·专家论坛·

DOI:10.16605/j.cnki.1007-7847.2021.04.0147

杨艳华,博士,副研究员、硕士生导师。近年来主要从事模式生物功能基因组、蛋白质组和植物次生物质代谢方面的研究工作,先后主持或参与国家级及省级课题10余项;共发表研究论文50余篇,其中SCI论文30余篇。

蛋样芽孢杆菌主要毒素及检测方法的研究进展

杨艳华*, 吕　鹏, 陈克平
(江苏大学 生命科学学院, 中国江苏 镇江 212013)

摘　要: 蜡样芽孢杆菌(*Bacillus cereus*)是一种普遍存在的食源性病原菌,可造成腹泻和呕吐两种类型的食物中毒和其他类型的疾病。根据蜡样芽孢杆菌引起食物中毒的症状,其毒素可分为致腹泻型肠毒素和致呕吐型肠毒素,其中溶血性肠毒素BL、非溶血性肠毒素和细胞毒素K是引起腹泻型食物中毒的3种主要毒素,cereulide为致呕吐型肠毒素。当前,蜡样芽孢杆菌污染导致的食物中毒事件时有发生,并严重危害人类健康。因此,了解蜡样芽孢杆菌及其毒素在细胞水平上的作用将有助于预防芽孢杆菌感染;与此同时,快速、准确地检测蜡样芽孢杆菌对有效控制食品污染和感染后治疗至关重要。本文综述了蜡样芽孢杆菌主要毒素的基本情况及检测方法的研究进展,旨在为研究人员开展相关工作提供一定的借鉴和参考。

关键词: 蜡样芽孢杆菌;毒素基因;检测方法
中图分类号: Q939.9, R155　　**文献标识码:** A　　**文章编号:** 1007-7847(2021)06-0471-08

Research Progress on Main Toxins and Detection Methods of *Bacillus cereus*

YANG Yan-hua*, LÜ Peng, CHEN Ke-ping
(*School of Life Sciences*, *Jiangsu University*, *Zhenjiang* 212013, *Jiangsu*, *China*)

Abstract: *Bacillus cereus* is a ubiquitous foodborne pathogen that can cause two types of food poisoning (the diarrhoeal type and the emetic type) and other illnesses. According to the symptoms of food poisoning, its toxins can be divided into diarrheal enterotoxin and emetic enterotoxin. Hemolysin BL, non-haemolytic enterotoxin and cytotoxin K are the three main diarrheal enterotoxins, and cereulide is an emetic enterotoxin. At present, food poisoning caused by *B. cereus* contamination occurs frequently and seriously endangers human health. Therefore, food safety problems caused by *B. cereus* have attracted extensive attention. Understanding the role of *B. cereus* and its toxins at the cellular level would be conducive to preventing the bacterial infection. Meanwhile, rapid and accurate detection of *B. cereus* is extremely important for effective control of food contamination and post-infection treatment. Herein, the research progress of major toxins and detection methods of *B. cereus* was reviewed. It may provide useful reference for researchers to carry out related work.

收稿日期: 2021-04-19; **修回日期:** 2021-07-09
基金项目: 国际(地区)合作与交流项目(31861143051); 国家自然科学基金面上项目(31872425)

作者简介: *通信作者: 杨艳华(1974—), 女, 山东济宁人, 博士, 江苏大学副研究员, 硕士生导师, 主要从事模式生物功能基因组和蛋白质组研究, Tel: 0511-88791702, E-mail: yanhuayang@126.com。

Key words: *Bacillus cereus*; toxin gene; detection method

(*Life Science Research*, 2021, 25(6): 471~478)

蜡样芽孢杆菌(*Bacillus cereus*)是一种在自然界中广泛分布的β溶血性杆状细菌，属于芽孢杆菌属、革兰氏阳性菌，经常在土壤和食物中被发现，在灰尘和污水中也可被分离鉴定。该菌大小为(1.0~1.2) μm × (3.0~5.0) μm，菌体杆状，表面粗糙、扁平、不规则，能够在 8~50 ℃的环境条件下生存，但在低温条件下生长较为缓慢。蜡样芽孢杆菌属于好氧型细菌，在特殊的环境条件下也能兼性厌氧生存，能形成热稳定的内生孢子[1]。芽孢具有高度耐热、耐有毒化学物质、耐 UV 射线、耐γ射线以及其他不利环境因素的特性，内生孢子的这些特点决定了它能够广泛存在于米饭、牛奶、肉类、新鲜蔬菜、海鲜等食物中[2]。蜡样芽孢杆菌是引发食源性肠道类疾病的一个主要病原微生物，对人类和动物的健康构成威胁，包括家畜和野生动物[3~7]。蜡样芽孢杆菌引起食物中毒的主要症状分为腹泻或呕吐[8]。此外，该菌还涉及许多严重并且可能致命的非胃肠道感染，例如严重的眼部感染、骨髓炎、肝炎和炎症反应[9~10]，甚至导致死亡[11]。因此，快速、准确地检测蜡样芽孢杆菌是控制食品污染和感染后治疗的关键环节。本文概述了蜡样芽孢杆菌主要毒素的基本情况及检测方法的研究进展，旨在为研究人员开展相关工作提供一定的借鉴和参考。

1 蜡样芽孢杆菌的主要毒素

蜡样芽孢杆菌能产生多种毒素，根据其引起食物中毒的症状可分为致呕吐型肠毒素和致腹泻型肠毒素。污染食品的蜡样芽孢杆菌可以通过产生这些毒素造成食物中毒。因此，蜡样芽孢杆菌产生的毒素引起广泛的关注。

1.1 致呕吐型肠毒素

致呕吐型肠毒素(cereulide)主要引起恶心、呕吐的症状，其中毒可在儿童和老年人中表现出严重或致命的症状[12~13]。17 岁瑞士男孩和 7 岁比利时女孩致死性的食物中毒均被鉴定由 cereulide 毒素引起，这种毒素会引起儿童恶心、呕吐和肝功能衰竭[14]。Cereulide 可在各器官中聚集[15]，导致线粒体毒性，并伴有肝毒性、脑病和β细胞功能异常等临床并发症[14, 16]。

Cereulide 的结构为[D-O-Leu-D-Ala-D-O-Val-D-Val]$_3$，是一种通过非核糖体途径合成的十二环形多肽，其环形结构使 cereulide 能够抵抗各种恶劣环境，表现出热稳定性(150 ℃, 60 min, pH<9.5)、酸碱稳定性(pH 2~11)以及对蛋白酶(如胰蛋白酶)的稳定性等[17~18]。Cereulide 由 pBCE 质粒上的 *ces* 基因簇合成，*ces* 基因簇由 *cesA*、*cesB*、*cesC*、*cesD*、*cesH*、*cesP* 和 *cesT* 构成[19~20]。其中，*cesA*/*cesB* 的 NADPH 亲和性对多肽的形成具有重要作用[21]；*cesC*/*cesD* 编码 ABC 转移体，对 cereulide 毒素的合成和运输起到一定的促进作用[22]；*cesH* 编码 31 kD 的水解酶，该水解酶属于α/β水解酶家族，可用自身的启动子启动表达；*cesP* 编码磷酸泛酰巯基乙胺基转移酶(phosphopantetheinyl transferase, PPTase)，主要参与非核糖体合成途径的起始。研究表明，pBCE 质粒编码的 cesH 水解酶可能负调节 *ces* 基因簇的合成；染色体基因 *Ppt chr* 和 pBCE 质粒基因 *cesP* 合成的 PPTase 均对 cereulide 的起始合成起促进作用[23]。

Cereulide 引发呕吐的机制目前尚不完全清楚，已有动物实验证实 cereulide 为缬氨霉素类似物，具有与缬氨霉素一样强的钾离子载体特性[17]。Cereulide 具有较高的疏水性，能够通过细胞膜进行扩散，并且对钾离子具有很高的亲和力，这两个特点使其成为最佳的离子载体，可破坏细胞膜的电化学梯度。研究证实，cereulide 的毒性作用正是由于它是一种钾离子载体，它是第一个被证明与食物中毒有关的非蛋白质离子载体[24]。进一步研究发现，2 nmol/L 的 cereulide 可以阻止 HepG2 细胞的增殖[25]，cereulide 也可以引起 HEp-2 细胞的空泡化[26]。当前，一般利用 HEp-2 细胞、鼠肝细胞和野猪精子运动性分析等方法检测 cereulide 毒素的生物活性[27~28]。有文献指出，可利用基质辅助激光解吸电离飞行时间(matrix-assisted laser desorption/ionization-time of flight, MALDI-TOF)质谱技术进行蜡样芽胞杆菌 cereulide 毒素的快速检测[29]。随着科学技术的进步，cereulide 的检测将会更为快捷、方便。

1.2 致腹泻型肠毒素

腹泻与一系列肠毒素有关，包括溶血性肠毒素 BL (hemolysin BL, Hbl)、非溶血性肠毒素(non-haemolytic enterotoxin, Nhe)、细胞毒素 K (cytotoxin

K, CytK)和肠毒素 FM (enterotoxin FM, EntFM)[20, 30]，以及潜在的肠毒素溶血素Ⅱ (enterotoxin hemolysin Ⅱ, HlyⅡ)和肠毒素 T (enterotoxin T, BceT)[31]。其中，Hbl、Nhe 和 CytK 是引起腹泻型食物中毒的 3 种主要毒素[32~33]。Hbl 和 Nhe 均由 3 个亚基组成[32]，而细胞毒素 CytK 由单一蛋白质组件构成，能够引起细胞毒性[34]。上述 3 种毒素的致病机制目前尚不完全清楚，一般认为是由于毒素可在细胞膜上形成孔洞，细胞膜上的孔洞造成细胞内的钠离子和氯离子流失，使细胞电位失去平衡。腹痛、腹泻的症状多在摄入污染的食物 8~16 h 后发生，持续时间为 12~24 h[35]。这种食物中毒主要由污染的牛奶、奶制品、蔬菜引起。不同蜡样芽孢杆菌菌株含有不同的毒素基因，其致病性也不相同[36~37]。

1.2.1　溶血性肠毒素(Hbl)

Hbl 最初从术后病人伤口分离出的蜡样芽孢杆菌菌株 F837/76 中分离得到，其由 HblB、L_1 和 L_2 三个蛋白质亚基构成，它们的产生和分泌独立进行，相对分子质量分别为 37.5 kD、38.2 kD 和 43.5 kD，只有 3 种亚基同时存在时，Hbl 才能产生最大的细胞毒性[38]。HblB、L_1 和 L_2 亚基分别由 *hblA*、*hblD* 和 *hblC* 基因编码，它们的操纵子相同，编码顺序依次为 *hblC*、*hblD* 和 *hblA*[39]。三种蛋白质亚基按一定的顺序结合到细胞膜上，依次为 HblB、L_1 和 L_2。当用对应的单克隆抗体进行检测时，通常会出现相对分子质量相差不大的两条带，这可能是由于各组件产生了同源异型的变构。Hbl 复合体中的 HblB 组件包含一个长的 α-螺旋束和 α/β 头部的结构域，该结构同大肠杆菌毒素蛋白细胞溶血素 A (cytolysin A, ClyA)的结构极其相似，说明它们可能具有相似的作用机制，可使细胞膜形成孔洞[40]。

hbl 基因的表达由 PlcR 蛋白调节，该蛋白质通过与特定序列的结合调节 *hbl* 操纵子的表达。*plcR* 基因的转录发生在稳定期，而在孢子形成期可被孢子形成因子 Spo0A 抑制[41]。三种蛋白质亚基的氨基酸序列分析结果表明，它们的末端氨基酸均含有信号肽序列，说明其通过一般分泌(Sec)途径进行分泌[33]。在细胞实验中，Hbl 能引起细胞膜的裂解，使血脂平板产生溶血现象[35]。Hbl 溶血活性的强弱与 3 种亚基的分子数目相关，只有当 HblB 亚基的相对分子质量高于 $HblL_1$ 和 $HblL_2$ 的相对分子质量时，Hbl 才具有最强的溶血活性。此外，Hbl 对不同细胞系呈现不同的细胞毒性，并且其毒性可以通过 HblB 亚基的抗体中和而降低[42]。已有研究证实，3 种蛋白质亚基构建成的无毒性融合蛋白所制备的抗体可用于 Hbl 毒素的检测[43]。

1.2.2　非溶血性肠毒素(Nhe)

Nhe 是蜡样芽孢杆菌的另外一种毒素，最早在 1995 年挪威发生的一场严重的食物中毒事件中被分离鉴定，引起该食物中毒事件的蜡样芽孢杆菌为 NVH0075/95 菌株[44]。Nhe 由 NheA、NheB、NheC 3 种蛋白质亚基组成，其相对分子质量依次为 41 kD、39.8 kD 和 36.5 kD；它们分别由 *nheA*、*nheB*、*nheC* 基因编码，并且由相同的操纵子调控。氨基酸序列分析发现，NheA、NheB 和 NheC 的末端氨基酸均含有信号肽序列，说明它们通过 Sec 途径进行分泌，但 3 种蛋白质亚基以独立的方式分泌到细胞外[33]。Nhe 和 Hbl 具有序列同源性，而 HblB 与 ClyA 具有较高的结构相似性，因此 Nhe 对来自不同物种的红细胞也具有溶血活性；由于共同的结构和功能特性，Hbl/Nhe 和 ClyA 家族的毒素可能构成一个成孔细胞毒素的超家族[45]。

细胞实验表明，Nhe 对多种细胞存在细胞毒性，但对不同细胞系的细胞毒性不同。Nhe 可在细胞质膜表面形成孔洞，导致上皮细胞和红细胞的裂解，进而造成食物中毒。Nhe 通过其单一组分 NheA、NheB 和 NheC 的有序结合对 Vero 和 CaCo-2 细胞产生细胞毒性，它们的结合顺序依次为 NheC、NheB 和 NheA[46]。研究发现，当 NheA、NheB、NheC 3 种蛋白质亚基的摩尔浓度比为 10∶10∶1 时，其对 Vero 细胞的毒性最强；增加 NheC 蛋白组分的浓度，则降低其细胞毒性；如果将等摩尔浓度的 NheC 添加到含有 NheA 和 NheB 的溶液中，则会完全抑制其细胞毒性[38]。当用NheB 蛋白的单克隆抗体去除 NheB 蛋白或通过加入与 NheB 相互作用的化合物时，Nhe 的细胞毒性几乎完全丧失[42]。上述结果表明，NheB 蛋白组件在Nhe 引起的细胞毒性中发挥着至关重要的作用，过高的 NheC 蛋白组件浓度则降低细胞毒性，其具体的机制有待进一步研究。

1.2.3　细胞毒素 K (CytK)

CytK 最初从蜡样芽孢杆菌菌株 391-98 中分离出来，该菌株引起严重的食物中毒，并造成 3 人死亡[34]。CytK 由单一蛋白质组件构成，其相对分子质量为 34 kD，具有高度细胞毒性，同时也具有坏死性和溶血性，能够在脂质双层中形成孔

洞。CytK蛋白由*cytK*基因编码，该基因启动子区存在PlcR的识别位点，PlcR通过结合到PlcR-box调节*cytK*基因的表达[34]。序列比对发现，CytK与金黄色葡萄球菌的白血球素、γ-溶血素和α-溶血素，产气荚膜梭菌的β-毒素，以及蜡样芽孢杆菌的溶血素Ⅱ相似，均属于β-桶成孔毒素家族；此外，CytK对人肠上皮细胞具有高毒性，并可能导致坏死性肠炎[47]。

Fagerlund等[30]研究发现，CytK-1与其变体CytK-2具有很高的序列同源性，CytK-1和CytK-2蛋白之间的差异主要集中在某些区域；在细胞水平上，CytK-1对Vero和Caco-2细胞具有较高的细胞毒性；CytK-2具有溶血性，并且对人肠道Caco-2细胞和Vero细胞具有毒性，但其毒性约为CytK-1的20%；CytK蛋白的细胞毒性是由于其能在上皮细胞形成孔洞，使细胞外液流入细胞，进而导致细胞的死亡。进一步研究发现，CytK蛋白的末端氨基酸含有信号肽序列，说明它也通过Sec途径进行分泌[33]。

1.2.4 其他腹泻型肠毒素

与3种主要致腹泻型肠毒素Hbl、Nhe和CytK相比，肠毒素EntFM、HlyⅡ和BceT为潜在的腹泻型毒力因子[48]。EntFM含有1个NlpC/P60结构域，具有细胞壁肽酶的特征。该蛋白质涉及细菌的形状、运动性以及上皮细胞的黏附、生物膜的形成、巨噬细胞的空泡化和毒力等，但是关于其毒性的研究相对较少[49]。HlyⅡ为寡聚β-桶成孔毒素，不耐热，对蛋白水解酶比较敏感，与金黄色葡萄球菌的α-毒素或产气荚膜梭菌的β-毒素有关[2]。毒素基因*bceT*含有1个开放阅读框，其编码的多肽由336个氨基酸组成。该基因在大肠杆菌中的表达产物表现出Vero细胞毒性，并且在血管通透性实验中呈阳性；同时，其在结扎的小鼠回肠环中可引起液体积聚，注射后对小鼠具有致死性[50]。

总的来说，腹泻型肠毒素复合物的细胞毒性占蜡样芽孢杆菌总毒性的90%以上。细胞实验表明，Vero和原代人脐静脉内皮细胞(human umbilical vein endothelial cell, HUVEC)对Nhe最敏感，HepG2、Vero和A549细胞系对Nhe和Hbl高度敏感；在大多数细胞系中，Nhe和Hbl对细胞毒性的贡献大致相同(40%~60%)，但是，在HUVEC细胞中Nhe的相对活性约为90%，在A549细胞中Hbl的相对活性约为75%；CytK对CaCo-2表现出最高的细胞毒性[51]。

2 蜡样芽孢杆菌的流行情况

在中国，涉及蜡样芽孢杆菌的食源性疾病通常通过乳制品或乳制品来源的食物发生[7, 52~57]。中国是人口大国，液态奶和奶粉的消费量较大，特定的市场需求和生理差异造成了蜡样芽孢杆菌在中国的流行。2011—2016年在中国主要城市进行的调查结果显示，蜡样芽孢杆菌广泛存在于巴氏消毒奶中，大约27%的巴氏消毒奶含有蜡样芽孢杆菌[53]。报告显示，分离出的100个蜡样芽孢杆菌菌株分布于香港、广州、深圳、哈尔滨、宁夏、北海、海口等城市或省份。总的来说，中国南方乳制品中蜡样芽孢杆菌的污染率相对低于北方地区，其在中国北方的污染率为31%，在南方的污染率为25%。例如，辽宁省婴儿配方奶粉中蜡样芽孢杆菌的污染率为42%，而云南省婴儿配方奶粉中蜡样芽孢杆菌的污染率仅为12%[53]。其他省份或地区，如北京、辽宁、甘肃、云南和东北地区，蜡样芽孢杆菌的污染率分别为30%、27%、19%、10%和16%[54, 58~59]。2012—2013年和2015年发布的两份关于中国市场婴儿配方奶粉的调查报告表明，蜡样芽孢杆菌的阳性率分别为14%和42%[7, 60]。Zhuang等[61]调查发现，中国婴儿配方奶粉样品中蜡样芽孢杆菌的污染率为8.2%。我国乳制品中蜡样芽孢杆菌毒素基因的种类及其检测情况详见表1。其他国家或地区同样也存在蜡样芽孢杆菌的流行情况。美国的一项全国性调查表明，202份生鲜混合牛奶样本中蜡样芽孢杆菌的阳性率为8.9%[62]，德国巴伐利亚测试的冰淇淋中蜡样芽孢杆菌的检出率高达62.7%，而墨西哥销售的手工奶酪中蜡样芽孢杆菌的检出率为28.4%[63]。比较而言，非洲国家由于卫生条件较差，其乳制品更易受到蜡样芽孢杆菌的污染。此外，米饭、凉皮、肉类及肉制品、即食蔬菜等其他食品中蜡样芽孢杆菌的污染也不容乐观，并对人类健康造成严重威胁[64~67]。因此，世界各国均迫切需要解决其境内蜡样芽孢杆菌的污染问题。

3 蜡样芽孢杆菌的主要检测方法

3.1 传统方法

传统的蜡样芽孢杆菌孢子或细胞检测方法包括：平板计数法、免疫学方法和液质联用法(liquid chromatography-mass spectrometry, LC-MS; LC-MS/MALDI-TOF)。平板计数法使用ISO7932标准

表 1　我国乳制品中芽孢杆菌毒素基因的种类及检测情况

Table 1　The positive rate of toxin genes in *Bacillus* samples from dairy products in China

Detection of toxin gene/(%)											Region	No. of *B. cereus* isolate/No. of sample	Origin	Year
hblA	*hblC*	*hblD*	*nheA*	*nheB*	*nheC*	*cytK*	*cesB*	*hly*Ⅱ	*entFM*	*bceT*				
79.0	79.0 (55.0)^a	79.0	100.0	100.0 (100.0)^b	100.0	ND	5.0	ND	ND	ND	Beijing	20/205	Raw milk	2013—2014
55.6	77.8	0	74.1	88.9 (74.1)^b	100.0	33.3	48.2	ND	40.7	73.4	China	43/–^{c}	Ultra-high temperature milk processing line	2014—2015
24.6	22.8	17.5	87.7	87.7	49.1	22.8	3.5	ND	71.9	7.0	Chinese markets	57/135	Infant formula	2015
47.0	68.0 (45.0)^a	68.0	99.0	99.0 (93.0)^b	94.0	73.0	5.0	54.0	96.0	75.0	Major cities in China	70/258	Pasteurized milk	2011—2016
35.5	29.0 (21.1)^a	21.1	75.0	100.0 (75.0)^b	90.8	44.7	2.6	ND	97.4	52.6	Wenzhou	76/400	Milk powder	2015—2016
0	59.1	54.5	90.9	72.7 (72.7)^b	100.0	68.2	ND	ND	ND	54.5	Liaoning	22/176	Milk-derived food	2016
36.0	38.4	38.4	87.2	81.6	86.4	36.8	3.2	ND	87.2	44.8	12 provinces of China	–/125	Milk powder	2018
57.4	68.5 (11.1)^a	16.7	94.4	94.4 (94.4)^b	100.0	75.9	11.1	53.7	85.2	77.8	Heilongjiang, Jilin, Hebei, Henan, Guizhou	54/500	Dairy products	2018—2019

注: a, 同时携带 *hblA*、*hblC* 和 *hblD*; b, 同时携带 *nheA*、*nheB* 和 *nheC*; ND, 未检测; c, 无具体数字。

Notes: a, *hblA*, *hblC* and *hblD* genes simultaneously positive; b, *nheA*, *nheB* and *nheC* genes simultaneously positive; ND, not detected; c, no data.

方法进行蜡样芽孢杆菌的计数和检测, 但该方法无法说明细菌产生毒素的能力, 也不能将蜡样芽孢杆菌与芽孢杆菌菌群中的其他细菌真正区分开来, 因而具有一定的局限性[68]。免疫学方法可针对营养细胞、芽孢和毒素的蛋白质进行检测, 包括鞭毛、细胞表面抗原及 NheA、NheB、NheC、HblL$_1$ 和 HblL$_2$ 等蛋白质[69-70]。酶联免疫吸附分析法(enzyme-linked immunosorbent assay, ELISA)通过靶向特异性单克隆抗体, 直接从蛋白质水平上检测蜡样芽孢杆菌的毒性成分。这种单克隆抗体能够纯化 NheB 和 NheB/C 复合物等毒素, 并中和细胞毒性, 如抗 NheB 组分的单克隆抗体能够中和 Nhe 的细胞毒性(高达 98%)[70-71]。液相色谱-质谱分析(LC-MS/MALDI-TOF) 通常用于快速检测呕吐食品中的芽孢杆菌分离物[7, 72-73]。质谱技术主要是通过细菌组分所拥有的特异峰图进行菌种鉴定, 该方法可用于菌种特异性的鉴定[74]。随着细菌质谱鉴定数据库的进一步完善, 质谱技术的应用也变的越来越简便、快捷、准确。相关报告显示, 从细菌提取物中鉴定出的 cereulide 在 m/z 1 191 处达到峰值, 检测限(limit of detection, LOD)为 30 ng/mL[29]。

3.2　PCR 和 LAMP 法

PCR 基因序列分析法包括普通 PCR、实时荧光定量 PCR (real-time PCR, RT-PCR)、多重 PCR (multiplex PCR, mPCR)、微滴数字 PCR 技术(droplet digital PCR, ddPCR)和交叉引物扩增法(cross-priming amplification, CPA)[75]等, 该方法主要通过靶向 16*S rRNA*[76]、*groEL*/*gyrB*[77]和 *panC*[78]等基因的实时荧光定量 PCR 或交叉引物扩增法等, 对蜡样芽孢杆菌菌株进行鉴定或分析。其中, 普通 PCR、RT-PCR、mPCR 是通过检测毒素基因来鉴定蜡样芽孢杆菌的主要分析技术[7]。普通 PCR 操作简单、耗时短, 但是检测结果的准确性较低, 需要进一步验证。与普通 PCR 相比, mPCR 更加灵敏、快速、准确, mPCR 可以同时检测同源性较高的芽孢杆菌菌群中的菌株, 对于病原菌的检测也更为便捷[79-80]。RT-PCR 的扩增和检测一步完成, 检测周期短, 且特异性强、灵敏度高、假阳性低。同时, RT-PCR 既可以定性也可以定量, 能更有效解决 PCR 的污染问题, 因而应用更为广泛[80]。研究人员将叠氮溴化丙锭染料和 RT-PCR 技术结合, 该方法能有效区分死菌和活菌。与 RT-PCR 相比, ddPCR 检测不需要标准曲线, 可以有效减小误差, 该方法适用于低浓度样本的检测, 灵敏度和稳定性更高。美中不足的是, ddPCR 定量的范围小, 不适合高丰度样本的检测, 并且价格昂贵、耗时[80-81]。事实上, 牛奶中通常含有较低浓度的孢子, 在进行 PCR 分析检测之前需要进行耗时的微生物富集

步骤。此外，牛奶中含有的高浓度离子和脂肪已被证实能抑制 RT-PCR。另一种核酸扩增技术是环介导等温扩增(loop-mediated isothermal amplification, LAMP)，该方法在等温环境下工作，可用于蜡样芽孢杆菌的快速、定点和便携式检测[82~83]。旋转反应芯片(rotate and react SlipChip, RnR-SlipChip) 借助 LAMP 技术被开发用于同时检测包括蜡样芽孢杆菌在内的多种细菌病原体[84]，该方法进样后通过一步旋转使靶菌与芯片上的 3 种试剂立即混合并反应，60 min 内可直接观察识别，成功率达 100%。但是，这项技术能否同时适用于蜡样芽孢杆菌孢子的检测尚有待进一步研究。

3.3 生物传感器

近年来，基于生物传感器和纳米技术的新方法因其简单、灵敏和快速而受到广泛关注。当前，能够有效并且特异性地靶向细菌孢子的识别元件(例如抗体、适体)数量相对较少，因此生物传感器的开发尚处于起步阶段[68]。与其他方法相比，电化学生物传感器因成本低、灵敏度高并且易于小型化而发展较为迅速[85~87]。Mazzaracchio 等[88]开发了一种无标记的阻抗式 DNA 适体传感器，其蜡样芽孢杆菌的检出限为 3×10^3 CFU (colony-forming unit)/mL，线性范围为 10^4~5×10^6 CFU/mL，该传感器的选择性好，被证实具备检测蜡样芽孢杆菌的能力。表面等离子体共振(surface plasmon resonance, SPR)是一种灵敏的无标记微生物检测方法。Kong 等[89]利用噬菌体内溶素修饰的 SPR 芯片检测了蜡样芽孢杆菌的营养细胞，其检测限为 10^2 CFU/mL，该方法采用消减抑制试验提高了 SPR 传感器检测蜡样芽孢杆菌孢子的敏感性。当前，研究人员需要加速开发更加灵敏、快速和高特异性的蜡样芽孢杆菌检测新技术、新方法，以提高蜡样芽孢杆菌的检测能力和准确性。

另外，还有其他方法可用于蜡样芽孢杆菌及其毒素基因的检测，由于篇幅限制这里不再一一阐述。为便于理解，表 2 列出了蜡样芽孢杆菌及其毒素基因检测的主要方法及相应的优缺点。

4 结论和展望

随着生活水平的提高，人们的食品安全意识和对优质食品的需求显著提高。然而，蜡样芽孢杆菌引起的食物中毒事件仍时有发生[7, 68]。蜡样芽孢杆菌是一种产生内生孢子的病原体，可引起食物中毒，症状为呕吐和腹泻，其排出的毒素也是

表 2 蜡样芽孢杆菌及其毒素基因的检测方法

Table 2 Detection methods of *B. cereus* and its toxin genes

Detection method	Detected target	Advantage	Disadvantage
Plate count method	*B. cereus* and its spores	Early official method	Give no indication of the ability of the bacteria to produce toxins
LC-MS/MALDI-TOF	Cereulide	High accuracy	Narrow application scope
Enzyme-linked immunosorbent assay, ELISA	*B. cereus* and its toxin proteins, polysaccharides	High specificity, sensitivity, and precision; low-cost	Complicated steps
Standard PCR	Universal, housekeeping and toxin genes	Rapid and simple	Low accuracy
Real-time PCR, RT-PCR	Toxin genes	Rapid, high sensitivity and specificity; low false positive, and not easy to be polluted	Unable to detect the size of amplified gene
Multiplex PCR, mPCR	Toxin genes	Rapid, accurate, sensitive; multiple bacteria can be identified simultaneously	Nonspecific amplification is easy to occur
Droplet digital PCR, ddPCR	Toxin genes	High sensitivity, accuracy, and stability; lower detection limit	Quantitative range is small, expensive and time-consuming
Cross-priming amplification, CPA	*B. cereus*	High sensitivity and specificity, easy to perform, and requires simple equipment	-
Loop-mediated isothermal amplification, LAMP	*B. cereus*	High sensitivity, specificity and rapidity, low-cost	Less versatile, easy to give false positive
Surface plasmon resonance, SPR	*B. cereus* and its spores	Rapid, specific, sensitive, and label-free	In early phase
Rotate and react SlipChip, RnR-SlipChip	*B. cereus*	Rapid, accurate, simple, economical and visual	-
Electrochemical biosensors	*B. cereus* spores	Low-cost, high sensitivity, and easy to miniaturize	The application is restricted because of low number of recognition antibodies and aptamers

损害肝组织和炎症性疾病(如胃肠炎和脑膜炎)的主要元凶[8, 90]。蜡样芽孢杆菌的毒力因子在细胞毒性中发挥着至关重要的作用, 了解蜡样芽孢杆菌及其毒素在细胞水平上的作用将有助于预防芽孢杆菌感染。在过去的几十年里, 虽然科研人员为确保食品安全付出了艰辛的努力, 但我国乳制品中蜡样芽孢杆菌的污染仍然是一个不容忽视的问题。另一方面, 无毒性的蜡样芽孢杆菌可作为药物用于改善肠道微环境, 因而作为人类的益生菌被利用, 并且这一领域正受到越来越多的关注[91~93]。但是, 有毒性的蜡样芽孢杆菌因携带各种毒素或抗生素耐药性的基因而可能给人类带来严重的健康风险。与其他致病菌相比, 蜡样芽孢杆菌因其内生孢子的高传递性、高活力和高耐受性而具有更高的风险[7, 93~95]。此外, 蜡样芽孢杆菌因可与其宿主细胞持续相互作用而难以控制。传统的微生物检测方法耗时、效率低、灵敏度偏低。近年来, 研究人员开发了基于分子生物学和生物传感器的新方法, 如 LAMP、电化学生物传感器和表面离子共振等。尽管新的便携式检测方法相比目前使用的方法更具优势, 但该方法不易实现, 因而只有少数几种方法可商业化。总的来讲, 我们要充分认识蜡样芽孢杆菌的危害, 了解其毒性因子的致病机制, 尽快开发高效、快速、准确的蜡样芽孢杆菌检测的新方法、新技术, 尽量将其危害降低到最低水平, 以保障人们的生命安全。

参考文献(References):

[1] SETLOW P. Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals[J]. Journal of Applied Microbiology, 2006, 101(3): 514–525.

[2] RAMARAO N, SANCHIS V. The pore–forming haemolysins of *Bacillus cereus*: a review[J]. Toxins, 2013, 5(6): 1119–1139.

[3] TUNICK M H, VAN HEKKEN D L. Dairy products and health: recent insights[J]. Journal of Agricultural and Food Chemistry, 2015, 63(43): 9381–9388.

[4] GODOS J, TIERI M, GHELFI F, *et al*. Dairy foods and health: an umbrella review of observational studies[J]. International Journal of Food Sciences and Nutrition, 2020, 71(2): 138–151.

[5] ROMERO–ALVAREZ D, PETERSON A T, SALZER J S, *et al*. Potential distributions of *Bacillus anthracis* and *Bacillus cereus* biovar anthracis causing anthrax in Africa[J]. PLoS Neglected Tropical Diseases, 2020, 14(3): e0008131.

[6] GUO R, TIAN Y X, ZHANG H, *et al*. Biological characteristics and genetic evolutionary analysis of emerging pathogenic *Bacillus cereus* isolated from Père David's deer (*Elaphurus davidianus*)[J]. Microbial Pathogenesis, 2020, 143: 104133.

[7] LIU X Y, HU Q, XU F, *et al*. Characterization of *Bacillus cereus* in dairy products in China[J]. Toxins, 2020, 12(7): 454.

[8] GRANUM P E. Spotlight on *Bacillus cereus* and its food poisoning toxins[J]. FEMS Microbiology Letters, 2017, 364(10): fnx071.

[9] BOTTONE E J. *Bacillus cereus*, a volatile human pathogen[J]. Clinical Microbiology Reviews, 2010, 23(2): 382–398.

[10] RISHI E, RISHI P, SENGUPTA S, *et al*. Acute postoperative *Bacillus cereus* endophthalmitis mimicking toxic anterior segment syndrome[J]. Ophthalmology, 2013, 120(1): 181–185.

[11] PÓSFAY–BARBE K M, SCHRENZEL J, FREY J, *et al*. Food poisoning as a cause of acute liver failure[J]. Pediatric Infectious Disease Journal, 2008, 27(9): 846–847.

[12] DIERICK K, VAN COILLIE E, SWIECICKA I, *et al*. Fatal family outbreak of *Bacillus cereus*–associated food poisoning[J]. Journal of Clinical Microbiology, 2005, 43(8): 4277–4279.

[13] NARANJO M, DENAYER S, BOTTELDOORN N, *et al*. Sudden death of a young adult associated with *Bacillus cereus* food poisoning[J]. Journal of Clinical Microbiology, 2011, 49(12): 4379–4381.

[14] MAHLER H, PASI A, KRAMER J M, *et al*. Fulminant liver failure in association with the emetic toxin of *Bacillus cereus*[J]. The New England Journal of Medicine, 1997, 336(16): 1142–1148.

[15] BAUER T, SIPOS W, STARK T D, *et al*. First insights into within host translocation of the *Bacillus cereus* toxin cereulide using a porcine model[J]. Frontiers in Microbiology, 2018, 9: 2652.

[16] VANGOITSENHOVEN R, RONDAS D, CRÈVECOEUR I, *et al*. Foodborne cereulide causes beta–cell dysfunction and apoptosis[J]. PLoS One, 2014, 9(8): e104866.

[17] LÜCKING G, FRENZEL E, RÜTSCHLE A, *et al*. Ces locus embedded proteins control the non–ribosomal synthesis of the cereulide toxin in emetic *Bacillus cereus* on multiple levels[J]. Frontiers in Microbiology, 2015, 6: 1101.

[18] EHLING–SCHULZ M, FRENZEL E, GOHAR M. Food–bacteria interplay: pathometabolism of emetic *Bacillus cereus*[J]. Frontiers in Microbiology, 2015, 6: 704.

[19] EHLING–SCHULZ M, VUKOV N, SCHULZ A, *et al*. Identification and partial characterization of the nonribosomal peptide synthetase gene responsible for cereulide production in emetic *Bacillus cereus*[J]. Applied and Environmental Microbiology, 2005, 71(1): 105–113.

[20] EHLING–SCHULZ M, GUINEBRETIERE M H, MONTHAN A, *et al*. Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*[J]. FEMS Microbiology Letters, 2006, 260(2): 232–240.

[21] ALONZO D A, MAGARVEY N A, SCHMEING T M. Characterization of cereulide synthetase, a toxin–producing macromolecular machine[J]. PLoS One, 2015, 10(6): e0128569.

[22] DOMMEL M K, FRENZEL E, STRASSER B, *et al*. Identification of the main promoter directing cereulide biosynthesis in emetic *Bacillus cereus* and its application for real–time monitoring of ces gene expression in foods[J]. Applied and Environmental Microbiology, 2010, 76(4): 1232–1240.

[23] HOTON F M, ANDRUP L, SWIECICKA I, *et al*. The cereulide genetic determinants of emetic *Bacillus cereus* are plasmid–borne[J]. Microbiology, 2005, 151(Pt 7): 2121–2124.

[24] MIKKOLA R, SARIS N E, GRIGORIEV P A, *et al*. Ionophoretic properties and mitochondrial effects of cereulide: the emetic toxin of *B. cereus*[J]. European Journal of Biochemistry, 1999, 263(1): 112–117.

[25] ANDERSSON M A, HAKULINEN P, HONKALAMPI–HAMALAINEN U, *et al*. Toxicological profile of cereulide, the *Bacillus cereus* emetic toxin, in functional assays with human, animal and bacterial cells[J]. Toxicon, 2007, 49(3): 351–367.

[26] STARK T, MARXEN S, RUTSCHLE A, *et al*. Mass spectrometric profiling of *Bacillus cereus* strains and quantitation of the emetic toxin cereulide by means of stable isotope dilution analysis and HEp–2 bioassay[J]. Analytical and Bioanalytical Chemistry, 2013, 405(1): 191–201.

[27] HOORNSTRA D, ANDERSSON M A, MIKKOLA R, *et al*. A new method for in vitro detection of microbially produced mitochondrial toxins[J]. Toxicology in Vitro, 2003, 17(5/6): 745–751.

[28] KAWAMURA–SATO K, HIRAMA Y, AGATA N, *et al*. Quantitative analysis of cereulide, an emetic toxin of *Bacillus cereus*, by using rat liver mitochondria[J]. Microbiology and Immunology, 2005, 49(1): 25–30.

[29] DUCREST P J, PFAMMATTER S, STEPHAN D, *et al*. Rapid detection of *Bacillus* ionophore cereulide in food products[J]. Scientific Reports, 2019, 9(1): 5814.

[30] FAGERLUND A, WEEN A, LUND T, *et al*. Genetic and functional analysis of the *cytK* family of genes in *Bacillus cereus*[J]. Microbiology, 2004, 150(Pt 8): 2689–2697.

[31] DIETRICH R, JESSBERGER N, EHLING–SCHULZ M. The food poisoning toxins of *Bacillus cereus*[J]. Toxins, 2021, 13(2): 98.

[32] STENFORS ARNESEN L P, FAGERLUND A, GRANUM P E. From soil to gut: *Bacillus cereus* and its food poisoning toxins[J]. FEMS Microbiology Reviews, 2008, 32(4): 579-606.

[33] FAGERLUND A, LINDBÄCK T, GRANUM P E. *Bacillus cereus* cytotoxins Hbl, Nhe and CytK are secreted via the Sec translocation pathway[J]. BMC Microbiology, 2010, 10: 304.

[34] LUND T, DE BUYSER M L, GRANUM P E. A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis[J]. Molecular Microbiology, 2000, 38(2): 254-261.

[35] SENESI S, GHELARDI E. Production, secretion and biological activity of *Bacillus cereus* enterotoxins[J]. Toxins, 2010, 2(7): 1690-1703.

[36] GUINEBRETIÈRE M H, BROUSSOLLE V, NGUYEN-THE C. Enterotoxigenic profiles of food-poisoning and food-borne *Bacillus cereus* strains[J]. Journal of Clinical Microbiology, 2002, 40(8): 3053-3056.

[37] JEßBERGER N, KREY V M, RADEMACHER C, *et al*. From genome to toxicity: a combinatory approach highlights the complexity of enterotoxin production in *Bacillus cereus*[J]. Frontiers in Microbiology, 2015, 6: 560.

[38] LINDBÄCK T, FAGERLUND A, RØDLAND M S, *et al*. Characterization of the *Bacillus cereus* Nhe enterotoxin[J]. Microbiology, 2004, 150(Pt 12): 3959-3967.

[39] RYAN P A, MACMILLAN J D, ZILINSKAS B A. Molecular cloning and characterization of the genes encoding the L_1 and L_2 components of hemolysin BL from *Bacillus cereus*[J]. Journal of Bacteriology, 1997, 179(8): 2551-2556.

[40] WALLACE A J, STILLMAN T J, ATKINS A, *et al*. *E. coli* hemolysin E (HlyE, ClyA, SheA): X-ray crystal structure of the toxin and observation of membrane pores by electron microscopy[J]. Cell, 2000, 100(2): 265-276.

[41] LERECLUS D, AGAISSE H, GRANDVALET C, *et al*. Regulation of toxin and virulence gene transcription in *Bacillus thuringiensis*[J]. International Journal of Medical Microbiology, 2000, 290(4/5): 295-299.

[42] PHUNG D, GRANUM P E, DIETRICH R, *et al*. Inhibition of cytotoxicity by the Nhe cytotoxin of *Bacillus cereus* through the interaction of dodecyl maltoside with the NheB component[J]. FEMS Microbiology Letters, 2012, 330(2): 98-104.

[43] KUMAR T D K, MURALI H S, BATRA H V. Construction of a nontoxic chimeric protein (L_1-L_2-B) of haemolysin BL from *Bacillus cereus* and its application in HBL toxin detection[J]. Journal of Microbiological Methods, 2008, 75(3): 472-477.

[44] LUND T, GRANUM P E. Characterization of a non-haemolytic enterotoxin complex from *Bacillus cereus* isolated after a foodborne outbreak[J]. FEMS Microbiology Letters, 1996, 141(2/3): 151-156.

[45] FAGERLUND A, LINDBÄCK T, STORSET A K, *et al*. *Bacillus cereus* Nhe is a pore-forming toxin with structural and functional properties similar to the ClyA (HlyE, SheA) family of haemolysins, able to induce osmotic lysis in epithelia[J]. Microbiology, 2008, 154(Pt 3): 1554-1554.

[46] LINDBÄCK T, HARDY S P, DIETRICH R, *et al*. Cytotoxicity of the *Bacillus cereus* Nhe enterotoxin requires specific binding order of its three exoprotein components[J]. Infection and Immunity, 2010, 78(9): 3813-3821.

[47] HARDY S P, LUND T, GRANUM P E. CytK toxin of *Bacillus cereus* forms pores in planar lipid bilayers and is cytotoxic to intestinal epithelia[J]. FEMS Microbiology Letters, 2001, 197(1): 47-51.

[48] CHOMA C, GRANUM P E. The enterotoxin T (BcET) from *Bacillus cereus* can probably not contribute to food poisoning[J]. FEMS Microbiology Letters, 2002, 217(1): 115-119.

[49] TRAN S L, GUILLEMET E, GOHAR M, *et al*. CwpFM (EntFM) is a *Bacillus cereus* potential cell wall peptidase implicated in adhesion, biofilm formation, and virulence[J]. Journal of Bacteriology, 2010, 192(10): 2638-2642.

[50] AGATA N, OHTA M, ARAKAWA Y, *et al*. The *bceT* gene of *Bacillus cereus* encodes an enterotoxic protein[J]. Microbiology, 1995, 141(Pt 4): 983-988.

[51] JEßBERGER N, DIETRICH R, BOCK S, *et al*. *Bacillus cereus* enterotoxins act as major virulence factors and exhibit distinct cytotoxicity to different human cell lines[J]. Toxicon, 2014, 77: 49-57.

[52] ZHAO S J, CHEN J L, FEI P, *et al*. Prevalence, molecular characterization, and antibiotic susceptibility of *Bacillus cereus* isolated from dairy products in China[J]. Journal of Dairy Science, 2020, 103(5): 3994-4001.

[53] GAO T T, DING Y, WU Q P, *et al*. Prevalence, virulence genes, antimicrobial susceptibility, and genetic diversity of *Bacillus cereus* isolated from pasteurized milk in China[J]. Frontiers in Microbiology, 2018, 9: 533.

[54] CUI Y F, LIU X Y, DIETRICH R, *et al*. Characterization of *Bacillus cereus* isolates from local dairy farms in China[J]. FEMS Microbiology Letters, 2016, 363(12): fnw096.

[55] 孙婷婷, 王伟杰, 韩佳敏, 等. 2016年辽宁省婴幼儿配方食品及谷类辅助食品中蜡样芽胞杆菌毒力基因的鉴定[J]. 中国微生态学杂志(SUN Ting-ting, WANG Wei-jie, HAN Jia-min, *et al*. Detection of virulence genes of *Bacillus cereus* in infant formula foods and cereal-based complementary foods in Liaoning in 2016[J]. Chinese Journal of Microecology), 2018, 30(7): 791-794.

[56] LIN Y Y, REN F Z, ZHAO L, *et al*. Genotypes and the persistence survival phenotypes of *Bacillus cereus* isolated from UHT milk processing lines[J]. Food Control, 2017, 82: 48-56.

[57] 谢爱蓉, 吴可可, 李毅, 等. 温州市市售奶粉蜡样芽胞杆菌污染情况调查及毒力基因分析[J]. 中国卫生检验杂志(XIE Ai-rong, WU Ke-ke, LI Yi, *et al*. Contamination survey and toxin genes distribution analysis of *Bacillus cereus* in milk powder in Wenzhou[J]. Chinese Journal of Health Laboratory Technology), 2018, 28(23): 2930-2932.

[58] FEI P, YUAN X J, ZHAO S J, *et al*. Prevalence and genetic diversity of *Bacillus cereus* isolated from raw milk and cattle farm environments[J]. Current Microbiology, 2019, 76(11): 1355-1360.

[59] MA H M, LI J, XI X X, *et al*. Evaluation of bacterial contamination in goat milk powder using PacBio single molecule real-time sequencing and droplet digital PCR[J]. Journal of Food Protection, 2018, 81(11): 1791-1799.

[60] 闫韶飞, 闫旭, 甘辛, 等. 我国市售婴儿配方乳粉中蜡样芽胞杆菌污染及其毒力基因调查[J]. 中国食品卫生杂志(YAN Shao-fei, YAN Xu, GAN Xin, *et al*. Survey on contamination of *Bacillus cereus* and its virulence gene profiles isolated from retail infant formula in China[J]. Chinese Journal of Food Hygiene), 2015, 27(3): 286-291.

[61] ZHUANG K J, LI H F, ZHANG Z W, *et al*. Typing and evaluating heat resistance of *Bacillus cereus sensu stricto* isolated from the processing environment of powdered infant formula[J]. Journal of Dairy Science, 2019, 102(9): 7781-7793.

[62] JACKSON E E, ERTEN E S, MADDI N, *et al*. Detection and enumeration of four foodborne pathogens in raw commingled silo milk in the United States[J]. Journal of Food Protection, 2012, 75(8): 1382-1393.

[63] ZHOU G P, ZHENG D S, DOU L N, *et al*. Occurrence of psychrotolerant *Bacillus cereus* group strains in ice creams[J]. International Journal of Food Microbiology, 2010, 137(2/3): 143-146.

[64] KONG L, YU S B, YUAN X M, *et al*. An investigation on the occurrence and molecular characterization of *Bacillus cereus* in meat and meat products in China[J]. Foodborne Pathogens and Disease, 2021, 18(5): 306-314.

[65] RODRIGO D, ROSELL C M, MARTINEZ A. Risk of *Bacillus cereus* in relation to rice and derivatives[J]. Foods, 2021, 10(2): 302.

[66] ELLOUZE M, BUSS DA SILVA N, ROUZEAU-SZYNALSKI K, *et al*. Modeling *Bacillus cereus* growth and cereulide formation in cereal-, dairy-, meat-, vegetable-based food and culture medium[J]. Frontiers in Microbiology, 2021, 12: 639546.

[67] 张慧娟, 张恩民, 贺金荣, 等. 蜡样芽胞杆菌在不同标本中的毒力基因分布特征[J]. 中国人兽共患病学报(ZHANG Hui-juan, ZHANG En-min, HE Jin-rong, *et al*. Distribution of virulence genes of *Bacillus cereus* in several specimens[J]. Chinese Journal of Zoonoses), 2019, 35(8): 683-687.

[68] VIDIC J, CHAIX C, MANZANO M, *et al*. Food sensing: detection of *Bacillus cereus* spores in dairy products[J]. Biosensors, 2020, 10(3): 15.

[69] DIETRICH R, FELLA C, STRICH S, *et al*. Production and characterization of monoclonal antibodies against the hemolysin BL enterotoxin complex produced by *Bacillus cereus*[J]. Applied and Environmental Microbiology, 1999, 65(10): 4470-4474.

[70] DIETRICH R, MORAVEK M, BÜRK C, *et al*. Production and characterization of antibodies against each of the three subunits of the *Bacillus cereus* nonhemolytic enterotoxin complex[J]. Applied and Environmental Microbiology, 2005, 71(12): 8214-8220.

(下转第 487 页)

[10] 杨仕华, 程本义, 沈伟峰, 等. 中国两系杂交水稻选育与应用进展[J]. 杂交水稻(YANG Shi–hua, CHENG Ben–yi, SHEN Wei–feng, *et al*. Progress of application and breeding on two–line hybrid rice in China[J]. Hybrid Rice), 2009, 24(1): 5–9.

[11] 牟同敏. 中国两系法杂交水稻研究进展和展望[J]. 科学通报(MOU Tong–min. The research progress and prospects of two–line hybrid rice in China[J]. Chinese Science Bulletin), 2016, 61(35): 3761–3769.

[12] 王际凤. 三系两系杂交水稻比较研究[J]. 山地农业生物学报(WANG Ji–feng. Comparative study on hybrid rice of three lines and two lines[J]. Journal of Mountain Agriculture and Biology), 2017, 36(4): 1–8.

[13] 牟同敏, 杨国才, 曹兵, 等. 8 个籼型水稻环境敏感核不育系的育性转换特性研究[J]. 植物学报(英) (MOU Tong–min, YANG Guo–cai, CAO Bing, *et al*. Characterization of fertility alteration in 8 environment–sensitive genic male–sterile lines of *indica* rice[J]. Journal of Integrative Plant Biology), 2001, 43(3): 238–242.

[14] 肖层林, 涂娥英, 刘逊. 我国水稻两用核不育系研究进展[J]. 种子(XIAO Ceng–lin, TU E–ying, LIU Xun, *et al*. Research progress of rice dual–use male sterile lines in China[J]. Seed), 1995 (2): 37–39.

[15] 邱振国.光温敏核不育水稻研究及利用进展[J]. 安徽农业科学(QIU Zhen–guo. Advances in research and utilization of photothermal–sensitive genic male sterile rice[J]. Journal of Anhui Agricultural Sciences), 2006, 34(20): 5228–5230, 5243.

[16] 舒志芬, 陈勇, 刘钊, 等. 5 个水稻光温敏核不育系柱头特性与异交结实率的关系[J]. 作物研究(SHU Zhi–Fen, CHEN Yong, LIU Zhao, *et al*. Correlation between outcrossing characteristics and stigma feature of 5 PTGMS lines[J]. Crop Research), 2015, 29:343–347.

[17] 田大成, 黄三奎, 段永国, 等. 水稻不育系花时和受粉时间与异交结实率的关系[J]. 杂交水稻(TIAN Da–cheng, HUANG San–kui, DUAN Yong–guo, *et al*. The relationship between flowering and pollination time and outcrossing rate of male sterile lines in hybrid rice seed production[J]. Hybrid Rice), 2004, 19 (3): 50–54.

[18] 杨保汉. 不育系柱头外露率及其结实率研究[J]. 杂交水稻(YANG Bao–han. Studies on stigma exsertion rate and outcrossing rate of CMS lines in rice[J]. Hybrid Rice), 1997, 12(1): 13–15.

[19] ZHOU H, ZHOU M, YANG Y Z, *et al*. RNase Z^{S1} processes Ub_{L40} mRNAs and controls thermosensitive genic male sterility in rice[J]. Nature Communications, 2014, 5: 4884.

[20] MEI M, CHEN L, ZHANG Z, *et al*. *Pms*3 is the locus causing the original photoperiod–sensitive male sterility mutation of ′Nong–ken 58S′[J]. Science in China. Series C, Life Sciences, 1999, 42(3): 316–322.

[21] FAN Y R, YANG J Y, MATHIONI S M, *et al*. *PMS1T*, producing phased small–interfering RNAs, regulates photoperiod–sensitive male sterility in rice[J]. Proceedings of the National Academy of Sciences USA, 2016, 113(52): 15144–15149.

(上接第 478 页)

[71] DIDIER A, DIETRICH R, GRUBER S, *et al*. Monoclonal antibodies neutralize *Bacillus cereus* Nhe enterotoxin by inhibiting ordered binding of its three exoprotein components[J]. Infection and Immunity, 2012, 80(2): 832–838.

[72] TAKAHASHI N, NAGAI S, FUJITA A, *et al*. Discrimination of psychrotolerant *Bacillus cereus* group based on MALDI–TOF MS analysis of ribosomal subunit proteins[J]. Food Microbiology, 2020, 91: 103542.

[73] YAMAGUCHI M, KAWAI T, KITAGAWA M, *et al*. A new method for rapid and quantitative detection of the *Bacillus cereus* emetic toxin cereulide in food products by liquid chromatography–tandem mass spectrometry analysis[J]. Food Microbiology, 2013, 34(1): 29–37.

[74] CARBONNELLE E, GROHS P, JACQUIER H, *et al*. Robustness of two MALDI–TOF mass spectrometry systems for bacterial identification[J]. Journal of Microbiological Methods, 2012, 89(2): 133–136.

[75] ZHANG J, DI B, SHAN H B, *et al*. Rapid detection of *Bacillus cereus* using cross–priming amplification[J]. Journal of Food Protection, 2019, 82(10): 1744–1750.

[76] HAKOVIRTA J R, PREZIOSO S, HODGE D, *et al*. Identification and analysis of informative single nucleotide polymorphisms in 16S rRNA gene sequences of the *Bacillus cereus* group[J]. Journal of Clinical Microbiology, 2016, 54(11): 2749–2756.

[77] WEI S, CHELLIAH R, PARK B J, *et al*. Molecular discrimination of *Bacillus cereus* group species in foods (lettuce, spinach, and kimbap) using quantitative real–time PCR targeting *groEL* and *gyrB*[J]. Microbial Pathogenesis, 2018, 115: 312–320.

[78] ANTOLINOS V, FERNÁNDEZ P S, ROS–CHUMILLAS M, *et al*. Development of a high–resolution melting–based approach for efficient differentiation among *Bacillus cereus* group isolates[J]. Foodborne Pathogens and Disease, 2012, 9(9): 777–785.

[79] PORCELLATO D, NARVHUS J, SKEIE S B. Detection and quantification of *Bacillus cereus* group in milk by droplet digital PCR[J]. Journal of Microbiological Methods, 2016, 127: 1–6.

[80] 黄晶菁, 罗静, 何宏轩. 蜡样芽孢杆菌检测方法研究进展[J]. 中国畜牧兽医(HUANG Jing–jing, LUO Jing, HE Hong–xuan. Research progress on detection methods for *Bacillus cereus*[J]. China Animal Husbandry & Veterinary Medicine), 2018, 45(3): 635–642.

[81] YU S, YAN L N, WU X, *et al*. Multiplex PCR coupled with propidium monoazide for the detection of viable *Cronobacter sakazakii*, *Bacillus cereus*, and *Salmonella* spp. in milk and milk products[J]. Journal of Dairy Science, 2017, 100(10): 7874–7882.

[82] VIDIC J, VIZZINI P, MANZANO M, *et al*. Point–of–need DNA testing for detection of foodborne pathogenic bacteria[J]. Sensors, 2019, 19(5): 1100.

[83] WONG Y P, OTHMAN S, LAU Y L, *et al*. Loop–mediated isothermal amplification (LAMP): a versatile technique for detection of micro–organisms[J]. Journal of Applied Microbiology, 2018, 124(3): 626–643.

[84] XIA Y, LIU Z H, YAN S Q, *et al*. Identifying multiple bacterial pathogens by loop–mediated isothermal amplification on a rotate & react slipchip[J]. Sensors and Actuators B: Chemical, 2016, 228: 491–499.

[85] MANZANO M, VIEZZI S, MAZERAT S, *et al*. Rapid and label–free electrochemical DNA biosensor for detecting hepatitis A virus[J]. Biosensors & Bioelectronics, 2018, 100: 89–95.

[86] VIDIC J, MANZANO M, CHANG C M, *et al*. Advanced biosensors for detection of pathogens related to livestock and poultry[J]. Veterinary Research, 2017, 48(1): 11.

[87] VIZZINI P, BRAIDOT M, VIDIC J, *et al*. Electrochemical and optical biosensors for the detection of *Campylobacter* and *Listeria*: an update look[J]. Micromachines, 2019, 10(8): 500.

[88] MAZZARACCHIO V, NEAGU D, PORCHETTA A, *et al*. A label–free impedimetric aptasensor for the detection of *Bacillus anthracis* spore simulant[J]. Biosensors & Bioelectronics, 2019, 126: 640–646.

[89] KONG M, SIM J, KANG T, *et al*. A novel and highly specific phage endolysin cell wall binding domain for detection of *Bacillus cereus*[J]. European Biophysics Journal, 2015, 44(6): 437–446.

[90] DAL PERARO M, VAN DER GOOT F G. Pore–forming toxins: ancient, but never really out of fashion[J]. Nature Reviews Microbiology, 2016, 14(2): 77–92.

[91] LEE N K, KIM W S, PAIK H D. *Bacillus strains* as human probiotics: characterization, safety, microbiome, and probiotic carrier[J]. Food Science and Biotechnology, 2019, 28(5): 1297–1305.

[92] DUC LE H, HONG H A, BARBOSA T M, *et al*. Characterization of *Bacillus* probiotics available for human use[J]. Applied and Environmental Microbiology, 2004, 70(4): 2161–2171.

[93] JEŻEWSKA–FRĄCKOWIAK J, SEROCZYŃSKA K, BANASZCZYK J, *et al*. The promises and risks of probiotic *Bacillus* species[J]. Acta Biochimica Polonica, 2018, 65(4): 509–519.

[94] HUANG Y Y, FLINT S H, PALMER J S. *Bacillus cereus* spores and toxins: the potential role of biofilms[J]. Food Microbiology, 2020, 90: 103493.

[95] SHAHEEN R, SVENSSON B, ANDERSSON M A, *et al*. Persistence strategies of *Bacillus cereus* spores isolated from dairy silo tanks[J]. Food Microbiology, 2010, 27(3): 347–355.