梁晋刚,刘鹏程,张秀杰. 基于 16S rDNA 高通量测序技术研究转基因作物对根际细菌群落结构的影响[J]. 江苏农业科学,2018,46(6):5 - 8.
doi:10.15889/j.issn.1002 - 1302.2018.06.002

基于 16S rDNA 高通量测序技术研究转基因作物对根际细菌群落结构的影响

梁晋刚, 刘鹏程, 张秀杰
(农业部科技发展中心,北京 100176)

摘要:近几年,随着分子生物学的发展,以 16S rDNA 基因为分子标记的高通量测序技术凭借低成本、高通量、流程自动化的优势为研究转基因作物对根际细菌群落结构的影响提供了新的技术平台。归纳介绍了基于 16S rDNA 扩增子高通量测序技术在评价转基因作物对根际细菌群落结构及多样性等方面应用的研究进展,总结了 16S rDNA 扩增子高通量测序技术应用中存在的问题,并分析展望其在土壤微生物多样性研究中的发展趋势。

关键词:转基因作物;根际细菌;16S rDNA 基因;高通量测序;土壤微生物
中图分类号: Q785　　**文献标志码**: A　　**文章编号**:1002 - 1302(2018)06 - 0005 - 04

几十年来,转基因作物释放对生态环境的影响一直是社会公众关注的一个重要问题,包括对土壤生态环境的影响[1-4]。土壤细菌是土壤中数量最丰富、分布最广泛的微生物类群,广泛参与有机物质分解、养分释放和能量转移等过程,其多样性和活性是保持土壤生态系统稳定的基础之一。根际细菌对外界干扰比较敏感,不仅受到植物基因型影响,也受土壤类型、农业耕作管理、季节变化等影响[5-9]。准确检测转基因作物对根际细菌群落组成和结构的影响,是评估转基因作物释放后土壤生态风险的重要基础[10-11]。

近年来,分子生物学的不断发展为根际细菌群落结构的研究提供了新思路,其中,以编码 rRNA 的 rDNA 基因为基础的高通量测序技术尤受青睐,该技术准确、灵敏,具有较高的通量,已经被广泛应用于土壤微生物遗传多样性的研究领域中[12-15]。目前,市面上常用的用于研究环境微生物的高通量测序平台有 Illumina MiSeq、Roche 454。针对细菌的 16S rDNA 基因序列分析,MiSeq 凭借其测序读段长、测序周期短、通量大等特点,成为使用最为普遍的测序平台[16]。

16S 核糖体 RNA(16S ribosomal RNA,简称 16S rRNA)是原核生物的核糖体中 30S 亚基的组成部分。由于不同种的真细菌与古细菌之间的 16S rRNA 编码基因 16S rDNA 是高度保守的,且 16S rDNA 序列长度适中(1 540 bp),包括 9 个高可变区(hypervariable region)和 8 个保守区(constant region),因此,常被用于对各种生物进行系统发生学方面的研究[17]。利用 16S rDNA 的通用引物进行 PCR 扩增,获得绝大部分微生物 16S rDNA 高可变区的扩增产物,构建扩增产物的文库,运用 Illumina MiSeq 平台或 Roche 454 平台进行高通量测序,然后比较分析测序数据,对土壤微生物群落的多样性进行研究[18-20]。

目前,已有研究者利用 16S rDNA 测序技术来研究各种环境样品中微生物的多样性[21-30]。迄今为止,大部分的研究结果均表明,转基因作物的释放对土壤微生物没有影响或有很小的影响,且影响是短暂的[31-42]。

1　转基因大豆对根际细菌群落结构的影响

Liang 等通过收集高产高蛋氨酸转基因大豆 ZD91 和其对应的非转基因大豆 ZD 在鼓粒期时的根际土壤样品,采用 Roche 454 焦磷酸测序技术对大豆根际土壤细菌 16S rDNA V4 区序列扩增子进行高通量测序及分析,发现在这种转基因大豆和其亲本的根际土壤中,均存在酸杆菌门(Acidobacteria)、变形菌门(Proteobacteria)、拟杆菌门(Bacteroidetes)、放线菌门(Actinobacteria)、厚壁菌门(Firmicutes)等细菌类群,但两者的细菌群落结构无显著性差异[43]。Lu 等采用 Illumina MiSeq 平台对 16S rDNA V4 区序列扩增子进行高通量测序,发现抗草甘膦除草剂转基因大豆

收稿日期:2017 - 10 - 16
基金项目:转基因生物新品种培育科技重大专项(编号:2016ZX08012003、2016ZX08011 - 003)。
作者简介:梁晋刚(1987—),男,山西阳泉人,博士,农艺师,主要从事转基因生物安全评价与检测研究。E - mail:382408162@qq.com。
通信作者:张秀杰,硕士,副研究员,主要从事转基因生物安全评价与检测研究。E - mail:zhxj7410@sina.com。

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ZUTS31 在鼓粒期对根际细菌群落的 α、β 多样性无显著性影响[44]。Lu 等利用同样的方法,发现抗除草剂转基因大豆 NZL06 - 698 对土壤微生物群落,特别是对固氮菌群产生的影响,表现为微生物物种丰度和多样性降低,固氮菌群丰度降低[45]。

2　转基因玉米对根际细菌群落结构的影响

Barriuso 等连续 4 年收集根际土壤样品,并采用 Roche 454 焦磷酸测序技术对根际土壤细菌 16S rDNA V6 区序列扩增子进行高通量测序及分析,发现抗虫玉米 MON810 与对照玉米品种相比,抗虫玉米 MON810 对根际细菌群落结构无显著性影响[46]。Barriuso 等采用相同的方法,连续 3 年监测喷施草甘膦对耐除草剂玉米 NK603 根际细菌群落结构的影响。结果表明,草甘膦对玉米 NK603 根际细菌群落结构无显著性影响[47]。Dohrmann 等利用 Roche 454 焦磷酸测序技术测定了细菌 16S rDNA V7 + V8 区序列,发现抗虫玉米 MON 89034 × MON 88017 对根际细菌群落结构无显著性影响[48]。

3　其他转基因植物对根际细菌群落结构的影响

Sohn 等应用 454 平台测序测定 16S rRNA 基因 V3 - V4 变异区序列,发现白藜芦醇强化转基因水稻 RS526 对根际土壤细菌群落结构无显著性影响[49]。Zhu 等利用 Illumina MiSeq 平台测定 16S rRNA 基因 V4 区序列,分析了种植 8 年转基因白杨树 D520、D521 后的根际土壤细菌结构,发现转基因白杨树对细菌多样性和群落结构无显著性影响[50]。Debruyn 等利用 Illumina MiSeq 平台测定 16S rRNA 基因 V4 区,发现木质素含量降低的转基因柳枝稷对根际细菌多样性、丰富度和群落组成均无显著性影响[51]。

4　总结与展望

16S rDNA 相对分子量大小适中,突变率小,基于 16S rDNA 的高通量测序技术已被广泛应用于微生物系统进化、分类及多样性分析研究中。在 16S rDNA 高可变区的选择方面还存在一定争议,由于目前二代高通量测序的读长限制,仅能针对 16S rDNA 的某一段可变区进行测序,有的选择测单可变区(V3、V4、V5、V6、V7),有的选择对连续可变区(V1 - V2、V1 - V3、V3 - V4、V3 - V5、V4 - V5、V4 - V6、V5 - V6、V6 - V7、V5 - V8、V6 - V8、V7 - V8、V1 - V8、V5 - V9、V6 - V9、nearly full - length)进行 16S rDNA 测序[52-65]。Sun 等研究比较了 16S 不同区域的测序结果,发现 V4 - V5 区(515F/907R 或 515F/926R)是最佳的测序区域,造成的基因组内异质性最小,该区域是 Roche 454 测序时代最常用的区域[66]。在 Illumina 时代,由于平台测序长度的限制,V4 单区测序(515F/806R)被更为广泛地使用,同时这对引物也是地球微生物组计划(Earth Microbiome Project,简称 EMP)中推荐使用的引物序列,它们将是 16S 测序的主力[67]。

16S rDNA 高通量测序技术对土壤微生物物种多样性、结构多样性、功能多样性和遗传多样性研究的迅猛发展起到了重要作用[68]。然而,这种方法对于转基因的研究来说,存在着不能检测受体、转化体、喷洒除草剂转化体对各类土壤微生物功能(包括氮循环、磷循环、碳循环等)的各类基因丰度等影响的缺点[16]。同样地,16S rDNA 扩增子高通量测序本身也常受到一些条件的影响,如 PCR 扩增体系对高通量测序的影响,PCR 扩增偏好性对测序的影响,PCR 扩增循环数对原始物种相对丰度的影响等[69]。在未来的研究中,16S rDNA 高通量测序技术在转基因作物对土壤细菌影响的研究中的运用会越来越普及,随着测序成本明显下降,土壤宏基因组测序为研究微生物群落结构与功能等提供了一种新的选择,相比 16S rDNA 高通量测序,该测序方法能够更全面地分析微生物的物种及基因等方面的多样性。由于土壤微生物群落组成复杂,在实际应用中,应该综合考虑各研究方法的优缺点,取长补短,以便在土壤微生物多样性研究中获得更为全面且准确的信息,尽快建立对土壤生态系统安全的评价技术体系,并对转基因作物的环境安全性作出全面、科学、客观的评价[70-72]。

致谢:感谢南京大学生命科学学院陆桂华副研究员、南京农业大学植物保护学院顾沁博士在论文写作过程中给予的指导和帮助。

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