the

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targeted design of Bt toxin anti-insect mimics based on the popular phage display antibody library technology in recent years, the innovative research and development and application strategies of new safe insecticidal proteins based on Bt toxins and the future potential development trends and feasible shortcuts of technological innovation in the detection of toxin protein residues were discussed. This paper can provide valuable literature and new ideas for further research on Bt toxin.

Key words: Bt toxin; insecticidal protein; protein expression; genetically modified crops; pesticide residue; immunoassay

Bt 毒素是苏云金芽孢杆菌(*Bacillus thuringiensis*)代谢产生的具有高特异性靶向抗虫功能的生物大分子蛋白质,现已认定命名的 Bt 毒素包括 Cry、Cyt、Vip 和 Sip 等 4 大类型总计超过1 000余种亚型,它们的相对分子量大多介于25 000~135 000 Da,杀虫谱涵盖鳞翅目、鞘翅目、双翅目、半翅目、膜翅目以及线虫和蜗牛等多种常见农林害虫和卫生媒介蚊虫[1]。尽管 Bt 毒素种类多、杀虫谱广,但它们中绝大多数亚型对靶标害虫的作用机制基本被锁定为与相应虫体中肠壁细胞膜上特异性膜蛋白受体的系列级联互作,从而导致虫体肠道功能消化吸收紊乱,最终引起虫体生长发育受阻直至死亡[2]。目前,Bt 毒素以微生物表达制剂和转基因抗虫作物形式被广泛用于害虫绿色防治,单是在中国登记的现行的有效成分中含 Bt 毒素的微生物农药就有 240 余种[3],而其商品化的转基因作物涉及水稻、玉米、大豆、马铃薯、棉花、烟草等重要作物类型,每年在全球的种植面积接近2×10^8 hm^2,带动产生了巨大的经济价值和社会生态效益[4]。不过,自 20 世纪 30 年代和 90 年代商品化的 Bt 毒素制剂和转基因抗虫作物先后面世以来,Bt 毒素产品在全世界连续推广应用已近百年,由其长期叠加蓄积诱发的靶标害虫抗药性[5]以及对非靶标生物的交互毒性[6]等潜在风险问题日益凸显,尤其是近年来,有关其转基因作物食品的安全性备受舆论关注[7],争议不绝于耳。中国早在 2001 年就由国务院颁布了《农业转基因生物安全管理条例》,2023 年农业农村部颁布最新修订的《转基因植物安全评价指南》,国家层面上一直高度重视转基因及其产品研发应用,同时也不断明确要求加大转基因及其产品的监督检查与安全评价力度。基于抗体-抗原特异性识别原理的免疫检测是在蛋白质层面上追踪和筛查 Bt 毒素的最常用方法,特别是酶联免疫分析法(Enzyme-linked immunosorbent assay,ELISA)和金标侧流免疫层析法(Lateral flow immunoassay, LFIA)已被纳入国家标准《转基因产品检测 蛋白质检测方法》(GB/T 19495.8-2004)用于 Bt 毒素检测。当前抗体已从传统多克隆抗体(Polyclonal antibodies, pAbs)和单克隆抗体(Monoclonal antibody, mAb)发展到了形式更为多样的人工基因工程抗体(Genetically engineered antibody, GEAb)阶段,依托这些抗体材料衍生出了借助比色(Colorimetric)、荧光(Fluorescence)、化学发光(Chemiluminescence)、电化学(Electrochemical)、光电化学发光(Photoelectrochemical)、表面等离子共振(Surface plasmon resonance)以及肉眼可视化试纸(特指 LFIA)等技术手段的可用于 Bt 毒素快速追踪筛查的免疫分析方法[8]。基于此,在系统梳理 Bt 毒素表达应用及其残留风险研究现状的基础上,重点概述了有关免疫检测方法在 Bt 毒素追踪筛查上的研究进展,并结合本研究团队近年来在基因工程抗体靶向设计与应用上的最新研究成果和相应研究经验,对围绕 Bt 毒素的杀虫蛋白质创新研发与应用策略以及相应毒素蛋白质追踪筛查技术的未来发展动向进行探讨,以期为开展相关研究提供新的、有价值的文献资料,同时为相关研究开拓新思路。

1 Bt 毒素表达应用研究现状

Bt 毒素在靶标害虫防治应用上主要依托基于微生物表达体系的制剂和基于植物表达体系的转基因抗虫作物两种形式。就微生物表达体系的制剂而言,商品化的 Bt 毒素制剂产品几乎都是依托苏云金芽孢杆菌无晶体突变株进行表达,相应配套的表达质粒载体较为成熟,毒素产物结构较为完整、构象相对稳定[9],蛋白质表达量及其活性受到 sigma 70 家族(Sig A/E/K/H)转录起始因子、Spo0A~P 孢子形成调控因子、sigma 54 家族(Sig L)和多聚磷酸盐激酶(PPK)代谢调控因子以及辅助蛋白质 P20 等多重因素的复杂协同调控[10]。而在实验室研究阶段,大肠杆菌(*Escherichia coli*)凭借其较为清晰的遗传背景和成熟的配套质粒载体[11],几乎成为了包括 Bt 毒素在内的外源

蛋白质室内小剂量表达分析和初步应用的首选菌株[12]。此外毕赤酵母(*Pichia pastoris*)[13]、球孢白僵菌(*Beauveria bassiana*)[14]、发光杆菌(*Photorhabdus temperata*)[15]、荧光假单胞菌(*Pseudomonas fluorescens*)[16]、乳酸链球菌(*Lactococcus lactis*)[17],甚至杆状病毒 baculovirus-sf 9 细胞表达体系[18]和噬菌体 phage-大肠杆菌表达体系[19]都有用于表达 Bt 毒素的研究报道,文献可查的涉及 Bt 毒素表达的微生物菌株及相应配套的质粒载体见表 1 所示。就植物表达体系的 Bt 毒素转基因抗虫作物而言,目前仅有 Cry1Ab、Cry1Ac、Cry1Fa2、Cry2Ab2、Cry2Ae、Cry3Bb1、Cry9C、Cry34Ab1、Cry35Ab1 和 Vip3Aa19、Vip3Aa20,以及人工改造的 Cry1A.105、mCry3A 和 eCry3.1Ab 等为数不多的亚型转基因抗虫作物实现了商品化推广应用[5,20]。不过涉及 Bt 毒素的转基因作物研发一直是业界持续关注的热点,供试的 Bt 毒素种类众多,相关作物现已涵盖水稻、玉米、小麦、大豆、花生、鹰嘴豆、豇豆、卷心菜、油菜、烟草、棉花等主要粮食作物和经济作物。涉及的 Bt 毒素蛋白质在相应转基因作物植株根、茎、叶以及花粉、果实等不同部位中的表达量差异较大,总体来说在叶片中的毒素蛋白质表达量相对较高,大多能达到 μg/g 级别,在花粉、果实中表达量普遍较低,一般都处于或低于 ng/g 级别[21-23]。文献[24]至文献[54]中报道的 Bt 毒素在鲜叶中的蛋白质表达量在μg/g级别(表 2)。这是因为在构建转基因抗虫作物品系过程中,往往会以靶标害虫对作物植株取食部位的偏好性(多数为叶茎)为导向,从而设计组织特异性驱动的启动子,如用于转基因水稻的 pGreen 启动子[21]、用于转基因玉米的 ubi 启动子[22]和用于转基因土豆的 Lhca3 启动子[23]等都是靶向定位在相应转基因作物植株叶片的特异性高效表达启动子,这种设计策略在有效防治害虫的同时,也能最大限度减少毒素蛋白质在这些农作物的食用组织部位的残留蓄积。

表 1 可用于表达 Bt 毒素的微生物及相应配套质粒载体

Table 1 Microorganism strains and corresponding plasmid vectors for expressing Bt toxin

主要表达体系	常见菌种类型	配套质粒载体
苏云金芽孢杆菌	4Q7⁻、BMB171、YBT020、Cry⁻B、HD73⁻、HD1⁻、SP41、XBU001、BFR1、Tt14、YG1、LG101、HDsigK⁻	pHT、pBMB31-304、pSTK、pSV、pHY300PLK、pSB909.4
大肠杆菌	BL21(DE3)、BL21(pLysS)、Rosetta (DE3)、HB2151、JM109	pET、pGEX、pT7-7、pMEx-B4A
其他微生物	*Pichia pastoris*	pPICZB、pPICZαA
	Beauveria bassiana GIM3.428、BbV28	pBARGPE1、pAN52
	Photorhabdus temperata K122	pBS、pBC
	Lactococcus lactis MG1363、KP1	pTRK
	Asticcacaulis excentricus	pSOD
	Pseudomonas fluorescens MB214	pMEKm12
	Baculovirus BmCPV、AcMNPV、AgMNPV	pDEST & pTrans-H1、pGEM-T & pBlueBacIII、pPolh-3006BiKTI、pOBII-T、pB(1-5)B & pAcUW-3006ProAaIT
	Phage	pHEN、pR2、pIT2、pCANTAB5E、pComb3、pIMS147

2 Bt 毒素残留风险研究现状

自 Bt 毒素制剂及其转基因抗虫作物推广应用以来,对其残留及暴露风险不间断持续性监测与评估是相关农业食品和生态环境安全研究的重要内容。长期跟踪研究结果表明,采用常规制剂喷洒和转基因作物表达应用方式,在自然条件下,Bt 毒素蛋白质在土壤和转基因作物组织中的残留量半降解期普遍在20~35 d,且 1 年内降解量均可达到 85%以上,完全降解则需要3~4 年甚至更长时间[55-56]。不过随着 Bt 毒素制剂和转基因抗虫作物长期广泛应用,其残留或叠加累积残留风险越发突出,其中驱动靶标害虫抗药性进化风险是包括 Bt 毒素在内的几乎所有农药在长期广泛使用后都会出现的问题。自 20 世纪 90 年代在田间自然环境中首次发现小菜蛾(*Plutella xylostella*)对 Bt 毒素产生抗药性以来,目前至少包括小菜蛾、草地贪夜蛾(*Spodoptera frugiperda*)、棉铃虫(*Helicoverpa armigera*)在内的 13 种常见鳞翅目害虫以及包括美洲玉米根萤叶甲(*Diabrotica virgifera virgifera*)和山杨叶甲(*Chrysomela tremulae*)

在内的 2 种鞘翅目害虫在野外已经被监测到对一种或多种 Bt 毒素产生了抗药性(表 3)。此外,遍布世界的相关研究机构在实验室条件下特异性筛选的各种靶标害虫抗性品系更是不胜枚举[5],这些品系的潜在逃逸风险极大地增加了野外环境中靶标害虫对 Bt 毒素抗性进化的不确定性。

表 2 Bt 毒素转基因作物及其毒素蛋白质在鲜叶中的表达量

Table 2 Bt toxin transgenic crops and the expression of their toxin proteins in fresh leaves

Bt 毒素亚型	表达作物	鲜叶中蛋白质表达量(μg/g)	参考文献
Cry1Ab	水稻	1.95~34.09	[24]
Cry1Ab/Cry1Ac	水稻	1.80~11.50	[25]
Cry1Ac/Cry1I-like	水稻	1.05~1.51	[21]
Cry1Ab/Vip3A	水稻	1.10~5.05	[26]
Cry1C	水稻	0.71~3.13	[27]
Cry2Aa	水稻	9.65~12.11	[28]
Cry2AX1	水稻	0.68~1.34	[29]
Cry1Ab	玉米	0.76~8.48	[30]
Cry1Ac	玉米	0.26~0.48	[31]
Cry1Ah	玉米	0.88~1.13	[32]
Cry1C	玉米	1.39~4.03	[22]
Cry1Ab/Vip3Aa	玉米	2.55~22.24	[33]
Cry3Bb1	玉米	3.80~228.40	[34]
Cry1Ca5/cry1Ba1	土豆	0.23~8.40	[35]
Cry1Ac	大豆	9.10~13.40	[36]
Cry8-like	大豆	5.00~16.00	[37]
Cry1Aa	鹰嘴豆	接近 0.15	[38]
Cry1Ac	鹰嘴豆	0.11~0.36	[39]
Cry1Ab/Cry1Ac	鹰嘴豆	5.00~40.00	[40]
Chimeric Cry1Aabc	鸽嘴豆	9.59~52.67	[41]
Cry2Aa	鸽嘴豆	25.00~80.00	[42]
Vip3Ba	豇豆	0.25~5.00	[43]
Cry1Ac	卷心菜	接近 0.23	[44]
Cry1C	油菜	接近 0.80	[45]
Cry1AcF	花生	接近 0.82	[46]
Cry1Ac	棉花	接近 0.25	[47]
Cry1F	棉花	1.21~33.18	[48]
Cry9C	棉花	39.70~50.20	[49]
Cry10Aa	棉花	4.05~19.57	[50]
Cry2Ah	烟草	4.41~40.28	[51]
Cry1Fa	牧草美洲雀稗	1.40~4.50	[52]
Cry1Aa	蓖麻	0.16~2.76	[53]
Cry1Ab/Cry1Ac	黄麻	19.00~25.00	[54]

非靶标生物的交互毒性也是 Bt 毒素在推广应用过程中关注的重点。大量研究结果表明,在科学理性剂量条件下的毒理试验中,Bt 毒素对包括人类、小鼠、兔子、羊在内的哺乳动物[20]以及鹌鹑[57]、斑马鱼[58]、蜜蜂[59]、蚯蚓[60]、捕食性天敌草蛉[61]等典型代表性实验生物均未观测到明显异常的毒副作用表征,但也无法完全排除其可能存在的潜在风险。有迹象表明,Bt 毒素残留可能会导致某些环境微生物多样性结构亚失衡[55,62],只是相关研究结果尚存争议。目前,有确切证据表明,部分 Bt 毒素对非靶标生物家蚕(*Bombyx mori*)、二星瓢虫(*Adalia bipunctata*)和秀丽隐杆线虫(*Caenorhabditis elegans*)具有较强的特异性交互毒副作用(表 3),部分 Bt 毒素还对个别寄生蜂种类如中红侧沟茧蜂(*Microplitis mediator*)[63]、内寄生小黄蜂(*Palmistichus elaeisis*)[64]、赤眼蜂(*Trichogramma chilonis*)[65]的卵孵化和幼虫发育存在一定毒副作用。总的来说,相较于化学农药,除了同样存在不可规避的驱动靶标害虫抗药性风险之外,Bt 毒素对非靶标生物的交互毒性风险仍然是现有可用的所有同等药效农药类型中相对最低的。Bt 毒素制剂及其转基因抗虫作物所带动产生的巨大经济价值和生态环境效益奠定了其在害虫绿色防控上的引领地位,并在可预知的未来相当时期内几乎不可替代。

3 Bt 毒素残留免疫检测研究现状

免疫检测是基于抗体-抗原特异性结合互作识别的分析方法,具有操作简便、反应快速、特异性强、灵敏度高等特点,现已广泛应用于包括 Bt 毒素在内的靶标抗原快速筛查监测中。免疫检测法的核心基础材料是抗体,而当前抗体形式已经从传统天然 pAbs 和 mAb 发展到了人工修饰的 GEAb 阶段。天然抗体中,除羊驼和鲨鱼等极少数为先天缺失轻链结构的特殊抗体外,其他高等级动物均为包含了典型双重-轻链结构的“Y”型抗体,而 GEAb 则为人工修饰而成的天然抗体的完整抗原结合片段,较为常见的如天然“Y”型抗体的单重-轻链由柔性短肽拼接而形成的单链抗体(scFv)及其单个重链或单个轻链的单域抗体(sDAb)和源于羊驼或鲨鱼的单重链纳米抗体(Nbs)[66]。目前,基于这些抗体形式,采用单抗体或双抗体组合等策略,结合特异性标记物及相应探测手段,衍生出了包括比色的酶联免疫分析(ELISA),肉眼可视化试纸 LFIA 以及荧光、化学

发光、电化学发光、光电化学发光、表面等离子共振等特征性发光探测乃至免疫 PCR 等形式多样的免疫检测方法,均可用于 Bt 毒素残留的追踪筛查,相关代表性研究实例见表 4。

表 3 Bt 毒素主要生物风险及相应 Bt 毒素亚型

Table 3 Main biological risks and corresponding subtypes of Bt toxins

风险形式	生物	目前发现已诱发生物风险的 Bt 毒素亚型
靶标害虫抗药性	小菜蛾	Cry1Ac、Cry1Ba、Cry1F、Cry1J、Cry2Ad
	草地贪夜蛾	Cry1Ab、Cry1Ac、Cry1F、Cry1Ca
	粉纹夜蛾	Cry1Aa、Cry1Ab、Cry1Ac
	甜菜夜蛾	Cry1C
	灰翅夜蛾	Cry1Ac、Cry2Ab、Cry2Ae
	谷实夜蛾	Cry1Ac、Cry2Ab、Vip3Aa
	烟芽夜蛾	Cry1Aa、Cry1Ab、Cry1Ac、Cry2Ab
	一点秘夜蛾	Cry1Ab
	棉铃虫	Cry1Ac、Cry2Ab、Cry2Ae
	棉红铃虫	Cry1Ac、Cry2Ab
	亚洲玉米螟	Cry1Ac、Cry1Ah
	欧洲玉米螟	Cry1F
	小蔗螟	Cry1Ab
	美洲玉米根萤叶甲	Cry3Bb、Cry34、35Ab1、mCry3A、eCry3.1Ab
	山杨叶甲	Cry3Aa
非靶标生物交互毒性	家蚕	Cry1Aa、Cry1Ab、Cry1Ac、Cry1Ai、Cry1Ba、Cry1Ca、Cry1Da、Cry1Fa、Cry1Ia、Cry2Aa、Cry8Ca、Cry9A、Cry9Da
	二星瓢虫	Cry1Ab、Cry3Bb
	秀丽隐杆线虫	Cry3Bb、Cry5Ba、Cry6Aa、Cry21Aa、Cry21Fa、Cry21Ha、Cry55Aa
	寄生蜂	Cry1Ac、Cry1F

ELISA 是基于酶标记(如辣根过氧化物酶 HRP)的特异性显色比色法,其中依托双抗体-抗原互作的夹心 ELISA(DAS-ELISA)和单抗体-抗原互作的竞争 ELISA(IC-ELISA)是 Bt 毒素最为经典的免疫检测方法,其检测灵敏度主要由抗体-抗原互作的亲和力决定,一般而言基于优质的抗体所建立的 ELISA 对 Bt 毒素检测的灵敏度能达到甚至略低于 ng/mL级或ng/mg级;如果进一步优化标记物,如将 HRP 与链霉亲和素(SA)及沸石咪唑盐骨架(ZIF-8)耦合形成 HRP&SA/ZIF-8 复合物标记抗体建立 DAS-ELISA,对 Cry1Ab 毒素检测的灵敏度就可达 pg/mL级或pg/mg级[67]。LFIA 也是 Bt 毒素最为常见的免疫检测方法之一,其中胶体金标记抗体的肉眼可视化试纸 LFIA 最具代表性,商品化产品也最为成熟,只是该方法灵敏度相对较低,一般在 100 ng/mL 级或 100 ng/mg级;不过随着量子点(QDs)[68]、多重荧光-生物素耦合放大效应物(如 FLPL-BSAS)[69]等荧光性标记物应用到 LFIA 上,借助荧光激发显色仪,可实现对 Bt 毒素检测的肉眼可视,检测灵敏度达到ng/mL级或ng/mg级甚至pg/mL级或pg/mg级。ELISA 和 LFIA 作为 Bt 毒素最基础也是最具代表性的两种免疫检测方法,相关研究较多,产品化开发也较为成熟,单是美国 EnviroLogix Inc 公司推出的 Bt-ELISA 试剂盒就涵盖了 Cry1Ab、Cry1Ac[70]、Cry1C、Cry1F、Cry2Aa、Cry2Ab、Cry3Bb1、Cry9C、Cry34Ab1、mCry3A 等亚型,检测限均低于 1 ng/mL或 1 ng/mg,同时其推出的 Bt-LFIA 试纸条也涵盖了 Cry1Ab、Cry1Ac、Cry1F、Cry2Ac、Cry2Ae、Cry3Bb、Cry9C、Cry34Ab1、Vip3A、CryBt11、mCry3A 等亚型,检测限均低于 0.1 μg/mL 或 0.1 μg/mg(http://www.envirotest-china.com/chan-pin-fen-lei/zhuan-ji-yin/)。

表 4 Bt 毒素主要免疫检测方法及相应代表性研究实例

Table 4 Main immunoassay methods of Bt toxin and corresponding representative examples

检测方法		检测对象	评价指标			参考文献
			最低检测限	50%抑制或饱和浓度	线性检测范围	
比色的酶联免疫分析	DAS-ELISA	Cry1Ab(mAb-mAb*)	0.40 ng/mL	7.70 ng/mL	1.00~40.00 ng/mL	[71]
		Cry1Ab(mAb-pAbs*)	0.47 ng/mL	-	2.50~100.00 ng/mL	[72]
		Cry1Ab(pAbs-pAbs*)	1.53 ng/mL	-	1.81~5.53 ng/mL	[73]
		Cry1Ab(scFv-pAbs*)	8.00 ng/mL		18.00~6 230.00 ng/mL	[74]
		Cry1Ac(pAbs-pAbs*)	5.00 ng/mL	-	16.00~250.00 ng/mL	[75]
		Cry1Ac(Nbs-Nbs*)	5.00 ng/mL	-	10.00~1 000.00 ng/mL	[76]
		Cry1B(Nbs-Nbs*)	3.46 ng/mL	-	5.00~1 000.00 ng/mL	[77]
		Cry1Ie(pAbs-mAb*)	0.27 ng/mL	-	0.45~15.71 ng/mL	[78]
		Cry2Aa(pAbs-mAb*)	10.76 ng/mL	358.00 ng/mL	9.00~2 572.00 ng/mL	[79]
		Vip3Aa(mAb-mAb*)	10.24 pg/mL		0.03~0.50 ng/mL	[80]
		Cry1Aa/1Ab/1Ac (V_L-V_L pAbs*)	10.50~16.00 ng/mL	-	-	[81]
		Cry1Aa/1Ab/1Ac/1B/1C/1E/1F(mAb-pAbs*)	6.37~11.35 ng/mL	-	18.00~100.00 ng/mL	[82]
		Cry1Aa/1Ab/1Ac/1B/1C/1F(scFv-pAbs*)	3.14~11.07 ng/mL	-	9.00~250.00 ng/mL	[83]
		Cry1Ab/1Ac/2Aa/2Ab (pAbs-receptor*)	5.03-30.83 ng/mL	53.00~254.80 ng/mL	5.30~696.97 ng/mL	[84]
	IC-ELISA	Cry1B(scFv*)	-	0.84 μg/mL	190.00~1 100.00 ng/mL	[85]
		Cry1C(scFv*)	-	0.39 μg/mL	20.00~4 400.00 ng/mL	[86]
		Cry1F(scFv*)	0.18 ng/mL	11.56 ng/mL	0.92~107.36 ng/mL	[87]
		Cry2Aa(mAb*)	1.05 μg/mL	10.65 μg/mL	1 100.00~60 700.00 ng/mL	[79]
		Cry1Ab、Cry1Ac、Cry1B、Cry1C、Cry1F (scFv*)	-	0.33~0.84 μg/mL	0.04~6.61 μg/mL	[88]
		Cry1Ab、Cry1Ac、Cry1B、Cry1C、Cry1F (sDAb*)	0.03~0.07 μg/mL	0.73~0.89 μg/mL	0.26~1.41 μg/mL	[89]
	HRP&SA、ZIF-labelled DAS-ELISA	Cry1Ab(mAb-mAb*)	4.80 pg/mL	-	0.05~16.00 ng/mL	[67]
	HRP&biotin-SA-labelled DAS-ELISA	Cry1Fa(Nbs-Nbs*)	0.88 ng/mL	-	1.00~100.00 ng/mL	[90]
侧向免疫分析法	Gold-labelled LFIA	Cry1Ab(mAb-pAbs*)	0.10 μg/mL	-	-	[91]
		Cry1Ab、Cry1Ac (mAb-pAbs*)	100.00 ng/mL	-	-	[92]
		Cry1Ac、Cry8Ka (mAb-mAb*)	0.10 μg/mL	-	-	[93]
		Vip-S(pAbs-mAb*)	100.00 ng/mL	-	-	[94]
		Vip3A(pAbs-mAb*)	100.00 ng/mL	-	-	[95]
	FLPL-BSAS labelled LFIA	Cry1Ab(mAb-pAbs*)	10.00 pg/mL	-	0~1 000.00 pg/mL	[69]
	QDs-labelled LFIA	Cry2Ab(pAbs-pAbs*)	2.91 ng/mL	-		[68]
荧光免疫分析法	DAS-TRFIA	Cry1B(scFv-pAbs*)	0.17 ng/mL		1.00~800.00 ng/mL	[96]
	IC-TRFIA	Cry1C(pAbs*)	0.07 ng/mL	60.16 ng/mL	0.96~1 633.60 ng/mL	[97]
		Cry1Ie(scFv*)	0.04 ng/mL	0.73 ng/mL	0.08~6.44 ng/mL	[98]
	INC-TRFIA	Cry1F(pAbs*)	0.03 ng/mL	150.00 ng/mL	0.04~2 000.00 ng/mL	[99]
	QDs-FLISA	Cry1Ab(mAb-pAbs*)	2.96 pg/mL		6.00~200.00 pg/mL	[100]
		Cry2A(Nbs-pAbs*)	0.41 ng/mL	-	2.60~1 000.00 ng/mL	[101]
		Cry3Bb(Nbs-Nbs*)	8.45 ng/mL		31.25~500.00 ng/mL	[102]

续表4 Continued4

检测方法		检测对象	评价指标			参考文献
			最低检测限	50%抑制或饱和浓度	线性检测范围	
	PDs-FLISA	Cry1Ab/1Ac (mAb-pAbs*)	0.25 ng/mL	-	0.50~12.00 ng/mL	[103]
	FDA&Cy3-labelled FLISA	Cry1Ac(mAb-mAb*)	0.01 ng/L	-	0.01~30 000.00 ng/L	[104]
	MNps&Cy3-labelled FLISA	Cry1Ac(mAb-mAb*)	0.10 pg/L	-	0.10~1 000 000 000.00 pg/L	[70]
	CEIA-based LIFIA	Cry1Ab(mAb-mAb*)	33.00 ng/mL	-	13.20~9 900.00 ng/mL	[105]
化学发光免疫分析法	DAS-CLIA	Cry1Ab(mAb-pAbs*)	3.00 pg/mL		8.00~2 000.00 pg/mL	[106]
		Cry2A(pAbs-sDAb*)	0.09 ng/mL	-	0.10~1 000.00 ng/mL	[107]
	IC-CLIA	Cry1Ab(mAb-sDAb*)	6.45 ng/mL	42.68 ng/mL	10.49~307.10 ng/mL	[108]
	HRP&AuNPs-labelled CLIA	Cry 1Ab(mAb*)	0.05 ng/mL	-	0.10~20.00 ng/mL	[109]
	AuNPs-labelled CLIA	Cry1Ab(pAbs*)	1.25 ng/mL			[110]
电化学免疫分析法	CNPs-labelled ECLIA	Cry1Ab(mAb*)	3.00 pg/mL	-	0.01~1.00 ng/mL	[111]
	HRP/Bi-SA based ECLIA	Cry1Ab(Nbs-Nbs*)	0.07 ng/mL	-	0.10~1 000.00 ng/mL	[112]
	Fe_3O_4-NPs、MXene based ECLIA	Cry1Ab(mAb-pAbs*)	1.00 pg/mL	-	5.00~100 000.00 pg/mL	[113]
	Immunomagnetic based ECLIA	Cry1Ab(mAb*)	0.10 ng/mL	-	0.25~4.00 ng/mL	[114]
	APTES、ITO based impedimetric ECLIA	Cry1Ab(pAbs*)	0.37 ng/mL	-	1.00~10.00 ng/mL	[115]
	Fe_3O_4-AuNPs-labelled ECLIA	Cry1Ac(mAb-pAbs*)	0.25 pg/mL	-	0~6.00 ng/mL	[116]
	Stacked Graphene Oxide、Thionine based ECLIA	Cry1C(Nbs-Nbs*)	3.20 pg/mL		0.01~100.00 ng/mL	[117]
光电化学免疫分析法	PAMAM-AuNPs based PECLIA	Cry1Ab(mAb*)	3.00 pg/mL	-	0.01~100.00 ng/mL	[118]
	AuNRs、QDs-MB、QDs based PECLIA	Cry1Ab(mAb-pAbs*)	1.40 pg/mL	-	0.01~100.00 ng/mL	[119]
	S1-AuNPs/QDs based PECLIA	Cry1Ab(mAb-pAbs*)	0.17 pg/mL	-	1.00~100 000.00 pg/mL	[120]
	ESCs based PECLIA	Cry1Ab	-	-	0.30~3 000.00 ng/mL	[121]
表面等离子共振免疫分析法	Au/Ag alloy nanoparticles based SPRIA	Cry1Ab(mAb*)	4.80 ng/mL	-	8.00~1 000.00 ng/mL	[122]
免疫 PCR 法		Cry1Ac(mAb*)	21.60 ng/mL	-	-	[123]
		Cry1Ac(Nbs*)	0.10 pg/mL	-	0~100.00 ng/mL	[124]

* 表示依托的抗体及组合形式,-表示原文未给出相应数据。

基于荧光、化学发光、电化学发光、光电化学发光、表面等离子共振等特征性发光信号探测的免疫分析法又被统称为免疫传感器(表 4),目前在 Bt 毒素检测上的创新研究较为热门。这些检测方法既有偏向的特征性,在标记物耦合材料、信号激发或信号探测上又有一些重叠,它们的共同特点是依托抗体-抗体互作的亲和力,进一步借助特殊发光标记物标记抗体,与 Bt 毒素特异性结合的同时,通过信号激发起到信号放大的作用,从而极大提高对 Bt 毒素检测的灵敏度。不过这些方法大多数处于实验室探索研究的初级阶段,目前还未出现相关成熟的商品化应用产品。其中,荧光免疫分析(FLISA)主要是借助荧光纳米材料标记到抗体上,在特异性结合 Bt 毒素后,通过荧光显色就能探测到 Bt 毒素,代表性的荧光标记物有稀土元素,如量子点、荧光聚合物点(PDs)、荧光素酯(FDA)、磁性纳米颗粒(MNps)荧光微球以及异硫氰酸荧光素(FITC)等,相关 FLISA 对 Bt 毒素的检测灵敏度可以达到ng/mL级或ng/mg级甚至个别可以突

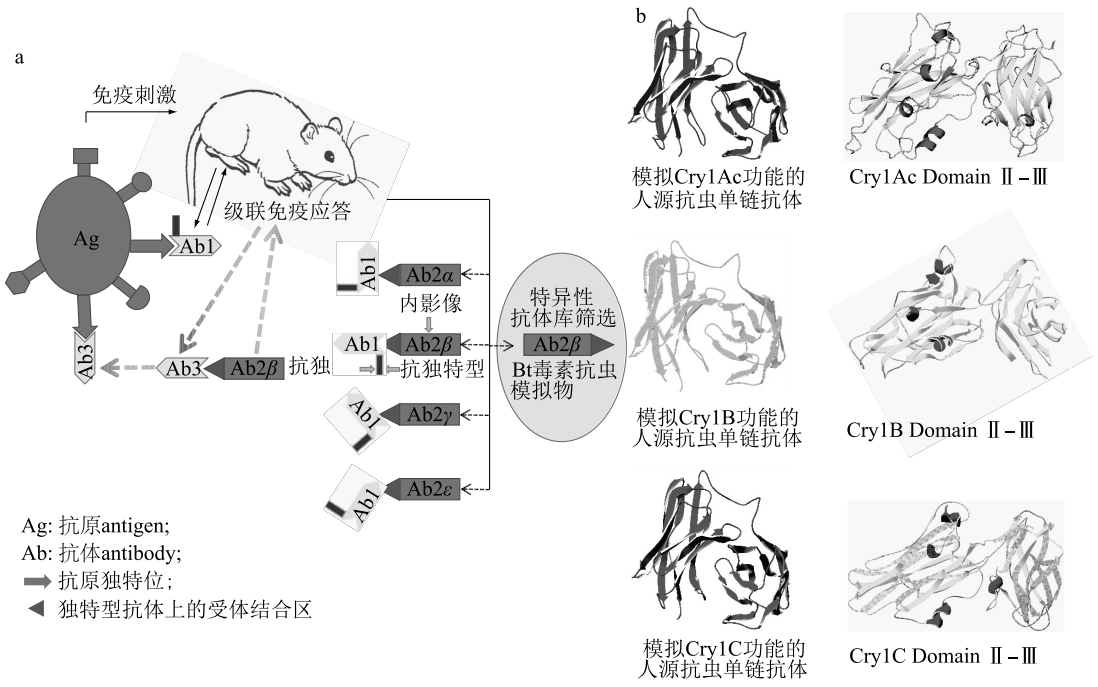
破pg/mL级或pg/mg级[70]。而化学发光免疫分析(CLIA)、电化学发光免疫分析(CLIA)、光电化学发光免疫分析(PECLIA)以及表面等离子共振免疫分析(SPRIA)则都是依托光、电或光电级联的以特征性发光纳米材料标记抗体为基础的,集材料标记、信号激发以及信号探测于一体的检测分析系统,其中金纳米颗粒(AuNPs)、铁离子磁性纳米颗粒(如 Fe_3O_4)和量子点纳米颗粒是较为常见的基础性耦合材料,而相应复合材料信号激发和信号探测方式较为多样,对 Bt 毒素的检测灵敏度也相对较高,一般在pg/mL级或pg/mg级甚至更低(表 4)。这类检测方法在信号材料耦合上较为复杂,对仪器要求较高,依赖性较强,在市场化推广应用上仍然任重道远。此外,基于抗体与特异性核苷酸耦合的免疫-PCR 也有零星涉及 Bt 毒素检测应用研究的报道,不过灵敏度差异较大,相关技术可能还不够稳定。

4 展 望

得益于 Bt 毒素对靶标害虫的高效广谱杀虫活性和对人类及生态环境的高安全性优势,其成熟的和在研的制剂产品及转基因抗虫作物品系几乎遍布全球,引发了害虫防控的绿色革命,也带动产生了前所未有的经济价值和生态环境效益。随着世界范围内害虫绿色防控理念深入人心,农药投入品从高危高毒高残留的化学农药向高效低毒低残留乃至高效无毒无残留的生物农药发展的趋势已成为必然。Bt 毒素作为当前最具代表性的蛋白质类生物材料,其表达应用策略不断创新的同时,对其残留的风险评估和追踪筛查也必将是农业食品和生态环境安全领域持续关注的重点。

围绕 Bt 毒素表达应用,目前尽管采用传统微生物制剂或转基因抗虫作物方式均能实现对相应靶标害虫的绿色防控,但随着靶标害虫抗药性进化,特别是一些单剂型 Bt 毒素产品已经无法满足生产上对害虫防治的需求。近年来,借鉴成熟的化学农药复配经验模式,针对不同农药对相同靶标害虫的不同作用模式特征,特别是设计 Bt 毒素与其他蛋白质类生物农药(如具抗虫功能的凝集素、蛋白酶抑制剂、动物毒素、植物防御素等)甚至其他亚型的 Bt 毒素进行复配的创新应用策略,有望提高对靶标害虫及其抗药性靶标害虫的治理能力,相关复配的制剂或基因融合表达的转基因抗虫作物品系已有部分研究成功的报道[125],但成熟的产品较少,相关研究值得继续推进。此外,近年来以植物内生菌为生防载体搭载外源抗病、抗虫蛋白质基因定殖于宿主作物协同防治靶标病虫害的策略也逐渐受到关注,如 Downing 等[126]以植物内生细菌 *Pseudomonas fluorescens* 搭载几丁质酶基因定殖于大豆防治立枯丝核菌引起的病害,Qi 等[127]以植物内生真菌 *Chaetomium globosum* 搭载半夏凝集素基因定殖于油菜防治蚜虫,均达到了预期效果。目前这种不同于传统微生物制剂和转基因抗虫作物模式的创新应用策略尚未涉及 Bt 毒素的相关研究,值得探索开发。

虽然 Bt 毒素也有残留风险,但是从目前已知可选的农药类型分析,综合靶标害虫抗虫活性、非靶标生物交互毒性以及生态环境危害性等因素考虑,Bt 毒素仍是当前安全系数最高的绿色生物抗虫材料,这基本上已经成为业界共识。但不容忽视的是,在 Bt 毒素制剂产品及其转基因抗虫作物的驱动下,近年来靶标害虫抗药性进化趋势正在加剧,同时存在交互毒性的非靶标生物,特别是经济物种家蚕的生境面临前所未有的胁迫压力。此外转基因跨物种基因漂移和转基因食品潜在安全风险等问题,在有限的时间内既无法肯定也不能完全排除,仍然需要长期跟踪调查和大数据综合评估。目前,除了借鉴化学农药采用交替或复配用药策略外,尚未发现更好的方式来有效缓解靶标害虫对 Bt 毒素的抗药性压力,不过针对非靶标生物的交互毒性,目前在室内条件下是可以通过人工定向突变受体基因(如家蚕中肠受体 ABC 转运蛋白[128])的方式来缓解甚至是抵御 Bt 毒素对其造成的交互毒性。本研究团队近年来借鉴抗体免疫网络理论中 Ab2β 类型抗独特型抗体具有模拟抗原结构乃至生物功能的特性(图 1a),设计以 Bt 毒素抗体为固相包被靶点,并结合相应靶标害虫中肠受体如钙黏蛋白质、碱性磷酸酶等蛋白质的关键功能片段互作信息,从人源化的噬菌体展示抗体库中靶向筛选获得一系列具备初步模拟相应 Bt 毒素部分关键结构和杀虫功能(图 1b)的人源抗虫抗体材料[129],Bt 毒素及其抗虫模拟物对靶标害虫幼虫饲喂 72 h 的校正死亡率如表 5 所示。这类全新的具备模拟 Bt 毒素杀虫功能的抗虫抗体材料,不仅有望缓解靶标害虫对 Bt 毒素的抗药性压力,同时由于其人源属性,理论上对人类免疫系统不会造成明显的异源排斥反应风险,因此与 Bt 毒素相比更具安全性,相关研究结果极具借鉴意义和探索价值。



a:机体级联免疫应答产生抗独特型抗体示意图;b:Bt 毒素杀虫功能模拟物抗虫抗体与相应原毒素关键活性区域结构。

图 1 基于抗独特型抗体模拟抗原功能原理的 Bt 毒素抗虫效应物抗体靶向设计

Fig.1 Anti-insect effector antibody targeting design of Bt toxin based on anti-idiotype antibody mimicking antigen function principle

表 5 Bt 毒素及其抗虫模拟物对靶标害虫幼虫饲喂 72 h 的校正死亡率

Table 5 The corrected mortality of target pest *Plutella xylostella* larvae fed with Bt toxins and its anti-insect mimics for 72 h

供试害虫	测试材料对靶标害虫的致死率(%)					
	Cry1Ac 毒素	Cry1Ac 毒素抗虫模拟物	Cry1B 毒素	Cry1B 毒素抗虫模拟物	Cry1C 毒素	Cry1C 毒素抗虫模拟物
小菜蛾	79.6±1.4	52.1±2.2	71.9±1.8	46.4±1.1	75.4±1.8	58.7±2.3

表中数据为平均值±标准差。

围绕 Bt 毒素免疫检测方面,抗体制备及信号物标记、探测分析是开展相关检测研究的重点,其中抗体是最为核心的基础性材料。目前 Bt 毒素免疫检测无论是产品研发还是技术创新设计,仍然以传统成熟的 pAbs 和 mAb 为主,而 GEAb 尽管受到热捧,但在抗原结合活性和功能稳定性方面普遍不尽如人意,还难以推进应用。值得注意的是,近年来一些新型“拟抗体”功能的生物材料有替代抗体与抗原特异性识别结合并用于 Bt 毒素免疫检测的发展趋势。如靶标害虫中肠受体钙黏蛋白质与部分 Bt 毒素的亲和力可达 1 nmol/L[130],由此 Shen 等[84]以钙黏蛋白质片段与 pAbs 组合建立 DAS-ELISA 对 Cry1Ab、Cry1Ac、Cry2Aa、Cry2Ab 毒素的广谱检测灵敏度达到 5.03~30.83 ng/mL,Wan 等[131]以钙黏蛋白质表位短肽聚合物偶联生物素建立 ELISA 对 Cry1Ab、Cry1Ac、Cry1C、Cry1F、Cry2Aa 毒素广谱识别能力的线性检测范围为 0~50 ng/mL,Wang 等[132]以小菜蛾 BBMV 与噬菌体展示短肽组合建立 DAS-ELISA 对 Bt Cry2Ad 毒素的检测灵敏度达到 8 ng/mL,Lu 等[133]则以噬菌体展示短肽与 mAb 组合建立 HRP/AuNPs 标记的 ECLIA 对 Cry1Ab 毒素的检测灵敏度高达 7 pg/mL,Jin 等[134]依托核酸适配体(Aptamer)建立的类 ECLIA 对 Cry1Ab 毒素的检测灵敏度达到 0.96 ng/mL,Chen 等[135]依托 DNA 探针建立的表面增强拉曼分析法对 Cry1Ab、Cry1Ac 的检测灵敏度高达 0.1 pg/mL。这些新型类抗体功能的生物材料为 Bt 毒素免疫检测创新研发提供了潜在的丰富的可组合甚至是可替代的基础性材料,值得进一步挖掘应用。

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